ApoA-1 in Diabetes: Damaged Goods

Daniel S. Ory and Jean E. Schaffer

Diabetes is a major risk factor for the development of atherosclerosis. In addition to increased risk of stroke, myocardial infarction, and peripheral vascular disease, diabetics suffer from a particularly aggressive form of atherosclerosis with greater in-hospital mortality following myocardial infarction and a higher incidence of heart failure, if they survive (1–5). While diabetics often have other accompanying risk factors for atherosclerosis (e.g., hypertension, hypercholesterolemia, obesity), the additional risk conferred by diabetes and the particularly aggressive vascular and myocardial disease that affects diabetics suggest that diabetes-associated atherosclerosis involves unique pathogenic mechanisms.

The systemic metabolic disturbances of diabetes, including hyperglycemia and hyperlipidemia, likely play a central role in the pathogenesis of diabetes-associated atherosclerosis through the generation of oxidative stress. Hyperglycemia causes increased flux through the polyol pathway, formation of advanced glycation end products, activation of protein kinase C isoforms, and increased hexosamine pathway flux, all of which may contribute to increased oxidative stress (4–6). Excessive free fatty acids delivered to nonadipose tissues can lead to reactive oxygen species (ROS) formation through cycles of oxidative phosphorylation, activation of NADPH oxidase, and alterations in mitochondrial structure that precipitate ROS production (7–9). In addition to evidence for activation of these pathways in cultured endothelial cells, human studies support the notion of increased systemic oxidative stress in diabetic subjects in whom increased circulating hexosamine pathway flux, all of which may contribute to increased oxidative stress (10). The effects of oxidative stress in diabetes on both the vascular wall and lipoproteins in the circulation may promote atherosclerosis.

In this issue of Diabetes, Jaleel et al. (11) provide intriguing evidence that poor glycemic control in type 1 diabetes is associated with accelerated oxidative damage to apolipoprotein (apo) A-1. These investigators adapted a pulse-chase approach, classically used in cell culture experiments, to label newly synthesized proteins with 13C-phenylalanine in human subjects. They then analyzed various plasma apoA-1 isoforms by two-dimensional gel separation and mass spectrometry. This approach enabled quantification of isotopic enrichment in newly synthesized forms of the protein containing the propeptide and in more mature cleaved forms, which together form a charge train of five spots in two-dimensional gel analyses. As expected, isotopic enrichment hours after the stable isotope pulse was highest in the immature forms, and over the course of 10 days, “chased” into more mature forms of the protein lacking the propeptide. Importantly, the older forms of apoA-1 accumulated significantly more evidence of damage including deamidation, oxidation, and carbonylation of amino acids, post-translational modifications that likely contributed to their altered migration in isoelectric focusing. Although the apoA-1 profile of type 1 diabetics during insulin infusion was indistinguishable from that of control subjects, type 1 diabetics deprived of insulin demonstrated increased oxidative damage to newly synthesized apoA-1 (Fig. 1).

These findings add to a growing body of molecular evidence for how the oxidative stress that accompanies poor metabolic control impacts physiology. It has long been appreciated that ROS can initiate damage to the nucleic acids, membranes, and proteins of cells. It should not be surprising then that similar damage can affect plasma proteins such as apoA-1. Transcriptional, post-translational, and signaling mechanisms have been well described in studies of the cellular response to oxidative stress (12–14). Given that apoA-1 is a major component of HDLs, which protect against atherosclerosis by facilitating the removal of cholesterol from macrophages in the artery wall and promoting reverse cholesterol transport, obvious extensions of this work will be to determine whether the changes observed by Jaleel et al. in apoA-1 forms are due directly to oxidative stress (e.g., attenuated following anti-oxidant treatment), with which HDL subclasses the damaged apoA-1 associates, and whether the altered forms of apoA-1 affect HDL clearance or function. The former will provide mechanistic insight into the etiology of these changes. The latter two aspects have the potential to functionally link the investigators’ biochemical observations to increased cardiovascular risk in diabetes.

