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## Dysfunctional HDL and atherosclerotic cardiovascular disease

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### Abstract

High-density lipoproteins (HDLs) protect against atherosclerosis by removing excess cholesterol from macrophages through the ATP-binding cassette transporter A1 (ABCA1) and ATP-binding cassette transporter G1 (ABCG1) pathways involved in reverse cholesterol transport. Factors that impair the availability of functional apolipoproteins or the activities of ABCA1 and ABCG1 could, therefore, strongly influence atherogenesis. HDL also inhibits lipid oxidation, restores endothelial function, exerts anti-inflammatory and antiapoptotic actions, and exerts anti-inflammatory actions in animal models. Such properties could contribute considerably to the capacity of HDL to inhibit atherosclerosis. Systemic and vascular inflammation has been proposed to convert HDL to a dysfunctional form that has impaired antiatherogenic effects. A loss of anti-inflammatory and antioxidative proteins, perhaps in combination with a gain of proinflammatory proteins, might be another important component in rendering HDL dysfunctional. The proinflammatory enzyme myeloperoxidase induces both oxidative modification and nitrosylation of specific residues on plasma and arterial apolipoprotein A-I to render HDL dysfunctional, which results in impaired ABCA1 macrophage transport, the activation of inflammatory pathways, and an increased risk of coronary artery disease. Understanding the features of dysfunctional HDL or apolipoprotein A-I in clinical practice might lead to new diagnostic and therapeutic approaches to atherosclerosis.

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Author contributions

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Competing interests statement

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High-density lipoproteins (HDLs) confer protection against atherosclerosis in several ways and, consequently, low levels of HDL cholesterol (HDL-C) are present in many conditions that are associated with an increased risk of cardiovascular disease (CVD). Particles of HDL and/or its main protein constituent, apolipoprotein A-I (apoA-I), have diverse antiatherosclerotic influences<sup>1</sup> that are determined by their physicochemical properties (FIG. 1)<sup>2</sup>. The most established functional property associated with HDL is macrophage cholesterol efflux<sup>3</sup>. Efflux of cholesterol through the ATP-binding cassette transporter A1 (ABCA1) is mediated most effectively by cholesterol-deficient and phospholipid-deficient apoA-I complexes<sup>1</sup> and by very small HDL particles (HDL-VS)<sup>4</sup>. By enhancing the endothelial synthesis of nitric oxide (NO), a potent vasodilator, HDL can also ameliorate endothelial dysfunction. Furthermore, HDL reduces coronary atherosclerosis by decreasing the expression of adhesion molecules on endothelial cells and thereby reducing inflammation, and by inhibiting the oxidation of low-density lipoprotein (LDL). HDL can also exert an endothelial antiapoptotic function that involves activation of phosphoinositide 3-kinase (PI3K)/Akt and upregulation of the apoptotic protein Bcl-2-like protein 1 (also known as apoptosis regulator Bcl-X)<sup>5</sup>.

In patients who present with acute coronary syndromes (ACS), the myeloperoxidase enzyme oxidizes particular residues on apoA-I, and thereby diminishes the efficiency of ABCA1-mediated macrophage cholesterol efflux<sup>6,7</sup>. Antioxidative properties of HDL particles are associated with compositional changes in the HDL proteome and lipidome<sup>8</sup>. However, alterations in HDL/apoA-I proteins and lipid constituents diminish the antioxidant, anti-inflammatory, antiapoptotic, and endothelial functional and reparative properties of HDL. Specifically, dysfunctional HDL results in increased content of oxidized phospholipids, triglycerides<sup>9</sup>, serum amyloid A (SAA), complement C3, and other inflammatory proteins<sup>7</sup>. Myeloperoxidase mediates oxidation of lipid-poor apoA-I in the vessel wall that creates a dysfunctional HDL particle, which activates nuclear factor NF- $\kappa$ B and promotes arterial inflammation<sup>6</sup>.

Under particular circumstances, HDL particles can become dysfunctional independently of the levels of HDL-C. Evidence that HDL and/or apoA-I exert proatherosclerotic functions as well as antiathero-sclerotic functions has increased interest in exploring the contribution of dysfunctional HDL to atherosclerotic CVD. We outline the concept of dysfunctional HDL in the context of available assays that might be clinically relevant<sup>1</sup>. Substantial efforts are required to improve the reproducibility of functional assays and to determine whether functional biomarkers provide accurate and reliable assessments of atherosclerotic CVD. An important requirement for such assays is the demonstration that they provide clinically useful information that is distinct from static measures of HDL-C and HDL particles, and that these tests provide a better assessment of CVD risk<sup>1</sup>, as shown with the macrophage cholesterol efflux assay<sup>10,11</sup>. Compositional features of HDL (the lipidome and proteome) might prove useful as reliable analytical biomarkers to be applied to clinical studies<sup>1,2,7</sup>. Post-translational modifications of apoA-I in human atherosclerotic lesions by distinct oxidative processes create dysfunctional HDL particles<sup>6,12</sup>, and prospective studies suggest that quantifying specific oxidized residues on circulating HDL might provide a useful indication of HDL dysfunction and CVD risk<sup>6,13</sup>.

In this Review, we summarize the accumulating evidence that, under particular circumstances, HDL/apoA-I loses its protective functions and might actually contribute to inflammatory processes that promote CVD in humans with atherosclerosis. We also consider the effect of therapeutic lifestyle changes and pharmacotherapies on HDL function, particularly under circumstances in which increases in HDL-C are not predictive of a reduced risk of atherosclerotic cardiovascular events, as well as considering the potential of dysfunctional HDL as a therapeutic target.

## Concept of dysfunctional HDL

Nearly 20 years ago, a team of investigators reported that HDL was converted from an anti-inflammatory particle to a proinflammatory particle during an acute-phase response in humans and in a croton oil model of inflammation in rabbits<sup>14</sup>. This report developed the concept of an 'inflammatory index' for HDL, which was defined as the relative capacity of the 'test' HDL to inhibit monocyte chemotaxis induced by oxidized LDL<sup>14-16</sup>. This monocyte chemotaxis assay has been used to distinguish between patients with coronary heart disease (CHD) and control individuals without vascular disease, with higher accuracy than the measurement of HDL-C levels. Indeed, 26 patients with CHD and normal HDL-C levels ( $57 \pm 13$  mg/dl) had an inflammatory index of  $1.38 \pm 0.91$ , compared with  $0.38 \pm 0.14$  ( $P < 0.001$ ) for 26 age-matched and sex-matched controls who were free from the disease and who had similar HDL-C levels ( $64 \pm 6$  mg/dl;  $P = 0.008$ )<sup>16</sup>.

The proinflammatory HDL particle was characterized by an altered protein composition: compared with its anti-inflammatory counterpart, it contained increased levels of ceruloplasmin and SAA, and decreased amounts of apoA-I, paraoxonase (PON), and platelet-activating factor-acetylhydrolase (PAF-AH)<sup>14</sup>. Concurrently, on the basis of results from a series of experiments in animal models<sup>17-20</sup>, investigators at the NIH described HDL as 'dysfunctional' with regard to the increased risk of developing atherosclerosis, rather than the decreased risk associated with increased plasma HDL-C levels. Overexpression in mice of human lecithin-cholesterol acyltransferase (LCAT), which catalyses the esterification of free cholesterol to cholesteryl esters in circulating plasma lipoproteins, increased the risk of atherosclerosis despite elevated levels of HDL-C and apoA-I in plasma<sup>19</sup>. This initial study and subsequent reports demonstrated that, in the absence of cholesteryl ester transfer protein (CETP), the HDL cholesteryl esters that are generated by LCAT overexpression cannot be transferred to triglyceride-rich lipoproteins, resulting in the formation of HDL with altered composition<sup>18</sup> and function in the intermediary and terminal aspects of reverse cholesterol transport. Reduced interactions between cholesteryl esters and CETP<sup>19</sup>, as well as with hepatic lipase<sup>20</sup>, also resulted in a reduced capacity to deliver the esters to hepatic scavenger receptor class B member 1 (SCARB1).

