commentary

HDL, lipid peroxidation, and atherosclerosis[1]

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Genetic, clinical, and pharmacological studies implicate elevated levels of LDL in the pathogenesis of atherosclerotic vascular disease, the leading cause of death in industrialized societies (1). Paradoxically, native LDL fails to exert potentially atherogenic effects in vitro, suggesting that it must be modified to promote vascular disease. Indeed, many lines of evidence support the LDL oxidation hypothesis, which suggests that oxidative damage to LDL is one important mechanism for rendering lipoproteins atherogenic (as reviewed in Refs. 2, 3).

In contrast to LDL, HDL, the beneficial form of blood cholesterol, protects the artery wall from atherosclerosis. Even in individuals whose LDL levels are low, HDL remains a strong independent predictor of coronary artery disease (CAD) risk (4, 5). The strong relationship between low levels of HDL and the risk for atherosclerosis and CAD has been attributed to several distinct mechanisms. More than 30 years ago, Glomset (6) proposed that HDL transfers cholesterol from peripheral tissues to the liver, where metabolites of the sterol are excreted into the bile. Subsequent studies showed that HDL accepts cholesterol from macrophage foam cells, the cellular hallmark of the atherosclerotic lesion. Therefore, HDL might be cardioprotective because it prevents cholesterol accumulation in cells of the artery wall (7).

Animal and human studies have raised the possibility that HDL also slows vascular disease by blocking inflammation (8). For example, hypercholesterolemic mice deficient in apoA-I develop systemic inflammation, and recombinant HDL blocks vascular inflammation in rabbits (9–11). One potential mechanism involves detoxification of lipid hydroperoxides, which are potentially atherogenic. Enzymes carried by HDL, including paraoxonase-1 (PON-1), LCAT, and lipoprotein-associated phospholipase A_2, have been proposed to degrade lipid oxidation products (12–14). Collectively, these observations suggest that HDL's antioxidant properties may make important contributions to its antiatherogenic and anti-inflammatory properties.

Lipid hydroperoxides are the initial products when lipids are damaged by 1-electron oxidants, such as tyrosyl radical and nitrogen dioxide radical. In plasma, HDL is their major carrier (15, 16). Several factors might account for the fact that HDL contains more lipid hydroperoxides than LDL, including: *i*) the greater susceptibility of HDL lipids to oxidation in vivo, *ii*) preferential accumulation of lipid hydroperoxides in HDL, and *iii*) impaired ability of HDL to degrade lipid-oxidation products.

Lipid peroxidation can also generate advanced products of oxidation, such as alkanes, aldehydes, and isoprostanes (17). Isoprostanes are prostaglandin-like compounds formed through peroxidation of arachidonic acid, and they contain F-type prostane rings. Quantifying F_2-isoprostanes is often considered the most accurate way to measure oxidative stress in vivo, and isoprostanes levels have been used extensively as biomarkers of lipid peroxidation as a risk factor for atherosclerosis and other diseases (18, 19).

These observations suggest that HDL might play major roles in the transport and metabolism of lipid hydroperoxides in vivo and that these processes contribute to its cardioprotective effects. Indeed, as reported in this issue of the *Journal of Lipid Research*, Proudfoot et al. (20) found that HDL is the major lipoprotein carrier of plasma F_2-isoprostanes. Using GS-MS, a sensitive and specific technique, they quantified F_2-isoprostanes levels in different lipoprotein fractions. Levels of F_2-isoprotanes were approximately twice as high in HDL as in LDL and were 50% higher than in VLDL (normalized to cholesterol). Moreover, levels in light HDL_2 and dense HDL_3 subfractions were respectively 2- and 3-fold higher than in total LDL. When lipoproteins were isolated by high-resolution size exclusion chromatography, the results were even more striking: F_2-isoprostanes in small and large HDL (corresponding roughly to HDL_2 and HDL_3) were 4- to 6-fold higher than in LDL; HDL_3-sized particles contained ~50% more F_2-isoprostanes than HDL_2. Importantly, the amount of arachidonic acid (the precursor of F_2-isoprostanes) was significantly higher in LDL than in HDL. Thus, increased availability of substrate (a factor that might contribute to increased susceptibility to oxidation) could not account for the different F_2-isoprostanes levels in LDL and HDL. Together with the pioneering work of Bowry, Stanley, and Stocker (15), these observations indicate that HDL is the major carrier of both early and late products of lipid oxidation.