While low plasma HDL is an independent risk factor for coronary artery disease (15,16), it is increasingly clear that perturbations in HDL metabolism can alter HDL function and promote atherosclerosis independent of plasma HDL levels (17–19). In fact, HDL cholesterol levels alone are insufficient to capture the functional variation in HDL particles and the associated cardiovascular risk for individual subjects (20). Together with the failure of HDL-raising therapy in recent clinical trials to reduce cardiovascular events (21), these findings suggest that the functional competence of HDL may be as important as absolute plasma HDL levels. It is likely that an important pathway for the generation of dysfunctional HDL is through oxidative damage, such as that precipitated by hyperglycemia and hyperlipidemia (22).

The damage to apoA-1 described in the accompanying original article adds to an expanding list of HDL alterations that may impair its function in vivo. HDL-associated
paraoxonase-1 (PON1), which is principally responsible for the anti-oxidant properties of HDL that prevent LDL oxidation, is reduced in diabetic subjects and is associated with defective anti-oxidant capacity (23,24). HDL anti-oxidant activity is further impaired by the formation of advanced glycation end products that interfere with PON1 activity and reduce cholesterol efflux to HDL (25,26).

In vitro oxidation of apoA1 has been shown to impair the ability of HDL to activate lecithin:cholesterol acyltransferase, the enzyme responsible for converting nascent HDL into mature, cholesteryl ester-rich HDL, and to interact with ATP-binding cassette transporter A1 to facilitate cholesterol export (27–29). Disruption of this critical step in the reverse cholesterol transport pathway is likely to have profound effects on the mobilization of cholesterol from vascular tissues. Beyond analyses such as these, proteomic examination of HDL is likely to identify changes in additional proteins that impact lipoprotein function in the setting of poor metabolic control in diabetes. Moreover, examination of the lipid constituents of the HDL particles, which are similarly susceptible to oxidation, is likely to provide equally important insights into HDL dysfunction and increased susceptibility to atherosclerosis in diabetes.