These early studies outlined the need to distinguish between the loss of HDL function and the gain of dysfunction. Indeed, HDL could completely lose its anti-inflammatory activities and acquire proinflammatory properties with a gain of dysfunction. However, in some circumstances, the capacity of HDL to deliver cholesteryl esters by SCARB1 might be attenuated, demonstrating only a partial loss of function. Subsequent studies have revealed that the degree of loss of normal HDL function and gain of dysfunction might differ with

respect to the different biological activities of HDL. In some studies, HDL displayed a complete loss of function and gain of dysfunction in terms of anti-inflammatory activity<sup>15–18,20</sup>, vasodilatory function<sup>21–25</sup>, and antiapoptotic activity<sup>5</sup>, but a deficiency in normal HDL function rather than complete dysfunction with regard to antioxidative activity<sup>26–30</sup> and cholesterol efflux capacity<sup>31–34</sup>.

## Genetics studies of HDL cholesterol

Human genome-wide association studies (GWAS) have identified a plethora of genetic loci that influence the levels of HDL-C<sup>35</sup>. Although some of these loci comprise genes that are already implicated in HDL metabolism from monogenic disorders, many are novel and the relationships between the associated genes and HDL functions are poorly understood. Voight *et al.* used a Mendelian randomization approach to study whether single-nucleotide polymorphisms (SNPs) associated with increased levels of HDL-C were also linked with changes in the risk of CHD, but found no significant association for the selected SNPs; by contrast, SNPs associated with high levels of LDL-C increased the risk of myocardial infarction<sup>36</sup>. Although this analysis excluded SNPs in genes with large effect sizes on the levels of HDL-C (such as those encoding CETP and lipoprotein lipase), which are associated with CHD but also affect other lipoprotein traits (such as the levels of triglycerides and LDL-C), SNPs in the gene encoding endothelial lipase, which promotes the turnover of HDL, showed considerable effects on the levels of HDL-C, but no association with CHD<sup>36</sup>. The investigators concluded that not all interventions that increase the levels of HDL-C, such as inhibition of endothelial lipase, are associated with reductions in the risk of CHD.

Subsequent GWAS have identified additional HDL-associated SNPs and have shown that the magnitude of the effect size of these SNPs on HDL-C levels does not correlate with the magnitude of the effect size on CHD<sup>37,38</sup>. By contrast, these relationships were positive for SNPs that influenced the levels of LDL-C and triglycerides<sup>39</sup>. These analyses led to the broad conclusion that HDL does not have a causal association with atherosclerotic CVD, or that a causal role for HDL-C — while possible — remains uncertain<sup>40</sup>.

However, although these studies benefit from large diverse populations and huge statistical power, some caveats to the broader conclusion might be warranted. First, the SNPs that were included in the analysis account for a minor part of the overall variation in HDL-C levels<sup>40</sup> and include many genes with unknown functions that might have pleiotropic effects, thereby affecting atherosclerosis independently of any HDL effects. Second, the distribution of effect sizes included in the analyses differed according to the particular lipoprotein traits. Thus, SNPs affecting LDL-C and triglycerides had larger effect sizes than those affecting HDL<sup>37</sup>. The analysis of the effect on CHD of SNPs that affect an intermediate phenotype such as HDL-C levels with very small effect sizes could be influenced by a confounding factor<sup>41</sup>. Triglyceride-associated SNPs with similarly small effect sizes to those of HDL-associated SNPs also seem to have no clear relationship to CHD<sup>37</sup>. Third, GWAS have also identified many SNPs that are associated with type 2 diabetes mellitus, several of which are associated with triglycerides and HDL-C<sup>41</sup>. However, this list of SNPs does not overlap with the list of SNPs associated with premature CHD<sup>42</sup>, further illustrating the complexity of this genetic

approach. Finally, the extent to which the SNPs that are associated with increases in HDL-C are also linked to relevant changes in HDL function is uncertain.

With regard to changes in HDL function, SNPs located near the *ABCA1* gene, which encodes the ATP-binding cassette transporter A1 involved in cholesterol efflux, were notably associated with small changes in the levels of HDL-C, but not with the risk of CHD in a large meta-analysis<sup>37,42</sup>. In contrast to these SNPs in *ABCA1*, which show small effect sizes, missense variants in *ABCA1* that are associated with large decreases in cholesterol efflux have been shown to correlate with increased carotid atherosclerosis using advanced carotid MRI<sup>43</sup>. Other studies of different *ABCA1* coding variants with smaller effects on cholesterol efflux have been reported, and show variable relationships to CHD<sup>43</sup>. However, these functional variants also cause a reduction in LDL-C levels, which could confound the analysis. So, most studies suggest that defective cholesterol efflux from *ABCA1* missense variants is associated with a proatherogenic role; however, these studies suffer from the problem of referral bias — that is, the patients who were studied were obtained from clinic populations and, therefore, were more likely to have CHD on that basis alone.

Overall, the results from GWAS cast some doubt on the causal relationship between HDL-C and CHD, and even on macrophage cholesterol efflux, the critical component of reverse cholesterol transport. Although not mutually exclusive with the involvement of HDL in reverse cholesterol transport, the results from these GWAS provide increased evidence for an atherogenic role of triglyceride-rich lipoproteins or their breakdown products, including oxidized phospholipids<sup>39</sup>. However, in view of the caveats stated above combined with strong evidence from preclinical animal models and limited human data in support of reverse cholesterol transport, we believe that firm conclusions are not yet warranted.

## HDL functionality and atherosclerosis

Data from both experimental and human studies support the involvement of HDL in reducing oxidative damage and inflammation, and enhancing endothelial function and macrophage-mediated cholesterol efflux. In this section, we provide an overview of HDL function (and dysfunction) in the context of available clinical measures that have been used to identify loss of HDL function, or HDL dysfunction (TABLE 1). Although these functional measures have previously been extensively reviewed<sup>1</sup>, a few important high-throughput assays have since been developed, and these advances facilitate the evaluation of dysfunctional HDL in large clinical trials (BOX 1).

## HDL composition and cardiovascular events

The anti-inflammatory and antioxidative activities of HDL can be impaired as a result of the accumulation in HDL of oxidized phospholipids, which possess potent proinflammatory and pro-oxidative properties<sup>14–16,44–46</sup>. In a case-control series of patients with clinical CHD despite supranormal HDL-C levels ( $118 \pm 24$  mg/dl), the monocyte chemotaxis assay inflammatory index of HDL was  $1.28 \pm 0.29$ , compared with  $0.35 \pm 0.11$  in the age-matched and sex-matched controls ( $P < 0.001$ )<sup>16</sup>. This finding was corroborated by similar results using a cell-free assay to evaluate the effect of phospholipid oxidation on HDL

particles, as well as by the results of studies in which both assays were used in animal models<sup>15</sup>.

In a proteomics study of patients with ACS, the HDL proteome had an increased abundance of SAA, complement C3, and other inflammatory proteins compared with control individuals without ACS<sup>47</sup>; however, this change in HDL composition did not seem to impair macrophage cholesterol efflux through ABCA1, ABCG1, or SCARB1-mediated pathways. By contrast, in another study, profound alterations in the HDL phospholipidome with an enrichment in lipolytic products (such as lysophosphatidylcholine and phosphatidic acid), as observed in patients with ACS, were linked to an impaired cholesterol efflux capacity from human THP-1 macrophages, which predominantly mediate cholesterol efflux through ABCA1 (REF. 48).

