REVIEW

HDLs and the pathogenesis of atherosclerosis

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Purpose of review
Plasma levels of HDL cholesterol are a biomarker of cardiovascular health but not a therapeutic target, as demonstrated by the failure of pharmacological modulation of HDL cholesterol to prevent or treat atherosclerotic cardiovascular disease. In health, HDL particles exert pleiotropic effects against atherosclerosis, including cholesterol removal from foam cells, vasodilatory effects through vascular endothelial cell nitric oxide production, decreased vascular inflammation and oxidative damage, endothelial cell proliferation and antiapoptotic effects.

Recent findings
These functional effects of HDL are independent of the cholesterol mass and are related to the proteome and lipidome. In disease states and with the ageing process, HDL components are extensively modified and may no longer play a beneficial role but are retained in the atheroma and contribute to atherosclerosis. We have recently shown that desmocollin 1 (DSC1) acts as an apolipoprotein (apo) A-I binding protein that is highly expressed in atherosclerotic plaques and inhibits atheroprotective HDL functions by retaining apoA-I. The apoA-I retention hypothesis proposes that macrophages express DSC1 in a maladaptive process that renders apoA-I inactive and contributes to atherosclerosis.

Summary
HDL loses their beneficial properties in ageing and disease states. Novel pathways may present new therapeutic avenues to restore their biological functions.

Keywords
apoA-I retention, atherosclerosis, ATP-binding cassette A1, desmocollin 1, HDLs

INTRODUCTION

The epidemiological association between plasma levels of HDL cholesterol (HDL-C) has been confirmed in large-scale prospective epidemiological studies and have withstood statistical scrutiny [1]. Yet, HDL-C, as a biomarker or surrogate end-point for atherosclerotic cardiovascular disease (ASCVD) does not fulfil Koch's (modified) postulates as a risk factor. The reasons for this are multiple: first, Mendelian randomization casts doubt on the link of causality between genetic determinants of HDL-C and ASCVD [2,3▪▪]; second, elevated HDL-C levels are not associated with longevity, but with a decreased lifespan [4,5▪]; third, HDL-C levels are no longer predictive of cardiovascular events in the secondary prevention of ASCVD [6]. Finally, pharmacological modulation of HDL-C using fibrates, niacin, inhibitors of cholesteryl ester transfer protein (CETP) [7] or human apolipoprotein (apo) A-I proteoliposome infusions [8] have failed to reduce ASCVD. The current concept, therefore, is that HDL-C represents a biomarker of cardiovascular health and attention has shifted to a better understanding of the function of HDL particles on the processes of atherosclerosis. Novel therapies aimed at replicating some of the effects of apoA-I are being investigated [9].

It is very likely that HDL exerts protective effects against atherosclerosis in youth and in health, but many of these functions are modified during ageing and in disease states. Here, we review the complexity and controversies surrounding HDL and ASCVD. Importantly, intimal HDL biogenesis, function and egress from the arterial wall may hold the key to unlocking the therapeutic potential of HDL.

HDLs are complex particles with size, proteome and liposome heterogeneity and remain incompletely understood. In health, HDL exerts beneficial effects on the cardiovascular system and prevents

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KEY POINTS

- HDL particles exert pleiotropic effects to prevent atherosclerosis in healthy individuals.
- Cholesterol removal from foam cells, vasodilatory effects through vascular endothelial cell NO production, decreased vascular inflammation and oxidative damage; endothelial cell proliferation and antiapoptotic effects are impaired in ageing, inflammatory states, cardiometabolic disorders and atherosclerosis.
- DSC1 is a novel apoA-I binding protein that is highly expressed in atherosclerotic plaques; it sequesters apoA-I and prevents HDL biogenesis.

ASCVD. Most of these pleiotropic effects are independent of the cholesterol mass within HDL particles. The protective mechanisms are multiple and can be distinguished by their biological processes and target tissues. These include cellular cholesterol efflux from macrophage foam cells, vasodilatory effects on vascular endothelial cells through the stimulation of nitric oxide (NO) production, anti-inflammatory effect on vascular inflammation through the repression of nuclear factor κB (NF-κB) activation and downstream expression of cell adhesion molecules, the prevention of oxidation of LDL particles through the action of several enzymes and proteins on HDL, and endothelial cell proliferation and antiapoptotic effects [10▪▪].

