Review

Antiatherogenic Functionality of High Density Lipoprotein: How Much versus How Good

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Plasma concentration of high density lipoprotein (HDL) is one of the most reliable negative risk factors for CVD. There is however convincing experimental and clinical evidence that plasma concentration of HDL does not convey the full picture of atheroprotective properties of HDL. HDL functionality, i.e. the ability of HDL to perform its many atheroprotective functions, is partly independent of HDL concentration and may be as important, if not more important, in determining the atheroprotective capacity of HDL. The capacity of HDL to support cholesterol efflux, its anti-inflammatory, anti-oxidant, anti-thrombotic and other atheroprotective functions are affected dramatically in conditions like coronary artery disease, chronic and acute inflammation, diabetes as well as through various interventions. The mechanisms connecting changes in HDL functionality to HDL structure are only beginning to emerge. Modifications of HDL proteins and lipids, such as advanced glycation and oxidation, changes in HDL composition and size of HDL particles, changes in abundance of various proteins and lipids carried by HDL are among factors affecting HDL functionality. A single common denominator reflecting the multiple HDL functions is yet to be found and may not exist leaving direct measurements of each HDL function as the way to assess atheroprotective capacity of HDL.


**Key words:** Atherosclerosis, Inflammation, Oxidation, Thrombosis

HDL Concentration Versus HDL Functionality

The plasma concentration of high density lipoprotein (HDL), which is primarily measured by the concentration of cholesterol in HDL (HDL-C), correlates inversely with the incidence of cardiovascular disease (CVD) and is one of the most reliable negative risk factors for CVD. This has been convincingly demonstrated in a number of observational¹) and interventional studies²) (for review of other studies see³, ⁴). Further, raised levels of HDL have been associated with slower progression and stabilization of the atherosclerotic plaques⁵). The mechanism of this association is likely to reflect the role of HDL in reverse cholesterol transport (RCT). Reverse cholesterol transport is a pathway for removing excessive cholesterol from cells and tissues and transporting it to liver, the major organ for degrading and excreting cholesterol. An imbalance between the amount of cholesterol delivered to cells, usually through poorly regulated uptake of modified low density lipoprotein (LDL), and the amount of cholesterol removed from cells, mostly, if not exclusively, through reverse cholesterol transport results in accumulation of excessive cholesterol. The tissue most affected is vasculature. Accumulation of cholesterol in vessel wall macrophages, and possibly to a degree also in smooth muscle and endothelial cells, is a trigger for, and a key element in the pathogenesis of atherosclerosis. In this context the role of HDL is seen as protective in removing excessive cholesterol and this was assumed to be proportional to its plasma concentration. Recent developments however demonstrated that the relationship between HDL and cardiovascular risk is more complex.
First, examples emerged of discordance between plasma HDL concentration and cardiovascular risk. One example is apolipoprotein A-I Milano (apoA-I Milano): carriers of this mutation had very low HDL levels (less than half of the normal values), but enhanced protection against atherosclerosis implying that the HDL of these subjects is superior compared to that of unaffected subjects. Another example is low fat/high carbohydrate intake that frequently leads to lower HDL levels, though the functional properties of HDL may improve. A less fortunate example is treatment with the cholesteryl ester transfer protein (CETP) inhibitor Torcetrapib: substantial elevation of HDL in a recent trial was accompanied with higher cardiovascular risk implying that a high HDL concentration need not always be protective. In general however, there is a clearly increased risk at lower HDL concentration although the case that high levels are protective has not been proven. Further, since low HDL levels may coexist with abnormal HDL functionality in certain disorders, the definitive contribution of each to the overall reduction of atheroprotection may be difficult to differentiate. It must also be noted that a substantial proportion of patients with CVD have normal LDL-C and HDL-C concentrations although normal or average may not be ideal for such individuals. Nevertheless it emphasizes that qualitative changes in lipoproteins such as “small LDL” may be as predictive as quantitative differences.

Second, whether the concentration of HDL is the major factor determining the rate of reverse cholesterol transport (RCT) has also been questioned. RCT is a state of flux and the concentration of an intermediate, such as HDL, is determined by the rates of its formation and its interconversion into multiple species. Arguably if higher concentrations of HDL result from slow catabolism, indicative of a slower rate of RCT, that would provide less protection against accumulation of cholesterol in arterial macrophages despite higher levels of HDL. However a higher concentration of HDL resulting from additional HDL generation as from a faster rate of RCT may arguably be more protective. Thus, a similar elevation HDL may have opposite effects on the rate of RCT depending on how it was achieved.