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

REFERENCES
1. Kannel WB, McGee DL. Diabetes and cardiovascular disease: the Framingham study. JAMA 1979;241:2035–2038
2. Abbott RD, Donahue RP, Kannel WB, Wilson PW. The impact of diabetes on survival following myocardial infarction in men vs women: the Framingham Study. JAMA 1988;260:3456–3460
3. Miettinen H, Lehto S, Salomaa V, Mäkinnen M, Mihailescu-Grohotan K, Wielage H. Diabetes and myocardial infarction: the FINMONICA Myocardial Infarction Register Study Group. Diabetes Care 1998;21:69–75
4. Kannel WB, McGee DL. Diabetes and cardiovascular disease: the Framingham study. JAMA 1979;241:2035–2038
5. Miettinen H, Lehto S, Salomaa V, Mäkinnen M, Mihailescu-Grohotan K, Wielage H. Diabetes and myocardial infarction: the FINMONICA Myocardial Infarction Register Study Group. Diabetes Care 1998;21:69–75
6. Kannel WB, McGee DL. Diabetes and cardiovascular disease: the Framingham study. JAMA 1979;241:2035–2038
7. Cacicedo JM, Benjachareewong S, Chou E, Ruderman NB, Ido Y. Palmitate-induced apoptosis in cultured bovine retinal pericytes: roles of NAD(P)H oxidase, oxidant stress, and ceramide. Diabetes 2005;54:1838–1845
8. Inoguchi T, Li P, Uneda F, Yu HY, Kakimoto M, Inamura M, Aoki T, Etoh T, Hashimoto T, Naruse M, Sano H, Utsumi H, Nawata H. High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C–dependent activation of NAD(P)H oxidase in cultured vascular cells. Diabetes 2000;49:1039–1045
9. Ostrander DB, Sparagana GC, Amoscasto AA, McMillin JB, Dowhan W. Decreased cardiolipin synthesis corresponds with cytochrome C release in palmitate-induced cardiomyocyte apoptosis. J Biol Chem 2001;276:38061–38067
10. Ceriello A, Quagliaro L, Piccoli L, Assaloni R, Da Ros R, Maier A, Esposito K, Giugliano D. Effect of postprandial hypertriglyceridemia and hyperglycemia on circulating adhesion molecules and oxidative stress generation and the possible role of simvastatin treatment. Diabetes 2004;53:701–710
11. Jaleel A, Henderson GC, Madden BJ, Kraus DS, Morse D, Gopal S, Nair KS. Identification of de novo synthesized and relatively older proteins: accelerated oxidative damage to de novo synthesized apoA1 in type 1 diabetes. Diabetes 2010;59:2366–2374
12. Houstis N, Rosen ED, Lander ES. Reactive oxygen species have a causal role in multiple forms of insulin resistance. Nature 2006;440:944–948
13. Han ES, Muller FL, Perea VI, Qi W, Liang H, Xi L, Fu C, Doyle E, Hickey M, Cornell J, Epstein CJ, Roberts LJ, Van Remmen H, Richardson A. The in vivo gene expression signature of oxidative stress. Physiol Genomics 2008;34:112–126
14. Bowerman B. Cell biology. Oxidative stress and cancer: a beta-catenin convergence. Science 2005;308:1119–1120
15. Gordon DJ, Rifkind BM. High-density lipoprotein–the clinical implications of recent studies. N Engl J Med 1989;321:1311–1316
16. Turner RC, Miller H, Neil HA, Stratton IM, Manley SE, Matthews DR, Holman RR. Risk factors for coronary artery disease in non-insulin dependent diabetes mellitus: United Kingdom Prospective Diabetes Study (UKPDS). BMJ 1998;316:823–828
17. Barter PJ, Nicholls S, Rye KA, Anantharamaiah GM, Navab M, Fogelman AM. Antiinflammatory properties of HDL. J Lipid Res 2006;116:1435–1442
18. Merva R, Rader DJ. Laboratory assessment of HDL heterogeneity and function. Clin Chem 2008;54:788–800
19. Rader DJ. Illuminating HDL: is it still a viable therapeutic target? N Engl J Med 2007;357:2180–2183
20. Daugherty A, Dunn JL, Rateri DL, Heinecke JW. Myeloperoxidase, a catalyst for lipoprotein oxidation, is expressed in human atherosclerotic lesions. J Clin Invest 1993;94:437–444
21. Boermeester MA, Vletter MM, van der Velden MT, van der Woude J, van Bavel B, van der Bijl PAM. Oxidative stress, inflammation, and atherosclerosis. J Intern Med 2005;258:28–49
22. Movva R, Rader DJ. Laboratory assessment of HDL heterogeneity and function. Clin Chem 2008;54:788–800
23. Rader DJ. Oxidation and inflammation. Annu Rev Med 2008;59:85–96
24. Boermeester MA, Vletter MM, van der Velden MT, van der Woude J, van der Bijl PAM. Oxidative stress, inflammation, and atherosclerosis. J Intern Med 2005;258:28–49
25. Moveva R, Rader DJ. Laboratory assessment of HDL heterogeneity and function. Clin Chem 2008;54:788–800
26. Rader DJ. Oxidation and inflammation. Annu Rev Med 2008;59:85–96
27. Zhou H, Tan KC, Shi SW, Wong Y. Increased serum advanced glycation end products are associated with impairment in HDL antioxidative capacity in diabetic nephropathy. Nephrol Dial Transplant 2008;23:927–933
28. Shao B, Pennathur S, Paganí I, Oda MN, Wittum JL, Oram JF, Heinecke JW. Myeloperoxidase impairs the initial interactions with ABCA1 required for signaling and cholesterol efflux. J Lipid Res 2010;51:1849–1858
29. Shao B, Cavigiolio G, Brot N, Oda MN, Heinecke JW. Methionine oxidation impairs reverse cholesterol transport by apolipoprotein A-I. Proc Natl Acad Sci U S A 2008;105:12224–12229