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REVIEW ARTICLE

HDL: More Than Just Cholesterol

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Abstract

BACKGROUND: Plasma concentration of high density lipoprotein cholesterol (HDL-C) are strongly, consistenly, and independently inversely associated with risk of atheroschlerotic cardiovascular disease (CVD). However, the last decade has seen several observations that do not follow this simple script.

CONTENT: A proteomic analysis of HDL has given us an intriguing glimpse into novel components of HDL. HDL isolated from normal humans contains several classes of proteins, including not only apolipoproteins, but also complement regulatory proteins, endopeptidase inhibitors, hemopexin, and acute phase response proteins. These observations raise the possibility of unsuspected roles for HDL. HDL delivery of complement proteins would implicate HDL in innate immunity. Serine proteinase inhibitors would enable HDL to modulate proteolysis of the vessel wall. HDL from patients with coronary artery disease was enriched in apoE, apoC-IV, apoA-IV, Paraoxonase (PON), and complement factor C3. Highlighted additional mechanisms through which HDL protects the vessel wall are: HDL improves vascular function, decreases vascular inflammation, detoxifies radicals, and limits thrombosis.

SUMMARY: Both inter- and intraorgan desynchrony may be involved in the pathogenesis of cardiometabolic disease attributable to effects in brain and multiple metabolic tissues including heart, liver, fat, muscle, pancreas, and gut. Efforts to dissect the molecular mediators that coordinate circadian, metabolic, and cardiovascular systems may ultimately lead to both improved therapeutics and preventive interventions.

KEYWORDS: HDL, Apo–A1, RCT, Inflammation, HDL Dysfunction, HDL Proteome, HDL & Apo–A1 Mimetics.

Introduction

Despite strong evidence for an inverse association between HDL-C levels and cardiovascular risk (1), successful therapeutic strategies to target HDL have remained elusive. A recent Phase III clinical trial failed to show clinical benefit with the cholesteryl ester transfer protein inhibitor torcetrapib despite markedly increased HDL-C levels (2,3). This outcome reinforced a growing consensus that measurement of HDL-C alone may be an incomplete surrogate for the in vivo functionality of HDL and the clinical efficacy of targeting HDL. The careful mechanistic assessment of HDL function has thus emerged as a potential way forward (4).

There is great interest in understanding the ability of HDL to mediate Reverse Cholesterol Transport (RCT) from macrophages because this ability underlies the antiatherogenic functions of the lipoprotein. Recent studies show that macrophage RCT mediated by HDL has important implications for the development of atherosclerosis, but the HDL contribution does not correlate well with the HDL-C levels, the increase in HDL-C due to elimination of Scavenger Receptor Class B type I (SR-BI) is associated with decreased RCT and increased atherosclerosis. These results indicate that the rate of macrophage RCT and the quality rather than the quantity of HDL are important in determining the antiatherogenic effects of HDL (5).

The ability of HDL to remove cholesterol and oxysterols from cells such as macrophages in the arterial wall is linked to the anti-inflammatory and immunosuppressive functions of this lipoprotein (6,7). These capabilities coupled with the antioxidant, antithrombotic and vasodilatory capabilities of HDL (8) give rise to the overall cardioprotective behavior of HDL. It is becoming increasingly apparent that these antiatherogenic effects of HDL are not only dependent on its concentration in circulating blood but also on its biological quality (9-11).

The functionality of HDL is impaired in humans with chronic inflammatory diseases, including atherosclerosis (12), and these deleterious effects can involve oxidative modification of Apolipoprotein A-I (apoA-I) (13). The HDL dysfunction involves reductions in both anti-inflammatory and cholesterol transport properties. Proteomic studies that have been conducted recently in various laboratories (14) exemplify the growing interest originating from clinical observations on the atheroprotective properties of HDL. What is emerging is that the HDL proteome contains, in addition to the previously recognized quantitatively "minor" apolipoproteins, enzymes, and transport proteins relevant to lipid metabolism and many others that are not in the lipid area but are involved in the processes of acute-phase response, proteinase inhibition, and complement regulation. The presence of small peptides and fragments of yet unknown function has also been reported (15). This unsuspected array of HDL-associated proteins with a marked diversity in biological function has opened new avenues of investigations that may provide a better understanding of the role played by HDL in atherogenesis and inflammatory processes in general and help identify novel biomarkers of cardiovascular disease (16).

HDL Heterogeneity

HDL is a complex macromolecule comprised of lipids (phospholipids and unesterified free cholesterol on the surface and cholesteryl ester and triglycerides in the core) and proteins (apolipoproteins and a variety of other proteins in smaller amounts). Multiple subfractions of HDL based on density, size, charge, and composition can be identified in plasma. A topic of considerable interest is whether specific subfractions of HDL confer greater ability to predict cardiovascular risk than HDL-C itself or may be of greater utility in assessing the benefits of a therapeutic intervention targeted to HDL. Patients with coronary disease generally have smaller, denser HDL particles, leading to the concept that larger HDL particles may be associated with greater protection from CHD. However, the data regarding the predictive ability of HDL subclasses for CHD risk are not conclusive. Recent study indicate that there is a direct relationship between HDL size, apoAI content, and Lecithin Cholesterol Acyl Transferase (LCAT) activation, that different subclasses of particles have distinct morphology and stability, and that spontaneous remodeling of HDL particles represents a potential mechanism for the generation of lipid-free/lipid-poor apoA-I *in vivo* (17).