Third, the atheroprotective properties of HDL are apparently not limited to its role in RCT. A number of other properties have emerged that may play an important role in atheroprotection (for review see). These functions include anti-inflammatory, anti-oxidation and anti-thrombotic functions affecting three of the most important elements of atherosclerosis: inflammation, oxidation and thrombogenesis. Recently several new functions of HDL have been described that may also affect the development of atherosclerosis. HDL stimulates endothelial nitric oxide synthase (eNOS) favorably diminishing endothelial dysfunction which is an element in atherogenesis. HDL is capable of stimulating glucose uptake and fatty acid oxidation thus opposing insulin resistance as in diabetes, another risk factor of atherosclerosis. Further, it was recently demonstrated that mice with selective impairment of ATP binding cassette transporter A1 (ABCA1) in β-cells have impaired glucose tolerance and defective insulin secretion, whereas HDL protected β-cells from damage induced by oxidized LDL. The probable mechanism connecting ABCA1 deficiency and β-cell impairment was found to be accumulation of cholesterol in β-cells that would normally be removed by apoA-I. Thus, hypoalphalipoproteinemia and/or impairment of HDL functionality would not only increase insulin resistance, but would also inhibit insulin secretion by pancreas. The contribution of each function to the overall atheroprotective capacity of HDL is currently unknown.

In conclusion there is convincing experimental evidence that the plasma concentration of HDL is not the only determinant of its atheroprotective capacity, that HDL functionality is largely independent from HDL concentration and is as important, if not more important, in determining the atheroprotective capacity of HDL. HDL contains a complex mixture of many different lipids besides cholesterol and contains over 40 different proteins and therefore, it is perhaps not surprising that our current assays for HDL, which are primarily based on the concentration of cholesterol on HDL, are inadequate for fully capturing all of its atheroprotective properties. Furthermore, it appears that different functions of HDL may change independently from each other. While the relationship between HDL plasma concentration and risk of atherosclerosis is well known, the effect of changes in HDL functionality that are independent of HDL plasma levels, have been less well studied. Below we analyze what is known about changes in HDL functionality.

**Reverse Cholesterol Transport**

It is usually assumed that the capacity of plasma to support cholesterol efflux is proportional to its HDL content. While under normal conditions and in healthy individuals that may well be the case, it may not apply in pathological conditions. The ability of HDL to support cholesterol efflux can vary dramatically depending on metabolic conditions. Generally
Table 1. HDL Functionality: Cholesterol Efflux

<table>
<thead>
<tr>
<th>Cholesterol efflux functionality</th>
<th>Modification</th>
<th>Disease or treatment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decrease</td>
<td>Larger particles (?)</td>
<td>High activity of PLTP</td>
<td>19)</td>
</tr>
<tr>
<td>Decrease</td>
<td>Larger particles</td>
<td>Statin</td>
<td>29)</td>
</tr>
<tr>
<td>Decrease</td>
<td>preβ1-HDL depletion</td>
<td>CAD, Transplantation, hemodialysis, ApoM deficiency</td>
<td>18, 22-25)</td>
</tr>
<tr>
<td>Decrease</td>
<td>Lower LpA-I to LpA-I/A-II ratio</td>
<td>Diabetes, CAD</td>
<td>33, 34)</td>
</tr>
<tr>
<td>Decrease</td>
<td>Partial hydrolysis</td>
<td>Inflammation</td>
<td>39)</td>
</tr>
<tr>
<td>Decrease</td>
<td>Oxidation</td>
<td>Inflammation</td>
<td>40, 41)</td>
</tr>
<tr>
<td>Decrease</td>
<td>Advanced glycation</td>
<td>Diabetes</td>
<td>42, 43)</td>
</tr>
<tr>
<td>Decrease</td>
<td>Chlorination</td>
<td>Diabetes, Inflammation</td>
<td>45, 46)</td>
</tr>
<tr>
<td>Decrease</td>
<td>Nitrilation</td>
<td>Diabetes, Inflammation</td>
<td>45, 46)</td>
</tr>
<tr>
<td>Increase</td>
<td>Tyrosylation</td>
<td>Inflammation</td>
<td>47, 48)</td>
</tr>
<tr>
<td>Increase</td>
<td>Smaller particles</td>
<td>Fibrates</td>
<td>20, 21)</td>
</tr>
<tr>
<td>Increase</td>
<td>Larger particles</td>
<td>CETP deficiency and inhibition</td>
<td>26, 27)</td>
</tr>
</tbody>
</table>

three factors determine the capacity of HDL to support cholesterol efflux: size, composition and modification. The examples of conditions associated with changes in HDL size and composition or its modification are shown in Table 1.

