EDITORIAL

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# Exposing the complexity of HDL

FOR MORE THAN 100 YEARS cholesterol has been known to play a role in the pathogenesis of atherosclerosis. This has led to the central role of measuring systemic levels of both total cholesterol and low-density lipoprotein (LDL) cholesterol in algorithms designed to predict the prospective risk of cardiovascular disease. Furthermore, therapeutic strategies that reduce levels of LDL cholesterol form the cornerstone of cardiovascular prevention. While a number of LDL subtypes are defined on the basis of particle size, they are all atherogenic. The relationship between LDL's counterpart—high-density lipoprotein (HDL) cholesterol—and atherosclerosis is more complicated.

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## HDL'S PROTECTIVE FUNCTIONS

Numerous lines of evidence support the concept that HDL protects against the formation of atherosclerotic plaque. Population studies consistently show that systemic levels of HDL cholesterol correlate inversely with the future risk of cardiovascular events.[1] Promoting the biological activity of HDL via direct infusion[2] or transgenic expression[3] has a protective influence on lesion size and composition in animal models of atherosclerosis.

In addition to its well-characterized role in promoting cholesterol efflux and reverse cholesterol efflux, HDL has been shown to be anti-inflammatory, antioxidant, and antithrombotic, and to increase the bioavailability of nitric oxide.[4]

It is therefore not surprising that raising levels of HDL cholesterol is an independent predictor of the clinical benefit observed in response to treatment with statins,[5] fibrates,[6] and niacin.[7] Moreover, infusing reconstituted forms of HDL containing either apolipoprotein A-I (apo A-I) Milano[8] or wild-type apo A-I[9] promotes regression of coronary atherosclerosis in humans.

## HDL IS NOT ALWAYS PROTECTIVE

However, HDL does not appear to be protective in all people. Many patients with high levels of HDL cholesterol present with cardiovascular disease. This suggests that the milieu of atherogenic risk factors is too overwhelming for HDL to be protective, or that in particular individuals HDL does not prevent the progression to clinical disease.

### HDL's 'relative functionality'

Considerable interest has recently focused on the "relative functionality" of HDL. In the systemic circulation, HDL is a heterogeneous population of particles, varying in size, shape, electrophoretic mobility, and composition of both lipid and protein. It would therefore not be surprising if these particles differed in their ability to have a beneficial impact on the artery wall.

**HDL is a complex of particles, each with a varied impact on the artery wall**

The relative functionality of HDL particles with regard to their role in promoting cholesterol efflux and inhibiting proinflammatory events in the arterial wall has been investigated by a number of groups. Altering the protein and phospholipid composition has been reported to impair the ability of reconstituted HDL particles to promote cholesterol efflux from macrophages.[10] One mechanism underlying reduced efflux capacity is likely to involve oxidative modification. Modification of HDL by oxidant[11] and glycation[12] systems results in impaired cholesterol efflux. This is further supported by the observation that apo A-I is a major target for modification by oxidant products of the leukocyte-generated factor myeloperoxidase.[13] Apo A-I contains more nitrotyrosine and chlorotyrosine in patients with coronary artery disease than in healthy

controls. Furthermore, the degree of oxidative modification of HDL correlates inversely with its ability to promote cholesterol efflux.

### HDL, oxidation, and atherosclerosis

The relationship between oxidative stress and impairment of HDL function has important implications for the relationship between oxidation and atherosclerosis. Despite a pivotal role played by oxidized LDL in the pathogenesis of atheroma formation, attempts to reduce oxidative stress have failed to result in an improved clinical outcome. The failure of potential antioxidant vitamins in large-scale clinical event trials[14–17] has brought the oxidation hypothesis of atherosclerosis unfairly into question.