ApoC-III is a proinflammatory protein that resides on the surface of very-low-density lipoprotein (VLDL), LDL, and HDL particles<sup>49</sup>. In an analysis of the Nurses' Health and Health Professionals Follow-Up Studies, the presence or absence of apoC-III in HDL subfractions from plasma could be used to identify participants with a risk of future CHD<sup>50</sup>. The relative risk of CHD per standard deviation of HDL without apoC-III was 0.66 (95% CI 0.53–0.93), whereas it was 1.18 (95% CI 1.03–1.34) for HDL with apoC-III; these findings indicate that HDL subtypes without apoC-III were inversely associated with the risk of CHD, whereas HDL subtypes with apoC-III showed a direct association with the risk of CHD. These data suggest that apoC-III interferes with the atheroprotective function of HDL.

### HDL/apoA-I oxidation

Increased oxidative stress is characteristic of established coronary atherosclerosis<sup>16,34</sup> and other inflammatory disorders<sup>14,15</sup>, and frequently leads to damaging oxidative modifications.

**Myeloperoxidase-mediated modifications.**—Myeloperoxidase, a haem protein that is expressed at high concentrations by macrophages, monocytes, and neutrophils in human atherosclerotic tissue<sup>51–54</sup>, can use hydrogen peroxide to generate a wide range of reactive intermediates, which can subsequently modify lipids, proteins, nucleic acids, and lipoproteins<sup>50</sup>. The expression of myeloperoxidase in myeloid cells of mice that lack the LDL receptor promotes atherosclerosis, suggesting that myeloperoxidase exacerbates atherosclerosis in this model of hypercholesterolaemia<sup>55,56</sup>.

Oxidation of apoA-I by myeloperoxidase occurs mainly within the subendothelial compartment<sup>57</sup> and results in the increased oxidation of multiple residues, which have been defined by mass spectrometric analysis<sup>58,59</sup>. Oxidative damage to HDL-associated apoA-I or lipid-poor apoA-I in the artery wall might limit the capacity of HDL/apoA-I to mediate cholesterol efflux from macrophages and thus promote the development of experimental and human atherosclerosis (FIG. 2)<sup>6,12,13,60,61</sup>. The distribution and biological function of apoA-I within the artery wall is distinct to that of HDL<sup>6,12</sup>. In contrast to circulating HDL, the majority of apoA-I in atheroma is not associated with HDL particles<sup>6,12,61</sup>. Post-translational modifications of lipid-poor apoA-I are common at sites of inflammation in atherosclerotic plaques.



Several mechanisms have been proposed to explain the impaired capacity of myeloperoxidase-oxidized HDL-associated apoA-I to mediate sterol efflux<sup>7,9,51–55,57–65</sup>. One mechanism involves methionine<sup>6,45,48,49,65</sup> oxidation and site-specific chlorination of Tyr192 on apoA-I, which impairs ABCA1-dependent cholesterol efflux and the activity of LCAT<sup>13,46</sup>. The levels of chlorinated Tyr192 and oxidized Met148 are higher in patients with stable coronary artery disease (CAD) or ACS than in control individuals, and were reported to be associated with reduced ABCA1-mediated macrophage cholesterol efflux and CAD status<sup>13</sup>. Another proposed mechanism for oxidative inactivation involves oxidation of tryptophan residues by myeloperoxidase. Substitution of all four tryptophan residues on apoA-I with phenylalanine conferred partial protection from myeloperoxidase-mediated dysfunction. However, substitution of phenylalanine residues for tryptophan residues also greatly increases the  $\alpha$ -helical content of lipid-free apoA-I, which raises the possibility that the resistance of the mutant protein to oxidative loss of efflux capacity reflects nonspecific structural changes.

In humans, the levels of protein-bound 3-chloro-tyrosine are markedly higher on circulating HDL from patients with established CHD than on circulating HDL from healthy individuals, and HDL isolated from coronary atherosclerotic lesions contains higher concentrations of 3-chlorotyrosine and 3-nitrotyrosine than plasma HDL<sup>52,60</sup>.

### HDL/apoA-I composition and inflammation

As well as the aforementioned changes that occur in response to increased oxidative stress during inflammation, HDL also undergoes substantial modification during the inflammatory response, which affects both the quantity and composition of HDL particles<sup>7,14,34,47</sup>. Circulating levels of HDL and apoA-I are markedly decreased during the acute-phase response of inflammation, and HDL particles become enriched in triglycerides and depleted of cholesteryl esters (FIG. 3)<sup>45</sup>. Several mechanisms are likely to contribute to these reduced levels of HDL-C and apoA-I during the acute-phase response<sup>7</sup>.

**SAA.**—HDL present in the acute phase contains a marked increase in SAA<sup>45</sup>. SAA secreted by hepatocytes might associate with existing spherical HDL particles through a remodelling process that results in the displacement of apoA-I (FIG. 4)<sup>66</sup>. Alternatively, in concert with endothelial lipase, SAA might reduce the levels of HDL-C by impeding the formation of nascent HDL<sup>67,68</sup>. Inflammatory cytokines such as tumour necrosis factor (TNF) and IL-6 increase the expression of hepatic SAA by as much as 1,000-fold. The vast majority of SAA secreted by the liver is found associated with HDL, where it can comprise the major apolipoprotein. However, decreases in the levels of circulating apoA-I often precede the increase in SAA, suggesting that the presence of SAA does not entirely account for reduced levels of HDL-C in inflammation. The levels of SAA and apoA-I seem to be reciprocally and coordinately regulated in the liver by inflammatory cytokines, such that the reduction of apoA-I during the acute phase might be partially attributed to transcriptional downregulation concomitantly with an increase in the expression of SAA<sup>68</sup>. In healthy humans exposed to endotoxin and in a mouse model of inflammation, enrichment of the HDL proteome with SAA1 and SAA2 reduced cholesterol efflux from J7774 macrophage cells<sup>69</sup>.

**HDL-associated enzymes.**—HDL remodelling during inflammation also leads to the loss of HDL-associated PON and PAF-AH. PON1 and PON3 are calcium-dependent HDL-associated enzymes that impede the peroxidation of LDL particles, so the loss of these enzymes decreases the antioxidative and anti-inflammatory capacity of HDL<sup>14,65</sup>.

PON1 and PON3 differ in their expression profile, protein localization, and enzymatic activity, but are considered to have similar effects on the hydrolysis of oxidized lipids and promotion of macrophage cholesterol efflux in experimental models<sup>70</sup>. Although low levels of PON1 activity were associated with a modest inverse risk of CHD in a population-based study, this relationship was abolished after adjustment for HDL-C and apoA-I, suggesting that low PON1 activity is not a causal factor in atherogenesis<sup>71</sup>. In a cross-sectional analysis of patients with autoimmune disease or type 1 diabetes mellitus with subclinical atherosclerosis, PON3 was depleted in HDL particles and the antioxidant function of HDL was reduced<sup>72</sup>. We are unaware, however, of prospective studies that have established a role for PON3 in human atherosclerosis. Given that PAF-AH readily hydrolyses proinflammatory, oxidatively fragmented short-chain phospholipids<sup>73</sup> and phospholipid hydroperoxides<sup>74</sup>, the loss of PAF-AH activity from HDL, as observed under inflammatory conditions, can contribute to HDL dysfunction.