HDL can be fractionated into discrete subclasses by different techniques according to their physicochemical properties. Human HDL can be separated by ultracentrifugation into two main subfractions on the basis of density, HDL_2 (1.063–1.125 g/ml) and HDL_3 (1.125–1.21 g/ml), which is relatively protein-rich. HDL_2 and HDL_3 can be further divided on gradient gel electrophoresis into $HDL_{2\beta}$ (10.6 nm), $HDL_{2\alpha}$ (9.2nm), $HDL_{3\alpha}$ (8.4 nm), $HDL_{3\beta}$ (8.0 nm) and HDL_{3c} (7.6 nm) in decreasing order of particle diameter (18). In addition, HDL can be separated into two main subpopulations on the basis of electrophoretic mobility: the major subfraction has a relatively high negative surface charge density and is called α-HDL, whereas the other fraction is called preβ-HDL (19,20). Most of the HDL particles in plasma are α-HDL (21) and preβ-HDL represents only approximately 5% of total apoA-I(22).

Preβ-HDL has been resolved into $pre\beta_1$, $pre\beta_2$ and $pre\beta_3$HDL particles according to increasing size by two-dimensional gel electrophoresis (20). ApoA-I is the only protein constituent of these nascent HDL particles but, interestingly, if apoM is also expressed by the cells, the particle size increases. ApoM may function intracellularly to transfer lipid onto the HDL particles during or after their formation by ATP-Binding Cassette A1 (ABCA1) (23). Depending upon cell type, α-HDL and/or preβ-HDL particles can be formed by the apoA-I/ABCA1 reaction (24).

Apolipoprotein F (ApoF) is a protein component of several lipoprotein classes including HDL. It is also known as lipid transfer inhibitor protein (LTIP) based on its ability to inhibit lipid transfer between lipoproteins *ex vivo*. ApoF overexpression reduces HDL cholesterol levels in mice by increasing clearance of HDL-CE. ApoF may be

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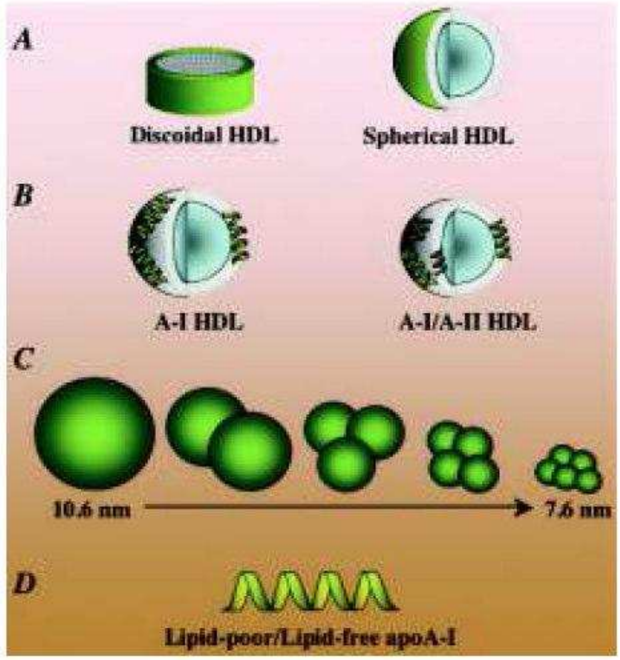

Figure 1. Heterogeneity of HDL subpopulation (Reproduced with permission, from F. Tabet and K.-A. Rye. Clinical Science 2009; 116: 87-98. © the Biochemical Society.)

an important determinant of HDL metabolism and reverse cholesterol transport (25).

Choline-containing phospholipids (phosphatidylcholine, lysolecithin, sphingomyelin) represent 90–95% of the phospholipids in HDL (26). The calculated lipid composition is 137 phospholipids, 50 free (unesterified) cholesterol, 90 cholesterol ester and 19 triacylglycerol molecules in an HDL_2 particle, and 51 phospholipids, 13 free cholesterol, 32 cholesterol ester and nine triacylglycerol molecules in an HDL_3 particle (27). Preβ-HDL contains mainly apoA-I and phospholipids with small amounts of cholesterol (22,28).

Indeed, there is considerable variation among individuals with similar HDL-C concentrations with regard to the ability of their serum to promote cholesterol efflux *ex vivo* (29,30), and some evidence that even after normalization, some HDL particles are more effective at promoting efflux than others (31). Thus the ability to assay human serum or isolated HDL for its cholesterol efflux capacity could be an important method of determining HDL function in assessing differences among individuals or in response to novel therapies.