The best studied of these effects is the removal of cholesterol from macrophages in atherosclerotic plaques as the initial step in the process of reverse cholesterol transport. The rate-limiting step of this pathway is the formation of nascent HDL particles through the interaction between apoA-I and the ATP-binding cassette A1 (ABCA1) – a process termed HDL biogenesis. The measurement of HDL biogenesis (HDL-C efflux capacity) has been shown in prospective studies to be strongly associated with ASCVD [11–13]. Subsequent cellular lipidation through the ATP-binding cassette G1 and scavenger receptor B1 (SR-B1), the enrichment of cholesteryl esters with the enzyme lecithin : cholesterol acyl transferase leads to the formation of larger HDL particles which can either transfer their core lipids to apoB-rich lipoproteins through CETP in the circulation or deliver their core lipids to the liver through hepatic SR-B1.

In vascular endothelial cells, HDL stimulates its receptor SR-B1 to initiate sequential activation of Scr tyrosine kinase, phosphoinositide 3-kinase (PI3K), Akt kinase and extracellular signal-related kinases leading to phosphorylation of endothelial NO synthase (eNOS) at Ser1177. The phosphorylation activates eNOS to produce the vasodilator NO. Also, sphingosine-1-phosphate (S1P) carried by HDL signals through its receptors $S1P_1$ and $S1P_3$ to modulate NO release via intracellular calcium mobilization and activation of the PI3K/Akt pathway [10▪▪,14,15].

HDL exerts anti-inflammatory effects in vascular endothelial cells by decreasing expression of intercellular adhesion molecule, vascular cell adhesion molecule and E-selectin. These actions are mediated by SR-B1 and S1P receptors [15,16]. Significantly, cholesterol depletion in plasma membrane lipid rafts modulates the inflammatory response in monocytes, macrophages and T cells [17].

The prevention of LDL oxidation is also considered a major function of HDL. Hypochlorous acid produced by myeloperoxidase oxidizes LDL. Oxidized LDL (oxLDL) is a major substrate for macrophage scavenger receptors, leading to the unregulated uptake of oxLDL by macrophages and the formation of macrophage foam cells. HDL contains several enzymes and specific proteins that prevent lipoprotein oxidation. The paraoxonase and aryl esterase activities of paraoxonase-1, platelet activating factor acyl hydrolase, apoJ, apoF and phospholipid transfer protein all have antioxidant properties. Endothelial cell apoptosis is prevented by HDL, in part through the content in S1P [18,19] and also by preventing the oxLDL-mediated cell apoptosis [3▪▪,20].

It must be emphasized that most of the effects of HDL occur in the subendothelial arterial layer and intima. Plasma HDL particles may not reflect arterial HDL [9].

HDL LOSES ITS PROTECTIVE EFFECTS IN AGEING AND IN DISEASE STATES

The concept of dysfunctional HDL is hardly a novel one [21]. Age itself may be a critical determinant of HDL function [22]. In disease states, such as diabetes and the metabolic syndrome, chronic kidney disease, chronic inflammation and atherosclerosis, many of the functions of HDL become impaired [20]. Extensive modifications of HDL proteins (post-translational modifications) and proteome (specific proteins) and lipids, especially glycerophospholipids and sphingolipids are seen in diabetes, chronic inflammation and increasing age. Elegant reviews have addressed the concept of dysfunctional HDL [10▪▪,20] (Fig. 1).