**Changes in the HDL lipidome.**—Changes in the lipid constituents of HDL might contribute to the reduced function of HDL during inflammation. Group IIA secretory phospholipase A<sub>2</sub> (sPLA<sub>2</sub>) is another prominent acute-phase reactant, the expression of which is highly induced in the liver by inflammatory cytokines<sup>75</sup>. Mice harbouring *PLA2G2A*, a transgene encoding human group IIA sPLA<sub>2</sub>, show reduced plasma levels of HDL-C, which are associated with increased rates of apoA-I catabolism<sup>76,77</sup>. In humans, endotoxaemia is associated with significantly reduced levels of plasma and HDL phospholipids<sup>78</sup>, which is consistent with enhanced phospholipase activity during inflammation.

Acute-phase HDL is also enriched in triglycerides<sup>45</sup>, which might lead to impaired HDL stability and enhanced apoA-I catabolism<sup>79</sup>. Oxidization of the acyl chains of phospholipids in HDL increases polarity of the lipid domains, which allows water to penetrate into the hydrophobic cellular lipid membrane. The increased polarity of the lipid domains of HDL particles, which alters the binding and orientation of apoA-I, results in a reduced capacity to stimulate the activity of PON 1 and LCAT<sup>80</sup>. Oxidization of phospholipids might, therefore, contribute to the generation of dysfunctional HDL in patients with ACS or other conditions involving high oxidative stress.

### Macrophage cholesterol efflux

Measuring the cholesterol efflux activity of HDL obtained by apoB precipitation from macrophage THP-1 cells was found to be superior to measuring the absolute levels of HDL-C in distinguishing between patients with incident coronary disease and control individuals<sup>81,82</sup>. This finding might, in part, be because the cholesterol efflux capacity of macrophages is predominantly mediated by very small, cholesterol-depleted HDL particles<sup>3,4</sup>, the circulating levels of which provide only a minor contribution to plasma



HDL-C<sup>3</sup>. Paradoxically, Hazen *et al.* reported that elevated cholesterol efflux activity was associated with an increased prospective risk of CVD events in patients within the stable angiographic cohort<sup>82</sup>. These unexpected findings might result from the inclusion of individuals with nonobstructive CAD at the time of arteriography, a low event rate in the overall cohort, and differences in baseline characteristics between patients and controls<sup>81</sup>. Among patients with ischaemic cardiomyopathy, the macrophage cholesterol efflux capacity of HDL was reduced compared with that of control individuals with preserved left ventricular function and no CHD<sup>83</sup>.

Macrophage cholesterol efflux capacity measurements have been performed in large, prospective studies using standard labelled cholesterol<sup>11</sup> and fluorescent BODIPY-cholesterol<sup>10</sup>. In a prospective study of 2,924 adults without CVD who were participants in the Dallas Heart Study<sup>10</sup>, the highest versus lowest quartile of macrophage cholesterol efflux capacity measured using the fluorescent BODIPY-cholesterol showed a 67% reduction in CVD risk in a fully adjusted model that included traditional risk factors as well as the levels of HDL-C and HDL particles. Investigators in the EPIC- Norfolk study<sup>11</sup> measured macrophage cholesterol efflux capacity using radiolabelled cholesterol. Although these two methods for assessing macrophage cholesterol efflux activity were not applied to the same population, the fluorescence-based assay<sup>11</sup> demonstrated more consistent associations with incident atherosclerotic CVD events than the radiolabelled cholesterol approach<sup>10</sup>.

With regard to the macrophage cholesterol efflux activity assays, the HDL isolated by ultracentrifugation of plasma samples and the HDL derived from the supernatant obtained after apoB precipitation are not equivalent biochemical entities, primarily owing to the presence in the supernatant of high amounts of albumin, immunoglobulin G, transferrin, and other major plasma proteins, which have multiple biological activities. In addition, apoB precipitation might cause the removal of apoE-containing HDL. These factors might inadvertently introduce changes in HDL composition. However, HDL obtained by ultracentrifugation also has its limitations — for example, ultracentrifugation methods that limit HDL density to 1.21 g/ml will not accurately represent the contribution of pre- $\beta$  HDL (HDL-VS) that is found in the density range 1.21–1.25 g/ml<sup>84</sup>. No data are available to suggest that one assay might be more physiologically relevant or better than the other for assessing the cellular cholesterol efflux activity of HDL.

### Endothelial function

HDL derived from patients with acute myocardial infarction is deficient in its capacity to stimulate the production of the vasodilator endothelial NO<sup>24</sup>, and to protect endothelial cells from apoptosis<sup>5</sup>. Such dysfunction might reflect alterations in the HDL proteome associated with acute myocardial infarction, including enrichment in inflammatory proteins, such as SAA, complement C3 and complement C9, and apoC-III, and/or a depletion of clusterin (apoJ) and apoA-IV<sup>5,7</sup>.

HDL particles perform an important and beneficial role in endothelial function. A single intravenous infusion of reconstituted HDL into humans with hypercholesterolaemia<sup>85</sup>, or with low levels of HDL secondary to partial deficiency of ABCA1 (REF. 86), normalizes endothelial function by a mechanism that involves restoration of the production of

endothelial NO. *Ex vivo* studies of endothelial cells incubated with HDL isolated from healthy individuals show that HDL induces the expression of endothelial NO synthase (eNOS), stimulates the production of NO in endothelial cells, and reduces endothelial oxidative stress<sup>87</sup>. The capacity of HDL to induce the expression of eNOS is partly dependent on SCARB1. Binding of apoA-I-containing HDL to SCARB1 in endothelial cells initiates a signalling cascade, which, through activation of Src family kinases, phosphatidylinositol 3-kinase, and Akt, leads to the phosphorylation and subsequent activation of eNOS<sup>88</sup>. This capacity of HDL to induce eNOS activity and enhance endothelial function is not apparent in preparations of HDL isolated from patients with type 2 diabetes mellitus<sup>23</sup> or CAD<sup>24</sup>.

During the acute phase of ST-segment elevation myocardial infarction (STEMI), the concentration of HDL particles is reduced, and these HDL particles are less protective against LDL oxidation and more susceptible to auto-oxidation; both of these changes are associated with a reduction in brachial artery endothelial vasomotor function<sup>89</sup>. Whether these HDL functional changes would remain significant after adjustment for reduced HDL particle number, however, is unknown.

## Other causes of dysfunctional HDL

### Diabetes mellitus

HDL dysfunction has been reported in patients with insulin resistance<sup>90,91</sup> or diabetes mellitus<sup>46,92</sup>. Alterations in HDL metabolism that accompany insulin resistance lead to the formation of small triglyceride-enriched and cholesteryl-ester-depleted HDL particles, glycation of apoA-I and other HDL-associated proteins, and oxidative modification of HDL lipids, apolipoproteins, and enzymes<sup>8,9,93</sup>. The compositional modification of the HDL lipid core (triglyceride enrichment and depletion of cholesteryl esters) can also alter the conformation of apoA-I, thereby reducing its surface binding affinity. These intrinsically unstable HDL particles are rapidly cleared from the circulation, which results in a reduced total concentration of HDL particles.

Chronic inflammation in patients with type 2 diabetes also contributes to the enhanced clearance of HDL from the plasma through alterations in the HDL proteome. For example, increased levels of SAA in these patients<sup>45,94</sup> displace apoA-I and other proteins from the surface of HDL, thereby accelerating clearance.

Enhanced oxidative stress in type 2 diabetes and prediabetes (metabolic syndrome) reduces the antioxidative capacity and antiapoptotic activity of small HDL particles by depleting the HDL-associated proteins apoA-I and PON1<sup>27,28,30,87,95,96</sup>. Altered phospholipid composition of HDL in patients with type 2 diabetes results in an elevated sphingomyelin-to-phosphatidylcholine ratio, which increases the rigidity of the HDL surface<sup>96</sup>, a major determinant of the antioxidative activity of HDL<sup>97</sup>. Inactivation of oxidized phospholipids requires transfer to HDL, and the rate of such transfer is inversely related to the rigidity of the HDL surface phospholipid monolayer.

