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 (1–3). 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 levels of adhesion molecules and oxidized lipids correlate with increases in A1C and hypertriglyceridemia (10). The effects of oxidative stress in diabetes on both the vascular wall and lipoproteins in the circulation may promote atherogenesis.

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 ^{13}C-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

From the Diabetic Cardiovascular Disease Center and the Department of Medicine, Washington University School of Medicine, St. Louis, Missouri.
Corresponding author: Jean E. Schaffer, jschaff@wustl.edu.
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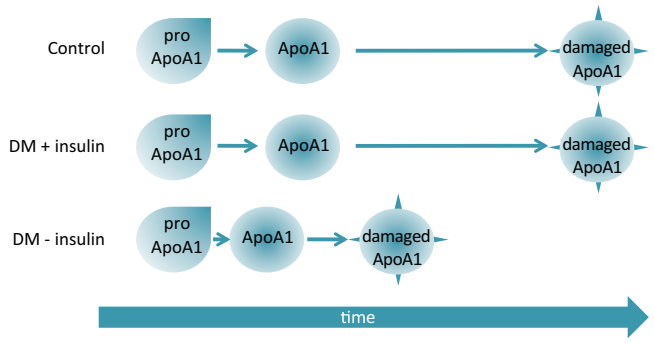

FIG. 1. Accelerated oxidative damage to apoA-1 in poorly controlled diabetes. ApoA-1 is initially synthesized with a propeptide that is cleaved (pro-apoA-1 to apoA-1). In the circulation, apoA-1 accumulates oxidative modifications (apoA-1 to damaged apoA-1). Stable isotope labeling demonstrates that in the setting of diabetes (DM) and acute insulin withdrawal, this process is accelerated.

paraoxanase-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 apoA-I 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.

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