ApoA-I is the major protein within HDL and is a target of several oxidative modifications, especially by myeloperoxidase within the atherosclerotic plaque. ApoA-I can undergo chlorination at residue Tyr192, sulfoxidation at Met148 and oxidation at

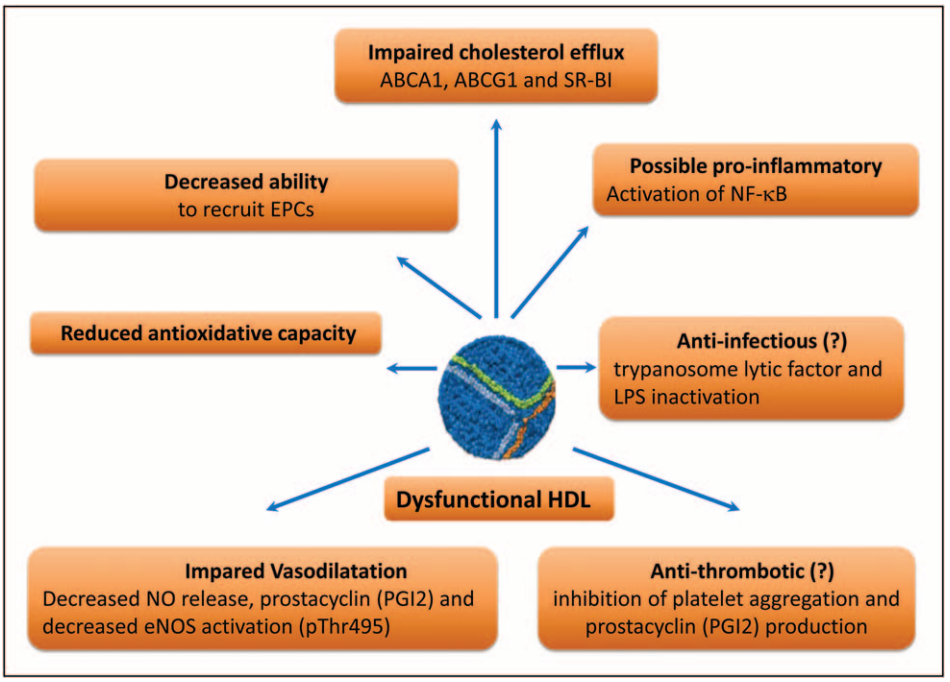

FIGURE 1. The modifications of the HDL proteome and lipidome leads to altered function. The pleiotropic beneficial effects of HDL particles in health are impaired in disease such as the ageing process, cardiometabolic conditions, chronic inflammation and atherosclerosis. Modified with permission from [9].

Trp72; these modifications are associated with impaired HDL biogenesis and decreased cellular cholesterol efflux.

Using a specific mAb targeted at an oxindolyl alanine moiety at Trp72 of apoA-I that recognizes myeloperoxidase-modified apoA-I and HDL, Huang *et al.* [23] showed that modified apoA-I is unable to mediate ABCA1-dependent cellular cholesterol efflux. Although oxTrp72-apoA1 is in low abundance in plasma, it accounts for ~20% of the apoA-I present in isolated atherosclerotic plaques. Furthermore, oxTrp72-apoA-I isolated from human atheroma is lipid poor, unable to promote cellular cholesterol efflux and has proinflammatory activity on endothelial cells.

The HDL proteome is also markedly altered in atherosclerosis and is enriched in inflammatory proteins such as apoCIII and serum amyloid A (SAA). HDL enriched with SAA impairs cellular cholesterol efflux and impairs the anti-inflammatory properties of HDL [24]. This is well in keeping with several studies showing that serum from patients with ASCVD have impaired cholesterol efflux capacity [11–13].

In patients with ASCVD, vascular endothelial cell NO production is not induced by HDL. This is associated with an increased affinity for the lectin-like oxLDL receptor-1 on endothelial cells for HDL from patients with coronary artery disease [14]. This promotes the phosphorylation of eNOS at Thr495, which inhibits eNOS activity.

In a surprising and somewhat controversial series of experiments, van der Vorst examined the effects of HDL-mediated cellular cholesterol depletion on macrophage response. The disruption of lipid raft in macrophage was shown to have proinflammatory effects by enhancing Toll-like-receptor-induced signalling via the activation of the protein kinase C–NF-κB/signal transducer and activator of transcription 1–interferon regulatory factor 1 cascade, which led to increased cytokine expression [25]. Although this effect may be beneficial in innate immunity against bacterial pathogens, enhanced innate immunity within the atherosclerotic plaque may have adverse consequences on atherosclerosis.

These data are consistent with the loss of protective functions of HDL in disease states.