HDL is comprised of a number of particles of different size. It is believed that small lipid-poor HDL particles are better acceptor than large lipid-rich HDL particles. Accordingly, conditions favoring formation of large lipid-rich HDL might be expected to impair HDL capacity for cholesterol efflux. However the evidence for this is not unequivocal. Remodeling of HDL with phospholipid transfer protein (PLTP) led to formation of large HDL particles that are less efficient as cholesterol acceptors. Conversely, shift from large to small HDL particles after treatment with fenofibrate or ciprofibrate increased the capacity of plasma to promote cholesterol efflux. A number of studies suggested that very small lipid-poor HDL particles, preβ-HDL, are especially potent cholesterol acceptors. Reduction in preβ-HDL content (found in transplant patients and patients after hemodialysis) reduced the capacity of plasma to support cholesterol efflux. On the other hand, enlarged HDL in patients with CETP deficiency or after treatment with CETP inhibitor also had increased capacity for cholesterol efflux. Treatment with statins, that mildly increases plasma HDL levels, most likely by inhibiting CETP, decreased capacity of plasma to support cholesterol efflux. One reason for variability may be the existence of different pathways of cholesterol efflux. Different pathways may play a predominant role in different cell types and at different metabolic or experimental conditions. For example, increasing a proportion of larger HDL increased the efflux through SR-B1 pathway, but reduced the efflux via ABCA1-dependent pathway. Since as it appears the ABCA1-dependent pathway is a predominant pathway in macrophages, it is likely that conditions associated with formation of large HDL particles are detrimental for the capacity of HDL to support cholesterol efflux from this cell type. However it should be noted that in population studies, larger HDL species appear to be most protective against future CVD events. Nevertheless several studies in smaller groups of subjects have shown that this may be an over-simplification. Asztalos and colleagues have shown that some HDL particles appear to reduce the risk of future events whereas other particles associate with increased risk. Clearly, it would be desirable to establish the comparative functions of individual HDL species.

Changes in HDL lipid and protein content also affect its functions. Enrichment of HDL with triglycerides and phospholipids increased its capacity to support cholesterol efflux, at least through SR-B1-dependent pathway, independently from the effect of the enrichment on HDL size. Clearly, it would be desirable to establish the comparative functions of individual HDL species.

A number of HDL modifications were shown to affect cholesterol efflux. Partial hydrolysis of apoA-I by chymase secreted from mast cells rendered HDL incapable of supporting cholesterol efflux. Oxida-
Table 2. HDL Functionality: Inflammation

<table>
<thead>
<tr>
<th>Anti-inflammatory functionality</th>
<th>Modification</th>
<th>Disease or treatment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decrease</td>
<td>Oxidation</td>
<td>Infusion of oxLDL</td>
<td>58, 65</td>
</tr>
<tr>
<td>Decrease</td>
<td>Nitration</td>
<td>Diabetes, CAD</td>
<td>44</td>
</tr>
<tr>
<td>Decrease</td>
<td>Advanced glycation</td>
<td>Diabetes</td>
<td>42</td>
</tr>
<tr>
<td>Decrease</td>
<td>Not known</td>
<td>Atherogenic diet</td>
<td>55</td>
</tr>
<tr>
<td>Decrease</td>
<td>Reduction of PON1 activity</td>
<td>Influenza, cholecystomy</td>
<td>56, 64</td>
</tr>
<tr>
<td>Decrease</td>
<td>Reduction of lipolysis</td>
<td>Not known</td>
<td>66</td>
</tr>
<tr>
<td>Decrease</td>
<td>Not known</td>
<td>Inflammation (systemic lupus erythematosus)</td>
<td>58</td>
</tr>
<tr>
<td>Decrease</td>
<td>Enrichment with SAA</td>
<td>Inflammation</td>
<td>15</td>
</tr>
<tr>
<td>Modulation</td>
<td>Changes in “inflammatory cargo”</td>
<td>Statins, LDL-apheresis</td>
<td>57, 68, 69</td>
</tr>
<tr>
<td>Increase</td>
<td>Not known</td>
<td>Low fat diet</td>
<td>7</td>
</tr>
</tbody>
</table>