Tandem mass spectrometry studies with internal standard peptides demonstrated a strong, negative correlation between the concentration of clusterin in HDL and both insulin sensitivity and BMI in two independent male populations<sup>98,99</sup>. The clusterin content of HDL was unrelated to elevated blood pressure, another component of the metabolic syndrome. However, levels of HDL clusterin were significantly decreased in patients with metabolic syndrome. These observations suggest that insulin resistance and the metabolic syndrome are associated with low clusterin levels in HDL. Clusterin confers a protective function in mouse models of tissue injury<sup>5</sup>. Moreover, obesity and insulin resistance are important CVD risk factors<sup>100</sup>. These observations suggest that depletion of clusterin in HDL might impair its cardioprotective function.

### **Cigarette smoking**

Increasing frequency and intensity of cigarette smoking is associated with lower HDL-C levels, lower HDL particle concentrations in female but not male smokers<sup>101</sup>, and compositional changes in HDL fractions that include phospholipid depletion in HDL<sub>2</sub> (HDL-VL, HDL-L) and HDL<sub>3</sub> (HDL-VS, HDL-S, and HDL-M) fractions, and triglyceride enrichment in the HDL<sub>2</sub> fraction<sup>102</sup>. Higher levels of CETP activity in smokers than in nonsmokers provide one explanation for these lipid compositional changes in HDL particles<sup>103</sup>. Smoking also causes the production of dysfunctional HDL<sub>3</sub> particles that are characterized by increased sensitivity to glycation and reduced antioxidative capacity, as measured by ferric-reducing capacity, and HDL<sub>2</sub> particles that are less effective at inhibiting the uptake of acetyl-LDL by THP-1 cells<sup>104</sup>.

### **Improving HDL functionality**

Interventions that have been shown to improve particular aspects of HDL functionality include lifestyle changes, weight loss achieved through bariatric surgery, and the use of statins and CETP inhibitors. Therapies that have been shown to improve or restore the functionality of some components of dysfunctional HDL include statins and niacin<sup>105</sup>.

### **Diet and exercise**

The effect of a short-term diet and exercise intervention was evaluated using the monocyte chemotaxis assay and HDL isolated from 22 patients with metabolic syndrome<sup>98</sup>. In contrast to the modest reduction in the levels of HDL-C that is typical of patients undergoing active weight loss, significant decreases in monocyte chemotaxis (from 1.19 to 0.94;  $P < 0.05$ ) were induced by this nonpharmacological intervention<sup>98</sup>. Conversely, Nicholls *et al.* showed that expression of intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) in human endothelial cells, as measures of vascular inflammation, persistently increased in response to treatment with HDL collected from humans after a meal containing predominantly saturated fat, in contrast to one consisting largely of polyunsaturated fat<sup>106</sup>.

### **Bariatric surgery**

The effect of Roux-en-Y gastric bypass surgery on HDL function was investigated in 34 morbidly obese women<sup>107</sup>. A total of 6 months after surgery, 20% weight loss was

accompanied by a 14% increase in the levels of HDL-C ( $P<0.04$ ), which resulted mainly from a 42% increase in the HDL<sub>2</sub> subfraction (HDL-L;  $P<0.01$ )<sup>107</sup>. As expected, this increase in HDL-L increased the cholesterol efflux capacity of macrophages through the SCARB1 pathway (+58%;  $P<0.001$ ) and the ABCG1 pathway (+26%;  $P<0.0001$ ).

## Statins

Statin therapy further improves the effects of HDL on cholesterol efflux from hepatoma cells, but has a variable effect on efflux from macrophages<sup>81,108</sup>. In patients with type IIb hyperlipoproteinaemia, treatment with atorvastatin was accompanied by a dose-dependent increase (<10%) in cholesterol efflux from Fu5AH hepatoma cells<sup>109</sup>. In dyslipidaemic individuals, pitavastatin increased the cholesterol efflux capacity from THP-1 macrophage cells<sup>108</sup>, but treatment of patients with CHD with statins had no effect on the capacity of J774 cells to mediate cholesterol efflux<sup>81</sup>.

Statins might interfere with HDL-mediated macrophage cholesterol efflux through ABCA1-induced pathways<sup>110</sup>. In THP-1 cells, statins increased the expression of miR-33, resulting in inhibited *ABCA1* expression, and incubation of J774 cells with statins (in particular, simvastatin) reduced the effect of human HDL on ABCA1-induced cholesterol efflux, suggesting a mechanism for the lack of efficacy of HDL-raising therapies in clinical trials in which all participants received statin therapy. However, miR-33 does not target the expression of *ABCG1* or *SCARB1* in human cells and, therefore, these pathways for HDL-mediated efflux would still be operative. Moreover, whether the effects of statins observed under cell-culture conditions are relevant *in vivo* is currently unknown.

Some therapeutic interventions using statins have been reported to increase HDL function — and, in some cases, dysfunction — as assessed by monocyte chemotaxis and other assays<sup>16,108,110</sup>. Investigators in the Statin HDL Improvement in Function Trial (SHIFT<sup>16</sup>) reported that, in patients with clinically evident atherosclerosis who had not previously received lipid-lowering medication, treatment with simvastatin improved the inflammatory index (as characterized by the monocyte chemotaxis assay and a cell-free assay) relative to baseline. Although the improvement was significant, the HDL from statin-treated patients retained a tendency towards a proinflammatory phenotype. Furthermore, the correlation between chemotactic activity and the lipid hydroperoxide content of HDL was significant both before ( $r = 0.214$ ,  $P = 0.039$ ) and after ( $r = 0.454$ ,  $P = 0.031$ ) simvastatin treatment. However, the activity of PON was unchanged after simvastatin treatment, suggesting that the lack of the statin effect on PON1 might have contributed to the incomplete normalization of the inflammatory index of HDL in this study. In patients with dyslipidaemia, pitavastatin increased the HDL phospholipid content and HDL-associated PON1 activity; however, no effect on PAF-AH activity was observed<sup>108</sup>.

## Niacin

Niacin elevates HDL-C levels, and reduces those of triglycerides and apoB-containing lipoproteins. However, the effects of niacin on various HDL functions are variable. For example, niacin treatment moderately enhanced the capacity of serum HDL to promote cholesterol efflux from cholesterol-loaded THP-1 macrophages<sup>111</sup>, but had no effect on

cholesterol efflux from J774 macrophages in statin-treated patients<sup>112</sup>. The increased macrophage cholesterol efflux in THP-1 macrophages was dependent on the expression of *ABCA1* and *ABCG1*, with *ABCG1* carrying out a more important role, consistent with an increase in large HDL particles<sup>112</sup>.

HDL isolated from niacin-treated individuals also showed a diminished macrophage inflammatory response to the activation of Toll-like receptor 4 by lipid A in THP-1 cells<sup>111</sup>. This anti-inflammatory effect of niacin was partly dependent upon the expression of *ABCA1* and *ABCG1*. In rodents, niacin also reduces acute vascular inflammation and improves endothelial function<sup>99</sup>. Niacin therapy (2 g daily) for 2 months restored the capacity of HDL to increase the production of endothelial NO, reduce oxidative stress, and facilitate endothelial repair mediated by endothelial progenitor cells in patients with type 2 diabetes<sup>23</sup>.