THE APOLIPOPROTEIN A-I RETENTION HYPOTHESIS

ApoA-I and HDL are retained in the arterial wall. In a series of elegant experiments, DiDonato *et al.* showed that lipid-free apoA-I and HDL are abundant in atherosclerotic plaques at concentrations more than 100-fold that found in normal aortas. Furthermore, most of the apoA-I isolated is lipid-poor or lipid-free and this apoA-I exhibited marked decrease in cellular cholesterol efflux [26]. The same group went on to demonstrate that an important proportion of apoA-I consists of oxTrp72 apoA-I, shown to

be unable to promote cellular cholesterol efflux via ABCA1 [23]. Once in the atherosclerotic plaque, apoA-I is subjected to many chemical modifications outlined above, and its beneficial pleiotropic actions are disabled. This begets the question by what mechanism are apoA-I and HDL retained within the arterial wall?

We propose a novel mechanism to explain the retention of apoA-I in atherosclerotic plaques. We sought to determine the proteins interacting with apoA-I in specific plasma membrane microdomains using an unbiased approach. There are technical hurdles to isolate plasma membrane microdomains in a sufficiently pure and intact state to characterize the domains at the molecular level. We set-up a technique to isolate plasma membrane microdomains by cell lysis using ball-bearing cell homogenization, fractionation of cell lysates by discontinuous sucrose gradient ultracentrifugation, sonication of the membrane-rich fractions to dissociate aggregates, immunoprecipitation to capture apoA-I-associated membraned microdomains and mass spectrometric analysis of the domains to identify protein and lipid composition. To maintain the structural and molecular integrity of plasma membrane microdomains, we applied detergent-free conditions during the isolation procedure. The lipidome of an isolated apoA-I-containing microdomain was closely matched to that of nascent HDL particles [27], suggesting that HDL biogenesis is related to the formation of the specific microdomain. The proteome of the microdomain contained 96 proteins; intriguingly, the major components of desmosomes [desmoglein (DSG) 1 and 3, desmocollin 1 (DSC1), plakophilin 1, plakoglobin and desmoplakin] were included [28▪▪].

We further identified DSC1 as a novel apoA-I binding protein. DSC 1–3 and DSG 1–4 are belong to the desmosomal cadherin family and each of DSC isoforms has two splice variants that differ in their cytoplasmic domains. DSG 1–4 and DSC 1–3 isoforms are encoded by separate genes clustered on chromosome 18. The extracellular domain of DSCs and DSGs is composed of five cadherin domains and interacts with each other to form desmosomal junctions at the cell–cell interface. Genetic mutations of the major desmosome proteins are associated with several severe skin disorders and arrhythmogenic cardiomyopathies. The precise role of DSC1 is not fully understood. *DSC1* gene mutations are not known to be associated with disease in man. Although a thorough analysis of genetic variants at the *DSC1* locus has yet to be performed, genetic variants in *DSC1* are not associated with human disease phenotypes (https://genome.ucsc.edu). Furthermore, human genetic databases shows a higher number of missense mutations in *DSC1* than expected 315 vs. 273; $z = -1.24$ (http://exac.broadinstitute.org/gene; >60 000 people), suggesting that missense mutations in *DSC1* may not contribute to genetic selection. It is also noteworthy that the *Dsc1* knock-out mice show that DSC1 may be dispensable for the formation of desmosomes.

Among the five extracellular cadherin domains (EC1–5) of DSC1, EC2 and EC5 domains play key roles in apoA-I–DSC1 interactions [28▪▪]. Once bound to DCS1, apoA-I remains sequestered (or, possibly, internalized) and is no longer available to interact with ABCA1 for HDL biogenesis and the removal of cellular cholesterol (Fig. 2). When DSC1 expression is decreased with short hairpin RNAs or its genetic expression abolished with CRISPR/Cas9 or when blocked by an antibody, apoA-I-mediated cellular cholesterol efflux is increased [28▪▪]. This pathway proposes a novel role for DSC1: the conservation of cholesterol in specific membrane-microdomains which competes with the removal of cellular cholesterol by ABCA1.