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Platelet-activating factor acetylhydrolase (PAF-AH) can degrade F_2-isoprostanes (21). Proudfoot et al. (20) found that LDL accounted for 88% of the PAF-AH activity measured in plasma, whereas the HDL_2 fraction accounted for only 12%. Moreover, HDL_3 had essentially no PAF-AH activity. PON-1, another enzyme carried by HDL, has also been proposed to degrade lipid oxidation products. The authors found that PON-1 activity associated mainly with the HDL_2 fraction, which may partly account for the lower level of F_2-isoprostanes in HDL_2 than in HDL_3. Thus it is possible that PAF-AH and PON-1 activities are important determinants of F_2-isoprostanes distribution in LDL and HDL.

These observations suggest that discrepant levels of enzymes that degrade lipid oxidation products account in part for the excess of oxidized lipids in HDL. To further investigate this issue, the authors monitored F_2-isoprostanes, lipid hydroperoxides, and PON-1 activity during oxidation of HDL or LDL by 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH). In this model system, the spontaneous breakdown of AAPH subjects LDL or HDL to a continuous radical flux, which promotes lipid peroxidation. The authors detected increased hydroperoxide levels in LDL and HDL, but accumulation in HDL was more rapid. Similarly, the levels of F_2-isoprostanes were significantly higher in HDL. Thus, despite containing less oxidizable substrate (arachidonic acid), HDL that was continuously bombarded with peroxyl radicals accumulated more lipid oxidation products than did LDL. One important factor is likely to be increased enzymatic scavenging of lipid peroxides in LDL. Another may be lower levels of lipid-soluble antioxidants in HDL (15).

The transfer of lipid hydroperoxides between HDL and LDL appears to be too slow to substantially influence the distribution of these compounds in plasma (15). Moreover, plasma is rich in antioxidant defense mechanisms, and LDL and HDL turn over rapidly in that compartment (half-lives of 3–4 days). These observations make it unlikely that either lipoprotein is oxidized in plasma or that LDL directly transfers lipid hydroperoxides to HDL in plasma.

So how might HDL's lipid oxidation products originate? One intriguing possibility is that HDL acquires them at sites of inflammation and then transports them back into plasma (22). Recent studies demonstrate that both macrophages and endothelial cells export 7-ketocholesterol (a cytotoxic cholesterol oxidation product) to HDL by a pathway involving the cholesterol transporter ABCG1 (23, 24). Also, model system studies indicate that hepatocytes can efficiently extract lipid hydroperoxides from HDL (15). Moreover, macrophages are the cellular hallmark of the atherosclerotic lesion, indicating that atherosclerosis is a chronic inflammatory disease. These observations suggest that HDL protects endothelial cells and macrophages from lipid-oxidation products by transporting those toxic substances to the liver, as originally proposed for cholesterol.

It is noteworthy that HDL isolated from humans with established CAD contains much higher levels of chlorotyrosine than does HDL of apparently healthy control subjects (22, 25). Chlorotyrosine is a specific oxidation product of the heme protein myeloperoxidase (26), and macrophages in human atherosclerotic lesions express high levels of that enzyme (27). Myeloperoxidase can trigger lipid peroxidation by a variety of pathways, by generating tyrosyl radical, for example (28). When apolipoprotein A-I (apoA-I), the major protein in HDL, is chlorinated by myeloperoxidase, it loses its ability to remove cholesterol from cells by the ABCA1 pathway (29), which normally is an important conduit for cholesterol efflux from macrophages (7). These observations suggest that macrophages could generate a dysfunctional form of HDL that contains oxidized lipids and proteins. Thus, the inflamed atherosclerotic lesion is one potential location where oxidation could be biologically and clinically important.

In future studies, it will be important to determine whether levels of F_2-isoprostanes in HDL can identify CAD subjects more effectively than total plasma F_2-isoprostanes levels. This possibility was suggested by studies of subjects with low HDL and established CAD (16). It will also be interesting to identify the tissue source(s) of F_2-isoprostanes and to establish whether so-called "antioxidant" treatments, such as dietary vitamin E (30), affect levels of lipid oxidation products in HDL. Another key issue is whether lipid oxidation products negate HDL's cardioprotective effects. Indeed, acrolein, a reactive carbonyl generated by lipid peroxidation, blocks apoA-I's ability to promote cholesterol efflux by the ABCA1 pathway (31). Finally, it is tempting to speculate that F_2-isoprostanes might exert cellular effects (32) that partly account for the proposed atherosclerotic properties of dysfunctional HDL.

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