Despite these observations, the results from the prospective, randomized AIM-HIGH trial<sup>113</sup> demonstrated that adding niacin to statin therapy had no further effect on the risk of CVD in patients with low HDL-C levels and established atherosclerosis. Data from the HPS2-THRIVE study<sup>114</sup> also showed no change in CVD risk after the addition of niacin-laropiprant therapy to statin-treated patients with low LDL-C and non-HDL-C levels and normal HDL-C levels. Unlike with niacin monotherapy, the concentration of HDL particles does not increase in statin-treated patients who receive niacin<sup>115,116</sup>. Furthermore, HDL from niacin-treated individuals enrolled in the AIM-HIGH trial showed only marginal benefits on cholesterol efflux in THP-1 cells and no effect in J774 cells<sup>112</sup>, despite inducing higher incremental HDL-C levels than achieved in either the AIM-HIGH<sup>113</sup> or HPS2-THRIVE<sup>114</sup> trials. Therefore, niacin might not confer the same atheroprotective properties in statin-treated patients as reported for niacin monotherapy. Moreover, it remains to be determined whether pretreatment with a statin actually interferes with the favourable niacin-mediated HDL functions that have been reported for niacin monotherapy.

### CETP inhibitors

A great deal of interest has been generated by the availability of CETP inhibitors, which markedly increase the levels of HDL-C. However, the first clinical trial with the CETP inhibitor torcetrapib was terminated owing to off-target toxicity<sup>117</sup>. Dalcetrapib, a weak CETP inhibitor, increased the levels of HDL-C by 30–35% and of HDL particle number by 9%<sup>118</sup>, but the phase III dal-OUTCOMES trial<sup>119</sup> with this agent in patients with previous ACS was terminated owing to lack of efficacy. However, the patients with ACS enrolled in this trial had compromised HDL functionality<sup>7,24</sup>. The possibility that these patients had dysfunctional HDL is further supported by the observation that the concentration of HDL-C in the placebo group was unrelated to CVD events<sup>120</sup>. So, the 30% increase in HDL-C levels induced by dalcetrapib in the dal-OUTCOMES study might have had either a minimal effect on HDL functionality or increased the proportion of dysfunctional HDL that had little capacity to protect. In cell-culture experiments, the enhanced HDL-induced macrophage efflux produced by dalcetrapib was abrogated by co-incubation with a statin<sup>110</sup>.

SNPs in the adenylate cyclase 9 (*ADCY9*) gene on chromosome 16 were reported to identify participants in the dal-OUTCOMES trial<sup>121</sup> who had lower or higher rates of cardiovascular events. Specifically, participants with the genotype AA at *rs1967309* showed a 39%

reduction in the composite cardiovascular end point after treatment with dalcetrapib versus placebo, whereas patients with the genotype GG showed a 27% increase in events<sup>121</sup>. Further prospective trials are needed to validate this pharmacogenomic approach with dalcetrapib.

Researchers in the dal-ACUTE trial<sup>122</sup> investigated the effects of dalcetrapib on markers of HDL function within 1 week of an ACS event. After 4 weeks of treatment with dalcetrapib, the total cholesterol efflux capacity had increased by 9.5% compared with placebo, primarily through an increase in non-ABCA1-mediated transport. The increased total efflux capacity correlated with increases in the levels of HDL-C ( $r = 0.43$ ) and apoA-I ( $r = 0.46$ ). Anacetrapib and evacetrapib are two newer CETP inhibitors, and both dramatically increase the levels of HDL-C and reduce those of LDL-C<sup>120,123</sup>. Clinical trials with these potent CETP inhibitors are currently ongoing<sup>124,125</sup>.

From the available reports, CETP inhibitors have been shown to improve particular aspects of HDL functionality<sup>111</sup>, although we recognize the need to express these functions on a per HDL particle basis<sup>1</sup>. Among patients with type IIB hyperlipoproteinaemia treated with atorvastatin (10 mg daily), the addition of torcetrapib (60 mg daily) improved the impaired functional capacity of HDL<sub>2</sub> (HDL-VL, HDL-L) and HDL<sub>3</sub> (HDL-M, HDL-S, HDL-VS) particles to mediate free cholesterol efflux through the SCARB1 and ABCG1 pathways<sup>126</sup>. HDL isolated from anacetrapib-treated patients as well as HDL from CETP-deficient patients increased cholesterol efflux when compared with control HDL<sup>111</sup>. This increase in HDL efflux capacity was attributed to the increase in apoE and LCAT present on HDL particles<sup>127</sup>. Anacetrapib effectively increased HDL-mediated macrophage cholesterol efflux through the ABCG1 pathway<sup>111</sup>, and HDL isolated from anacetrapib-treated individuals reduced macrophage inflammatory responses to the activation of Toll-like receptor 4, similar to the results obtained after niacin treatment. Ongoing trials with CETP inhibitors will provide additional information on HDL and CVD events<sup>124,125</sup>.

## Conclusions

The measurement of HDL-C has been useful for assessing CVD risk in epidemiological surveys, and does correlate with macrophage cholesterol efflux. However, the available data suggest that functional measurements might contribute important information on HDL activities that are involved in atherogenesis, inflammation, infection, and immunity<sup>1,8</sup>. The importance of HDL functionality in cardiovascular events has now been confirmed in the Dallas Heart Study<sup>9</sup> and EPIC-Norfolk study<sup>11</sup>. As previously discussed, the use of reproducible, clinically available, and cost-effective measures of HDL function is an essential step for future exploration of functional and dysfunctional HDL<sup>1</sup>. These measures require validation in trials of atherosclerosis imaging and cardiovascular events.

Several compelling proof-of-concept studies currently illustrate the heterogeneity of various HDL functions among different HDL subpopulations, provide a correlation between inflammation and HDL dysfunction in atherosclerotic CVD, and offer some promise for existing and emerging therapies aimed at improving or maintaining the anti-inflammatory properties of HDL/apoA-I. However, no consensus exists about optimal testing to characterize HDL function and, although particular post-translationally-modified variants of



apoA-I have been identified as risk markers for atherosclerosis, no assays are currently available for routine clinical use<sup>6,12</sup>.

The development of monoclonal antibodies that identify specific forms of dysfunctional apoA-I is a promising area of investigation for monitoring pathophysiological processes within the arterial wall and plasma, and for the evaluation of HDL/apoA-I therapies that are directed at mitigating the proatherogenic effects of dysfunctional HDL. As further research expands our knowledge in this arena, testing of HDL function could allow for better clinical risk stratification, optimization of individual clinical treatments, and the evaluation of novel therapeutics.

MRI with a gadolinium-based sensor has been used in a rabbit model of atherosclerosis to detect myeloperoxidase-rich areas that contained an abundance of macrophages on histopathological examination<sup>128</sup>, and a gadolinium-based activatable sensor has been used to detect myeloperoxidase activity in neutrophils and macrophages in ischaemic myocardium in mice after myocardial infarction<sup>129</sup>. Nanoparticles that detect macrophage activity in atherosclerotic plaques in mice have been developed<sup>130</sup>, and future advances in nanotechnology methods might enable the identification of specific myeloperoxidase-induced or other chemically-induced apoA-I modifications in the arterial wall that could serve as clinically useful tools in atherosclerosis imaging. These advanced imaging modalities might be used to investigate the effects of potential therapeutic agents designed to inhibit the detrimental effects of dysfunctional HDL. Owing to the diverse contributions of dysfunctional HDL to various stages of atherosclerosis, pending investigations of dysfunctional HDL/apoA-I will require the evaluation of multiple plasma and vascular HDL/apoA-I functions in diverse populations of patients, the results of which will need to be incorporated into randomized, double-blind clinical trials of atherosclerosis and CVD events. Only through these efforts can it be determined whether particular HDL/apoA-I-based measures, in addition to and beyond the measurement of HDL-C levels, might evolve into critical biomarkers of disease and therapeutic targets.