Several groups have shown that apoA-I is not present in healthy or minimally diseased arteries, but its concentration is markedly increased in arteriosclerosis. We found that DSC1 is also abundantly expressed in human carotid and coronary atherosclerotic plaques, especially in foam cells expressing CD68 [28▪▪]. Immunohistochemistry reveals colocalization of DSC1 and apoA-I (Fig. 3).

The expression of DSC1 in atherosclerotic plaques is surprising. Macrophages do not form desmosomes and little is known about the role of DSC1 at sites of inflammation and tissue injury, as found in complex atherosclerotic plaques. We postulate that the expression of DSC1 may represent a maladaptive process by which stressed macrophage initiate a response to injury with conservation of cholesterol for membrane biosynthesis. In human atherosclerosis, this response may counteract the removal of excess cholesterol from macrophage foam cells in ABCA1-created plasma membrane microdomains by sequestering cholesterol and apoA-I in a raft-like domain that contain DCS1. ApoA-I bound to DCS1 would be subjected to biochemical alterations outlined above and making it dysfunctional. The sequestration of apoA-I in the arterial wall likely contributes to the lower plasma levels of apoA-I and HDL-C seen on atherosclerosis.

It will be very intriguing to determine whether other desmosomal components are abnormally expressed in macrophages and atherosclerotic plaques and, if so, whether desmosomes are assembled, or whether DSC1 has novel associates and forms novel cholesterol-binding structures in the plasma membrane.