Inflammation

Inflammation is a major element in the pathogenesis of atherosclerosis. In general HDL has potent anti-inflammatory properties. HDL inhibits expression of pro-inflammatory adhesion molecules and stimulates TGFβ expression in endothelial cells. It neutralizes the pro-inflammatory activity of CRP, inhibits production of pro-inflammatory prostaglandins by monocytes and prevents and/or neutralizes pro-inflammatory effects of oxidized LDL phospholipids on endothelium. At the same time the extent of anti-inflammatory capacity of HDL may vary widely even becoming pro-inflammatory. The examples of changes in anti-inflammatory properties of HDL are presented in Table 2.

In experimental models, pro-atherogenic diet, infusion of oxLDL and infection converted HDL from anti-inflammatory to pro-inflammatory. The proportion of subjects carrying pro-inflammatory HDL among patients with CAD has been reported to be much higher than in healthy populations. In patients with systemic lupus erythematosus almost half had pro- rather than anti-inflammatory HDL, probably contributing to the high risk of CAD in such patients.

The exact mechanism of changes in the anti-inflammatory status of HDL is not known but it appears to be part of an acute phase phenomenon. One hypothesis suggests that it results from the substitution of apoA-I in HDL by serum amyloid A (SAA), since level of SAA is elevated as part of an acute phase response. In kidney failure, incorporation of SAA into HDL affects HDL structure and may render HDL at least partially dysfunctional. Reduction in paraoxonase (PON1) and LCAT activities may also reduce anti-inflammatory properties of HDL. Modifications of HDL, such as oxidation, nitration or advanced glycation has been demonstrated to impair the anti-inflammatory properties of HDL; moreover, oxidized HDL stimulated expression of a number of pro-inflammatory genes in endothelium. Another possibility is that at least some anti-inflammatory properties of HDL are derived from phospholipids released from HDL after hydrolysis by endothelial lipase. Consequently, inhibition of the lipolysis would render HDL dysfunctional. Further, HDL has been shown to be a carrier of endotoxin and the ability to sequester endotoxin (due to variations in HDL levels and composition) may contribute to the anti-inflammatory properties of HDL. The capacity of HDL to bind and transport potential pro-inflammatory factors is not however limited to endotoxin since it was found that HDL carries an array of proteins involved in inflammatory response, including acute-phase response proteins. Changes in
the “inflammatory cargo” of HDL may play a major role in determining its anti-inflammatory properties. It is however not clear what may be the contribution of changes in properties of HDL particles (such as size and/or charge that may affect binding of these factors to HDL) versus the levels of expression of the inflammatory factors and their release at the sites of synthesis.

Examples of improved anti-inflammatory capacity of HDL were shown after treatment with statins and/or exercise. The mechanism of the effect of these interventions on anti-inflammatory properties of HDL is not known.

### Oxidation

HDL prevents oxidation of LDL. Anti-oxidant properties of HDL are determined by both apoA-I and several anti-oxidant enzymes, such as paraoxonase (PON1), which resides in HDL. As oxidized LDL (oxLDL) is taken up by macrophages in an unregulated fashion, the degree of LDL oxidation has a direct influence on cholesterol accumulation in macrophages and formation of foam cells. Thus, preventing LDL oxidation and indeed LDL modification in general may contribute significantly to the atheroprotective properties of HDL. Furthermore, HDL diminishes apoptosis in macrophages caused by oxLDL by facilitating the efflux of oxidized lipids.

Similar to the anti-inflammatory function of HDL, its anti-oxidant function varies among individuals and may become impaired. Examples of changes in anti-oxidant functionality are presented in Table 3.

Persegol et al. have shown that HDL from obese patients were incapable of preventing vascular dysfunction induced by oxidized LDL. This impairment was associated with lower apoA-I content and higher triglyceride to cholesteryl ester ratio; however, it is not clear whether changes in HDL composition were responsible for altered functionality. HDL from transplant patients were less able to maintain normal endothelial function, presumably by the same mechanism; these patients had larger HDL. Similarly, HDL from patients with coronary atherosclerosis, obstructive sleep apnea and Crohn’s disease were less potent in preventing oxidation, the structural basis of this impairment is not known. Under experimental conditions HDL from PLTP-deficient mice was more active in preventing LDL oxidation; HDL from these mice were protein enriched but phospholipid deficient (changes in composition consistent with smaller HDL particles). Kontush et al. and Graham et al. investigated the anti-oxidant capacity of HDL subfractions of various sizes. They found that small dense HDL exhibit higher anti-oxidant capacity than larger less dense particles.