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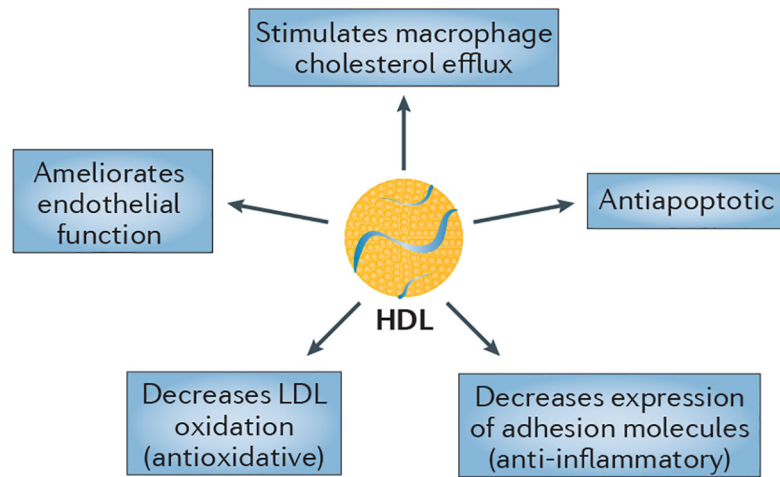
**Key points**

- HDL protects against atherosclerosis through multiple mechanisms that include amelioration of endothelial dysfunction, removal of excess cholesterol from macrophages, and antioxidative, anti-inflammatory, and antiapoptotic effects
- Under particular circumstances, HDL loses its atheroprotective properties, resulting in the formation of dysfunctional HDL particles
- Dysfunctional HDL particles increase proinflammatory signalling and reduce the efflux of cholesterol from macrophages by the ATP-binding cassette transporter A1
- In prospective studies, myeloperoxidase-mediated oxidation of particular residues on apolipoprotein A-I creates a dysfunctional HDL particle that is associated with an increased incidence of cardiovascular events

**Box 1|****Assays to evaluate dysfunctional HDL**

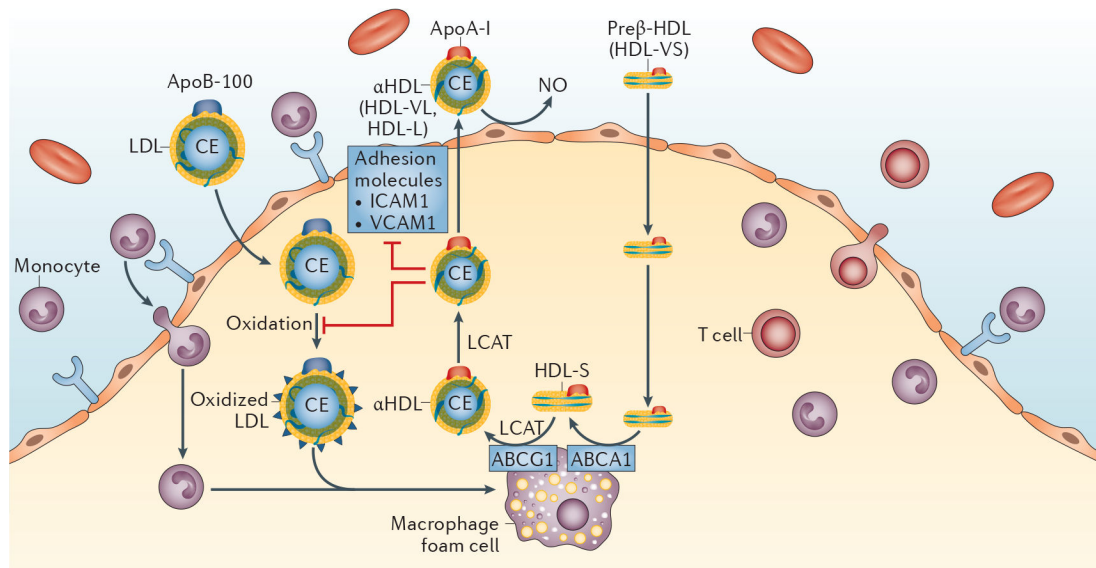
The capacity of high-density lipoprotein (HDL) to prevent the formation of, or to inactivate, oxidized phospholipids can be assessed in cell-based assays and cell-free assays to measure the effect of HDL on the production of reactive oxygen species; however, this approach has been impeded by the oxidative instability of the fluorogenic probe dichlorodihydro-fluorescein diacetate (DCFH-DA), which is currently used to measure oxidation to fluorescent 2',7'-dichlorofluorescein (DCF) by cellular reactive oxygen species. An alternative assay can be used to measure the rate of oxidation by cellular reactive oxygen species of the fluorogenic probe dihydrorhodamine-123 (DHR) to fluorescent rhodamine; however, this method does not accurately capture matrix-lipid probe interactions that occur with circulating inflammatory mediators that are characteristic of systemic inflammation.

A new method uses polyethylene glycol precipitation and immunocapture to isolate HDL, and measures HDL lipid peroxidation fluorometrically to assess HDL function. In the presence of horseradish peroxidase, the conversion of the fluorochrome Amplex Red to highly fluorescent resorufin is used specifically to quantify the endogenous lipid hydroperoxides present in the captured HDL-cholesterol sample. This cell-free, fluorometric, high-throughput assay correlated modestly with the currently used cell-based assay ( $r=0.47$ ,  $P<0.001$ ) and cell-free assays ( $r=0.46$ ,  $P<0.001$ )<sup>131,132</sup>



**Figure 1 | Particles of HDL and/or its main protein constituent, apolipoprotein A-I, have diverse anti-atherosclerotic influences.**

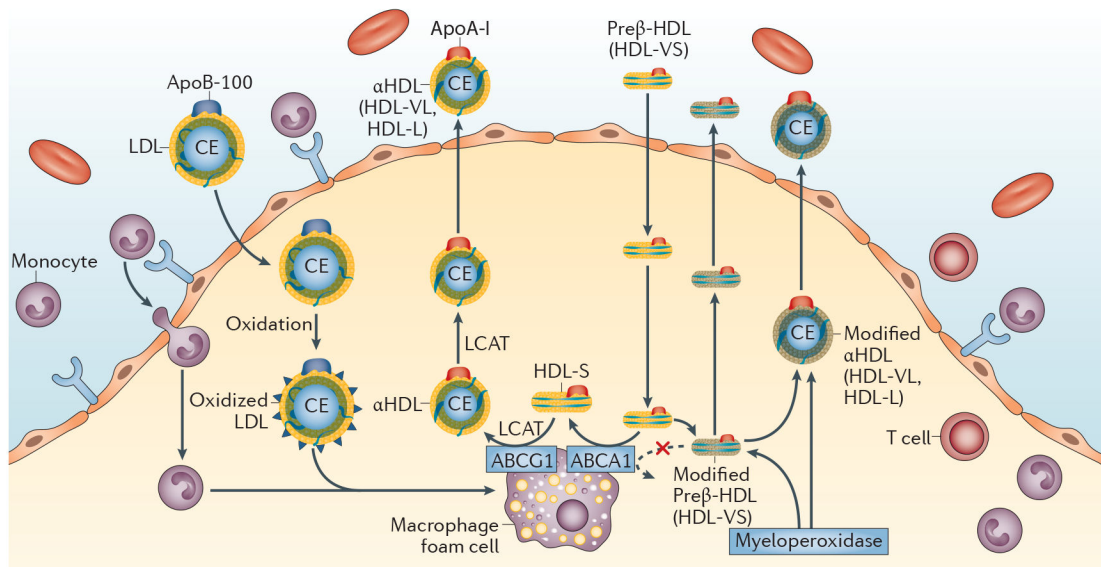
Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein.