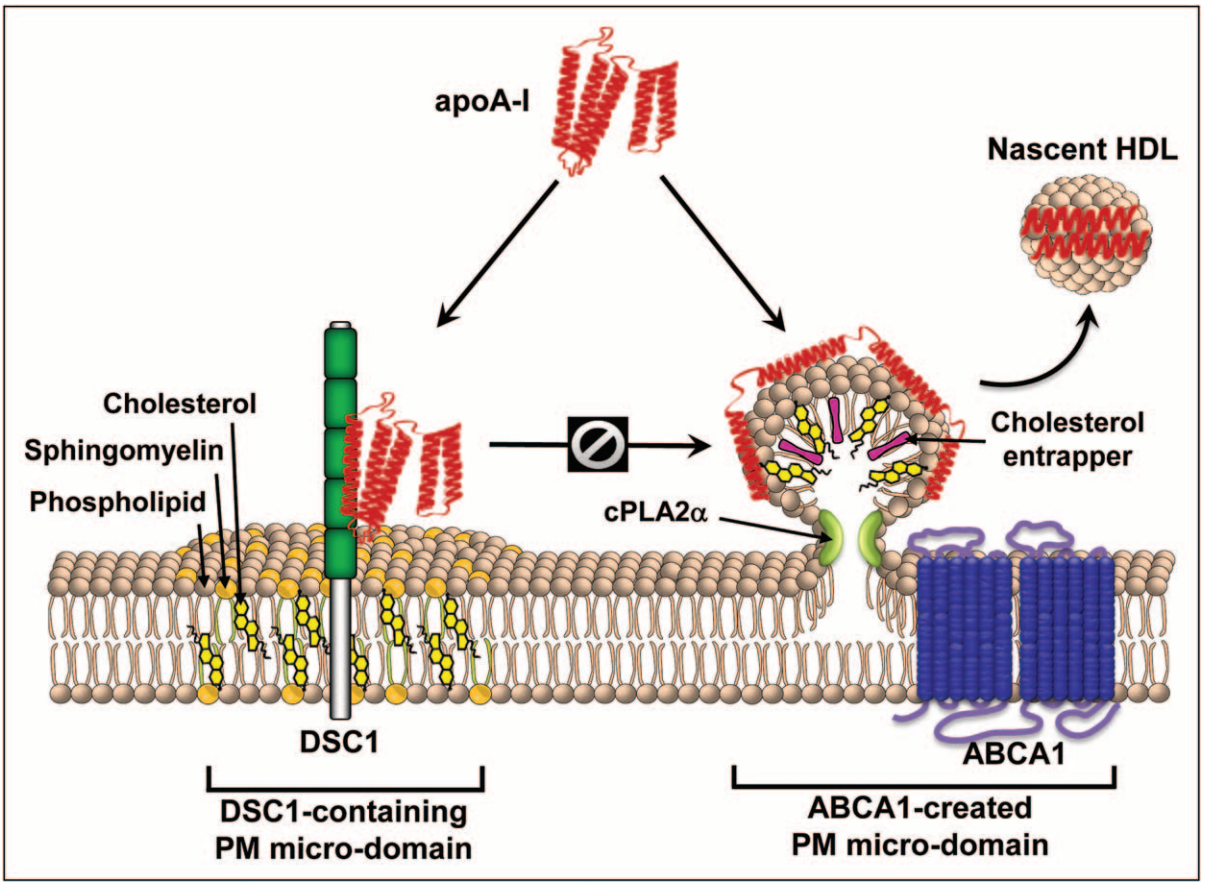

FIGURE 2. Current model of HDL biogenesis. ATP-binding cassette transporter A1 creates a specific plasma membrane microdomain that facilitates a high-affinity interaction with apoA-I for the formation of HDL particles, the rate-limiting step in the reverse cholesterol transport. ATP-binding cassette transporter A1-created microdomains may contain a cholesterol entrapper to keep cholesterol within the domains. Cytosolic Ca^{2+}-dependent phospholipase A2 may increase the local concentration of lysophospholipids to help the release of nascent HDL particles from the plasma membrane. In contrast, desmocollin 1-containing plasma membrane microdomains prevent HDL biogenesis by sequestering apoA-I and cholesterol from the ATP-binding cassette transporter A1-created domains. Inhibition of apoA-I–desmocollin 1 interactions promotes HDL biogenesis, suggesting that a functional interaction between the two counteracting plasma membrane microdomains may be important for the regulation of HDL biogenesis and plasma membrane cholesterol levels. Adapted with permission [29] by Wolters Kluwer Health, Inc. and Copyright Clearance Center. apoA-I, apolipoprotein A-I.

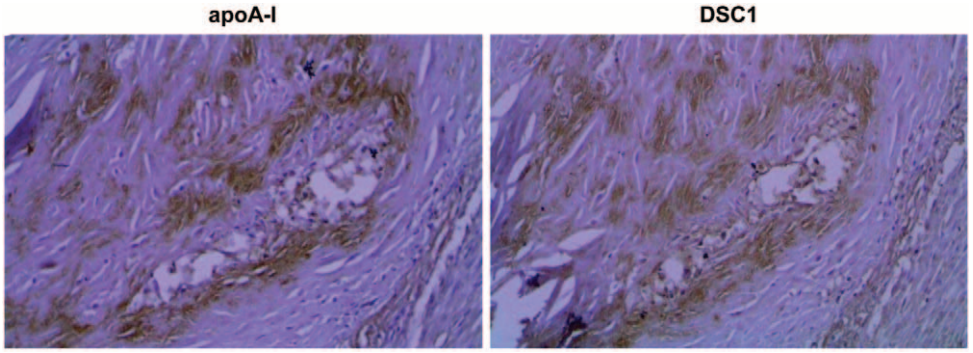

FIGURE 3. Colocalization of desmocollin 1 and apoA-I in human atherosclerotic plaques. Immunohistochemical staining of two serial coronary artery sections obtained from patients with coronary atherosclerosis shows that apoA-I and desmocollin 1 immunoreactivities (brown colour) overlap in atheroma. apoA-I, apolipoprotein A-I.

CONCLUSION

Whether modified HDL particles can promote atherosclerosis remains unproven. To date, the evidence suggests that ageing, diabetes and the metabolic syndrome, chronic inflammation and the presence of atherosclerosis impairs many of the beneficial effects of apoA-I and HDL. Considering the apparent failure of our current approaches at modulating HDL to prevent ASCVD, we must rethink the basic steps of HDL biogenesis. We have shown that apoA-I binds to the extracellular domain of DSC1 and blocking DSC1 expression or apoA-I–DSC1 interactions increased HDL biogenesis and cellular cholesterol efflux *in vitro* [28▪▪]. As a potential therapeutic target, blocking DCS1 to improve apoA-I-mediated cellular cholesterol efflux is appealing.

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Conflicts of interest

There are no conflicts of interest.

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