Concentration and activity of PON1 is another strong determinant of anti-oxidant capacity of HDL. Activity of PON1 was impaired in patients with diabetes and CAD as was the anti-oxidant capacity of HDL. Similar findings were described in LDL receptor KO/ob/ob mice, where lower levels of PON1 were associated with impaired ability of HDL to protect LDL against oxidation.

Finally, genetic modifications also affect anti-oxidant properties of HDL. The best investigated examples of genetic modification of anti-oxidant function of HDL are carriers of apoA-I Milano and apoA-I Paris variants of apoA-I with cysteine substitutions. The carriers of these variants have low HDL concentration without concomitant elevation of risk of CAD. Free thiols in proteins can act as strong nucleophiles and are known to quench oxidation events. Both variants were considerably more active in preventing lipoygenase-mediated oxidation of phospholipids compared to apoA-I wt.

### Table 3. HDL Functionality: Oxidation

<table>
<thead>
<tr>
<th>Anti-oxidant functionality</th>
<th>Modification</th>
<th>Disease or treatment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decrease</td>
<td>Lower apoA-I content, higher TG/CE ratio</td>
<td>Obesity</td>
<td>76)</td>
</tr>
<tr>
<td>Decrease</td>
<td>Larger particles</td>
<td>Cardiac transplantation</td>
<td>18)</td>
</tr>
<tr>
<td>Decrease</td>
<td>Not known</td>
<td>CAD, Obstructive sleep apnea</td>
<td>72, 77, 78)</td>
</tr>
<tr>
<td>Decrease</td>
<td>Not known</td>
<td>Inflammation (Crohn’s disease)</td>
<td>79)</td>
</tr>
<tr>
<td>Decrease</td>
<td>Decrease in PON1 activity</td>
<td>Diabetes, CAD</td>
<td>83, 84)</td>
</tr>
<tr>
<td>Decrease</td>
<td>Blocking apoA-I with auto-antibodies</td>
<td>Systemic lupus erythematosus, CAD</td>
<td>88)</td>
</tr>
<tr>
<td>Increase</td>
<td>Higher apoA-I content, lower PL content (smaller particles)</td>
<td>PLTP-deficiency</td>
<td>80)</td>
</tr>
<tr>
<td>Increase</td>
<td>Extra thiol groups</td>
<td>Genetic mutations (apoA-I Milano, apoA-I Paris)</td>
<td>87, 81, 82)</td>
</tr>
<tr>
<td>Increase</td>
<td>Smaller particles</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### References

- Persegol et al.
- Kontush et al.
- Graham et al.
- Paris
- Milano
- ApoA-I Milano
- ApoA-I Paris
- Lipoygenase-mediated oxidation of phospholipids
- Free thiols in proteins
- Strong nucleophiles
properties of these variants contribute to their enhanced atheroprotective capacity. Anti-oxidant capacity of HDL may be also blocked by auto-antibodies against apoA-1 found in patients with systemic lupus erythematosus and CAD(88).

**Thrombosis**

It is accepted that at later stages of atherosclerosis, plaque disruption triggers thrombosis promoting the sudden expansion of atheromatous lesions leading to vessel occlusion. HDL has well-characterized anti-platelet adhesion and anti-thrombotic properties(89). HDL can affect thrombosis at several levels: antagonizing the activation of factor X, inhibiting the formation of the prothrombinase complex on the surface of platelets and platelet activation, and also inhibiting the secretion of PAI-1 and tPA by the endothelium (for review see(10, 89, 90)). Although low levels of HDL are known to be associated with pro-thrombotic status(91), the data on anti-thrombosis HDL functionality are very limited; few available examples are shown in Table 4.

Low levels of HDL are associated with enhanced platelet activation. The mechanism is most likely through interaction of oxidized phospholipids with platelet CD36(92) suggesting a protective role of HDL. It was however found that unmodified HDL shows only a weak protective effect against platelet activation by oxidized phospholipids. Paradoxically, the anti-platelet effect of HDL was enhanced dramatically after oxidation of HDL; the effect was found to be mediated through SR-B1(93, 94).