A number of important caveats are noted on review of these studies. Given the lack of inclusion of measures of oxidation, it is unknown if these patients had increased levels of oxidative stress or if these therapies had an antioxidant effect. In fact, these vitamins likely have little antioxidant activity in humans, with the suggestion that vitamin E may possess pro-oxidant properties in vivo in humans.[18] The development of reliable markers of oxidative stress in humans would provide an opportunity to evaluate the impact of therapies that truly act as antioxidants to determine if this is an effective therapeutic approach, resulting in more effective prevention of cardiovascular disease.

**New therapies should consider the impact on the quality of HDL generated**

## POTENTIAL STRATEGIES BASED ON HDL'S HETEROGENEITY

The heterogenous ability of HDL to inhibit proinflammatory events is the focus of a review by Dr. Ansell[19] in this issue of the *Journal*. Given the role of inflammation in the formation and propagation of atherosclerotic plaque, strategies that inhibit migration of inflammatory cells into the arterial wall may have a substantial impact on the incidence of coronary heart disease. HDL isolated from patients with elevated plasma levels of HDL cholesterol and coronary heart disease promotes rather than inhibits monocyte chemotaxis in an ex vivo assay, which[20] supports the concept that some people have circulating HDL that is not protective.

The finding that this activity is decreased in acute sepsis[21] and is improved after taking either statins[20] or mimetic peptides of apo A-I[22] suggests that HDL function can be influenced by external factors. This is supported by the observation that the ability of HDL to inhibit expression of proinflammatory adhesion molecules is impaired after consumption of a meal rich in saturated fat, and that it is improved with polyunsaturated fat.[23]

## IMPLICATIONS FOR PREVENTING ATHEROSCLEROSIS

The finding that the protective activities of HDL are variable has major implications for the prevention of atherosclerotic cardiovascular disease. Considerable interest has focused on the development of therapeutic strategies that raise levels of HDL cholesterol. However, that the impact of therapies on the functional properties of HDL may be at least as important. Relatively small increases in HDL cholesterol are independent predictors of benefit of established therapies.[5,6] Further analysis revealed that increasing levels of small, but not large, forms of HDL predicted the clinical benefit of fibrates.[24]

Development of new therapies will need to consider their impact on the quality of HDL that is generated. Furthermore, the need to measure the functional activity of HDL provides the impetus to develop new biomarkers to predict cardiovascular risk and to assess the response to therapies.

The impact of therapies on HDL function has received particular attention in the setting of pharmacologic inhibition of cholesteryl ester transfer protein (CETP). A beneficial impact in animal models of atherosclerosis[25] and an ability to substantially raise HDL cholesterol levels in humans[26] stimulated immense interest in CETP inhibition as a preventive therapy. Failure of the most advanced inhibitor under development, torcetrapib, to slow progression of carotid intimal-medial thickness[27] or coronary atherosclerosis[28] and reports of an increase in the mortality rate in a large clinical-event trial raised concerns regarding the mechanism responsible for the lack of clinical efficacy. Concerns regarding excess mortality prompted discontinuation of ongoing studies of torce-

trapib by the manufacturer in December 2006. In particular, it remains uncertain whether the lack of efficacy, despite substantial raising of HDL cholesterol and incremental lowering of LDL cholesterol, is due to the generation of HDL particles with impaired function, to the elevation of blood pressure, or to some other form of vascular toxicity.

The generation of large, cholesterol-enriched HDL particles has raised concerns that CETP inhibition may impair cholesterol efflux and reverse cholesterol transport. Evidence that different forms of HDL promote efflux via different mechanisms and that overall efflux capacity did not appear to be impaired after administration of torcetrapib in humans suggests that the HDL generated retains its functionality.[29] However, further investigation evaluating the impact of CETP inhibition on all of the biological activities of HDL will provide further insight into whether this therapeutic strategy has promise.

## UNFINISHED BUSINESS

Considerable advances have been made in our understanding of HDL and its role in the prevention of atherosclerotic cardiovascular disease. What has become most apparent is the complexity of HDL and its biological activity. Further research is required to maximize its beneficial effects on the arterial wall. ■

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