**Figure 2 | Role of HDL in the modulation of coronary atherosclerosis.**

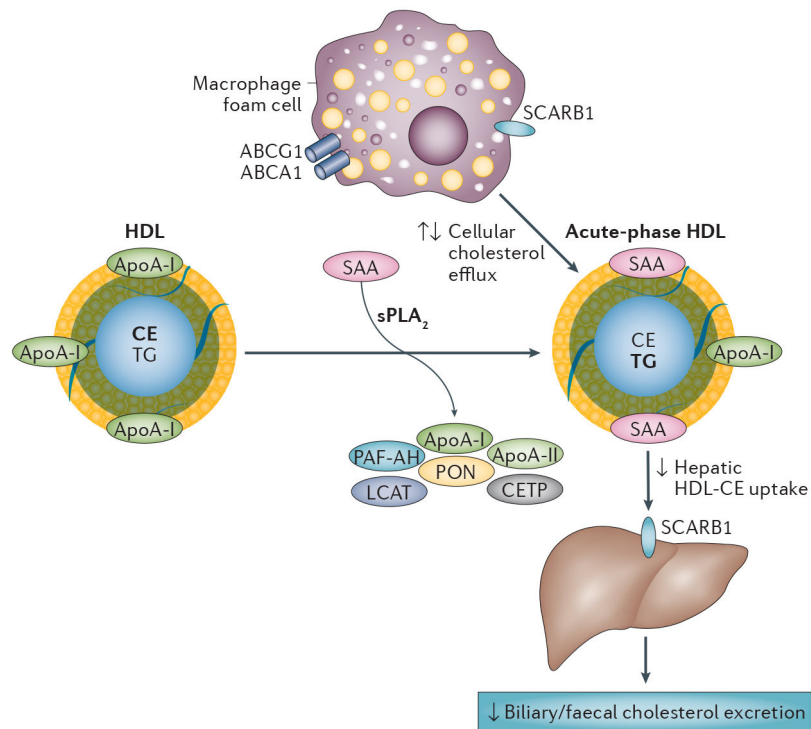
HDL protects against atherosclerosis through multiple mechanisms, as illustrated. HDL performs a pivotal role in removing cholesterol from cholesterol-loaded macrophages by binding to ABCA1 and stimulating the process of reverse cholesterol transport. In reverse cholesterol transport, pre $\beta$ -HDL (HDL-VS) binds to the ABCA1 transporter and initiates cholesterol efflux with the conversion of pre $\beta$ -HDL (HDL-VS) to HDL $\alpha$  (HDL-S). LCAT catalyses the esterification of cholesterol to CEs and the maturation of HDL to CE-rich mature HDL, which can transport cholesterol back to the liver or exchange CEs for triglycerides with the apolipoprotein B-containing lipoproteins. HDL also enhances the endothelial synthesis of NO, a potent vasodilator, to ameliorate endothelial dysfunction, and might also reduce coronary atherosclerosis by decreasing the expression of adhesion molecules on endothelial cells to reduce inflammation, and by decreasing LDL oxidation. Abbreviations: ABCA1, ATP-binding cassette transporter A1; ABCG1, ATP-binding cassette transporter G1; apoA-I, apolipoprotein A-I; apoB-100, apolipoprotein B-100; CE, cholesteryl ester; HDL, high-density lipoprotein; HDL-L, large HDL particle; HDL-S, small HDL particle; HDL-VL, very large HDL particle; HDL-VS, very small HDL particle; ICAM1, intercellular adhesion molecular 1; LCAT, lecithin-cholesterol acyltransferase; LDL, low-density lipoprotein; NO, nitric oxide; VCAM1, vascular cell adhesion protein 1.





**Figure 3 | Myeloperoxidase-mediated modification of apoA-I and sterol efflux.**

In the absence of myeloperoxidase activity, pre $\beta$ -HDL (HDL-VS) binds to the ABCA1 transporter and initiates the efflux of cholesterol from macrophage foam cells with the concomitant conversion of pre $\beta$ -HDL (HDL-VS) to HDL $\alpha_4$  (HDL-S). Oxidation of apoA-I on multiple residues by the proinflammatory enzyme myeloperoxidase might limit the capacity of HDL or apoA-I to mediate cholesterol efflux from macrophages and thus promote the development of experimental and human atherosclerosis. Abbreviations: ABCA1, ATP-binding cassette transporter A1; ABCG1, ATP-binding cassette transporter G1; apoA-I, apolipoprotein A-I; apoB-100, apolipoprotein B-100; CE, cholesteryl ester; HDL, high-density lipoprotein; HDL-L, large HDL particle; HDL-S, small HDL particle; HDL-VL, very large HDL particle; HDL-VS, very small HDL particle; LCAT, lecithin-cholesterol acyltransferase; LDL, low-density lipoprotein.



**Figure 4 |. Acute-phase HDL.**

HDL undergoes substantial modification during an acute-phase response. Inflammatory cytokines induce the hepatic expression of acute-phase SAA and group IIa sPLA<sub>2</sub>, which leads to the formation of HDL particles that are relatively enriched in SAA and depleted of apoA-I and phospholipid. Increased oxidative stress during inflammation generates HDL that contains oxidatively modified apoA-I. In addition, HDL remodelling during inflammation generates an abundance of triglycerides and a loss of HDL-associated proteins such as apoA-II, CETP, LCAT, PAF-AH, and PON. Results from studies investigating the effect of inflammation on steps in the reverse cholesterol transport pathway have been conflicting. During inflammation, cellular cholesterol efflux by ABCA1 and ABCG1 might be unchanged, decreased, or increased, and the uptake by SCARB1 of CEs from acute-phase HDL into the liver for subsequent excretion is either decreased or unchanged. Abbreviations: ABCA1, ATP-binding cassette transporter A1; ABCG1, ATP-binding cassette transporter G1; apoA, apolipoprotein A; CE, cholesteryl ester; CETP, cholesterol ester transfer protein; HDL, high-density lipoprotein; LCAT, lecithin-cholesterol acyltransferase; PAF-AH, platelet-activating factor acetylhydrolase; PON, paraoxonase; SAA, serum amyloid A; SCARB1, scavenger receptor class B member 1; sPLA<sub>2</sub>, secretory phospholipase A<sub>2</sub>; TG, triglyceride.

**Table 1 |**Clinically available tests for HDL function and dysfunction<sup>131,132</sup>

<b>Test</b>	<b>Population</b>	<b>Disease association</b>
Macrophage cholesterol efflux	Patients with stable CHD (case-control)	CAD <sup>81</sup>
Monocyte chemotaxis assay	Patients with stable CHD (case-control)	CAD <sup>16</sup>
Cell-free assay	Patients with stable CHD (case-control)	CAD <sup>16</sup>
<b><i>Proteomics</i></b>		
Oxidized tryptophan	Patients with stable CHD or ACS (case-control)	CVD <sup>6</sup>
Chlorinated tyrosine-192	Patients with stable CHD or ACS (case-control)	CAD <sup>13</sup>
Oxidized methionine-148	Patients with stable CHD or ACS (case-control)	CAD <sup>13</sup>
Apolipoprotein C-III on HDL	Prospective population	CVD events <sup>50</sup>

Abbreviations: ACS, acute coronary syndrome; CAD, coronary artery disease; CHD, coronary heart disease; CVD, cardiovascular disease; HDL, high-density lipoprotein.