The size of HDL particles was another characteristic to affect the ability of HDL to inhibit platelet activation: smaller HDL(3) had little effect on platelet activation, while inhibition was observed by bigger apoE containing HDL(2) particles(90). Finally, an HDL modification that affected anti-coagulation property of HDL was the apoA-I Milano mutation. Experimental infusion of apoA-I Milano led to substantial inhibition of thrombosis despite a little change in HDL levels(95).

**Other Anti-Atherogenic Properties of HDL**

Other atheroprotective functions of HDL include stimulation of eNOS and protection of endothelial function, stimulation of glucose uptake, fatty acid oxidation and insulin secretion. There are almost no data available on how these functions may be affected under pathological conditions and how such impairment may be related to HDL structure. Existing examples include a finding that HDL from cardiac transplant patients was unable to support endothelial function despite a high plasma level(18). Advanced glycation of HDL impaired capacity to preserve endothelial function and to stimulate eNOS(96). Conversely, carriers of apoA-I Milano had normal endothelial function and concentration of soluble cell adhesion molecules despite much lower levels of HDL, again implying superior functionality of HDL in the carriers of apoA-I Milano(97).

**What Determines HDL Functionality?**

Collectively the examples presented above leave little doubt that HDL functions can be affected, and that those changes are not always correlated with changes in concentration. Further, altered functionality are linked to cardiovascular risk. An obvious conclusion is that measurements of HDL-C levels, albeit simple and well established, may not be sufficient for assessing cardiovascular risk fully. It may even be misleading; however, assessing HDL functionality is not an easy task. It is further complicated by the multiplicity of the functions and lack of clear evidence of the respective contributions of each function to the overall atheroprotective effect of HDL. The question has been raised whether there may be a single factor rendering HDL more or less functional? Further, is it possible that one function influences the others? For example would cholesterol efflux through e.g. changing fluidity of the plasma membrane determine other functions of HDL and therefore changes in cholesterol efflux would be an underlying mechanism for changes in other properties of HDL? Or would the ability of HDL to bind to cells (greatly impaired by e.g. nitration) determine how all its functions may be executed? There is no definitive answer to these ques-

### Table 4. HDL Functionality: Thrombosis

<table>
<thead>
<tr>
<th>Anti-thrombosis functionality</th>
<th>Modification</th>
<th>Disease or treatment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase</td>
<td>Oxidation</td>
<td>Experimental</td>
<td>93, 94)</td>
</tr>
<tr>
<td>Increase</td>
<td>Enlarged apoE-rich particles</td>
<td></td>
<td>90)</td>
</tr>
<tr>
<td>Increase</td>
<td>Dimerisation, extra thiol group</td>
<td>Genetic mutation (apoA-I Milano)</td>
<td>95)</td>
</tr>
</tbody>
</table>
tions. It is likely however that although some functions may be determined by a common factor and their mechanisms may be interconnected, others function independently.

One common feature in the impairment of most HDL functions is chemical modification of HDL: oxidation, nitration and glycation. Binding of chemically modified HDL to cells is impaired\(^\text{38}\), its capacity as acceptor of cholesterol becomes less effective and it manifests pro-inflammatory and pro-oxidative characteristics. Chemical modifications of HDL usually occur within metabolic states that are associated with oxidative stress, such as diabetes and chronic inflammation, and at these states chemical modification of HDL may impair HDL functionality across the board. However other modifications characteristic for the same metabolic states, such as tyrosylation, increase the functionality of HDL\(^\text{47, 48}\).

Another common feature influencing HDL functionality is particle size. Generally, small HDL is more active in promoting cholesterol efflux and has greater anti-inflammatory and anti-oxidant properties, but increased small HDL in serum may indicate an aberration in the maturation of HDL and decreased flux of cholesterol back to the liver and may be associated with increased risk of cardiovascular disease\(^\text{24, 31, 99, 100}\). Thus metabolic conditions or treatments associated with redistribution of HDL among different size particles may affect several aspects of HDL functionality in ways that are still not predictable.

Finally the anti-inflammatory and anti-oxidant properties of HDL are likely to be influenced to a significant degree by the cargo of pro- and anti-inflammatory and anti-oxidant factors bound to and carried by HDL.

It appears therefore that there is no definitive common structural feature that determines HDL functionality. Furthermore, the outcome is often a balance of negative and positive effects of a disease or treatment on different HDL functions complicating predictions of the overall effect on clinical outcomes. This makes functional tests measuring individual functions the best currently available option to assess possible impairment of the atheroprotective capacity of HDL. Complexity and cost of measuring all functions preclude these tests from becoming routine clinical laboratory tests for assessing the cardiovascular risk for an individual patient. Ultimately, the composition of HDL, however, has to relate to its function, but the linkage between the composition of HDL and its many functions has to be first established before new clinical laboratory tests based on composition besides HDL-C can be developed. Currently, however, functional tests of HDL may be very valuable for assessing specific disease or treatments in clinical trials: if there are indications that specific functions may be impaired, they may then be assessed and targeted. Functional tests for HDL will also likely be critical in the development of new HDL raising drugs to insure that any changes in HDL metabolism triggered by new drugs improve and do not antagonize the atheroprotective functions of HDL.

Acknowledgements

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References

9) Swiridov D and Nestel P: Dynamics of reverse cholesterol transport; protection against atherosclerosis. Atherosclerosis, 2002; 161:245-254
32) Yancey PG, de la Llera-Moya M, Swarnarak S, Monzo P, Klein SM, Connelly MA, Johnson WJ, Williams DL, and Rothblat GH: High density lipoprotein phospholipid composition is a major determinant of the bi-direc-
tional flux and net movement of cellular free cholesterol mediated by scavenger receptor BI. J Biol Chem, 2000; 275:36596-36604
41) Morel DW: Reduced cholesterol efflux to mildly oxidized high density lipoprotein. Biochem Biophys Res Comm, 1994; 200:408-416
56) Van Lenten BJ, Wagner AC, Nayak DP, Hama S, Navab
60) Cai L, de Beer MC, de Beer FC, and van der Westhuizen DR: Serum Amyloid A is a Ligand for Scavenger Receptor Class B Type I and Inhibits High Density Lipoprotein Binding and Selective Lipid Uptake. J Biol Chem, 2005; 280:2954-2961
63) Cho KH, Park SH, Park JE, Kim YO, Choi I, Kim JY, and Kim JR: The function, composition, and particle size of high-density lipoprotein were severely impaired in an oliguric phase of hemorrhagic fever with renal syndrome patients. Clin Biochem, 2008; 41:56-64
69) Opole IO, Belmont JM, Kumar A, and Moriarty PM: Effect of low-density lipoprotein apheresis on inflammatory and noninflammatory high-density lipoprotein cholesterol. Am J Cardiol, 2007; 100:1416-1418
70) Banka CL: High density lipoprotein and lipoprotein oxidation. Curr Opin Lipidol, 1996; 7:139-142
75) Mackness MI, Abbott C, Arrol S, and Durrington PN: The role of high-density lipoprotein and lipid-soluble antioxidant vitamins in inhibiting low-density lipoprotein oxidation. Biochem J, 1993
77) Navab M, Hama SY, Hough GP, Subbanagounder G, Reddy ST, and Fogelman AM: A cell-free assay for detecting HDL that is dysfunctional in preventing the formation of or inactivating oxidized phospholipids. J Lipid Res, 2001; 42:1308-1317
80) Yan D, Navab M, Bruce C, Fogelman AM, and Jiang X-C: PLTP deficiency improves the anti-inflammatory properties of HDL and reduces the ability of LDL to induce monocyte chemotactic activity. J Lipid Res, 2004; 45:1852-1858
87) Bielicki JK and Oda MN: Apolipoprotein A-I (Milano) and Apolipoprotein A-I (Paris) Exhibit an Antioxidant Activity Distinct from That of Wild-Type Apolipoprotein A-I. Biochemistry, 2002; 41:2089-2096
90) Korporaal SJ and Akkerman JW: Platelet activation by low density lipoprotein and high density lipoprotein. Pathophysiol Haemost Thromb, 2006; 35:270-280
91) Naqvi TZ, Shah PK, Ivey PA, Molloy MD, Thomas AM, Panicker S, Ahmed A, Cercek B, and Kaul S: Evidence that high-density lipoprotein cholesterol is an independent predictor of acute platelet-dependent thrombus formation. Am J Cardiol, 1999; 84:1011-1017
98) Duell PB, Nachman JI, and Bierman EL: Nonenzymatic glycosylation of HDL resulting in inhibition of high-affinity binding to cultured human fibroblasts. Diabetes, 1990; 39:1257-1263