Robust laboratory assays of HDL subfractions and functions and validation of the usefulness of these assays for predicting cardiovascular risk and assessing response to therapeutic interventions are critically important and of great interest to cardiovascular clinicians and investigators and clinical chemists (11).

Dysfunctional HDL

RCT is the process by which cholesterol in peripheral cells (eg, lipid-laden foam cells) is effluxed onto circulating HDL and transported back to the liver for secretion into bile and feces (32,33). Promotion of RCT is considered a major antiatherogenic function of HDL (34).

It is well established that HDL-C concentration in the blood is independently and inversely associated with an increased risk of CVD (35,36). However, it is apparent that many patients with 'normal' or even 'elevated' plasma HDL experience clinical events (37). Furthermore, the recent clinical trial ILLUMINATE, which targets to increase plasma HDL levels with a new selective Cholesterol Ester Transfer Protein (CETP) inhibitor, Torcetrapib, was prematurely terminated because of an increase in all-cause mortality despite an increase in HDL-C levels. These disappointing results suggest that HDL may not always be atheroprotective (38,39), and in some conditions, it paradoxically enhances the process of atherosclerosis (40).

Fogelman and colleagues developed an assay to measure the antiinflammatory activity of HDL by determining the extent to which HDL inhibits monocyte chemotaxis induced by LDL using an *in vitro* reconstituted artery wall model by the coculture of smooth muscle cells and endothelial cells (41). Other assays for HDL function include cell free antioxidant activity (42), inhibition of endothelial cell adhesion molecule expression (43), and the ability of HDL to act as an acceptor of cellular cholesterol. Navab, Fogeleman, and colleagues using this artery wall model system have demonstrated several conditions in which HDL can lose its antiinflammatory properties, including during the acute phase response when serum amyloid A partially replaces apoAI in HDL, during influenza A infection, and in apolipoprotein AII transgenic mice (44-46).

HDL from Coronary Artery Disease (CAD) subjects is dysfunctional. In fact, HDL from many CAD patients was actually proinflammatory, thus increasing monocyte chemotaxis in response to LDL, unlike the HDL from healthy controls that reduced monocyte chemotaxis. There are many possible alterations between this dysfunctional HDL and normal functional HDL (42).

Inflammation induces major changes in HDL levels and composition. Mediators of inflammation such as tumor necrosis factor (TNF)-α and interleukin (IL)-6 induce expression of serum amyloid A (47) and group IIA secretory phospholipase A2 (sPLA2-IIA) (48), which dramatically alter HDL apolipoprotein content and levels, respectively. Acute phase Serum Amyloid A (SAA) in the plasma is associated with HDL, where it can comprise the major apolipoprotein (49). The increase in sPLA2- IIA activity results in hydrolysis of HDL surface phospholipids and a decrease in HDL particle size (50). The plasma CETP is an integral component of reverse cholesterol transport and regulates HDL cholesterol concentrations. By promoting the transfer of cholesteryl esters (CE) from HDL to apoB-containing lipoprotein particles, HDL-derived CE is taken up via the LDL receptor and cleared by the liver (51). An additional result of CETP action is the generation of lipid-poor apoA-I (52), a key acceptor in ATP-binding cassette transporter AI (ABCA1)- mediated lipid efflux (53). The presence of SAA on HDL holds the potential to impact both the CE transfer and the apoA-I liberating ability of CETP. sPLA2-IIA could also impact the latter action of CETP as apoA-I was shown to dissociate more readily from CETP-remodeled reconstituted HDL after hydrolysis by bee venom phospholipase A2 (54).

Teleologically, the dramatic changes in HDL composition and metabolism during inflammation must serve a short-term purpose to allow the organism to survive a noxious assault. Acute tissue injury results in cell death with large quantities of cell membranes rich in phospholipids and cholesterol generated. Macrophages are mobilized to such sites, ingest these fragments, and acquire considerable lipid load (55).

Another factor that might make HDL dysfunctional is a change in the HDL-associated lipids. HDL may acquire oxidized lipids from cells and by exchange with other particles, or HDL lipids may be oxidized in situ. Lipid peroxides may interfere with HDL antioxidant, antiinflammatory, and cholesterol acceptor activities. Fully functional HDL may promote lipid hydroperoxide metabolism and its uptake and clearance by the liver, which would be protective (56). In contrast, dysfunctional HDL might promote the transfer of lipid hydroperoxides to apoB-containing lipoproteins and promote VLDL and LDL oxidation (57).

Myeloperoxidase (MPO) is an enzyme found in neutrophils, monocytes, and some macrophages that uses hydrogen peroxide to generate chlorinating and nitrating oxidants, which play an important role in killing microorganisms. However, these same reactive species can also modify host proteins and lipids. MPO is enriched in human atheroma (39), and its presence may promote lesion progression, by increasing LDL oxidation, and block plaque regression, by modifications of apoAI/HDL that impair RCT. Bergt *et al*. demonstrated that incubation of HDL with MPO, in a chlorinating reaction (MPO/H_2O_2/Cl^-) that produces HOCl (chlorine bleach), or with reagent HOCl (at a very high 100:1 ratio of HOCl:apoAI) leads to a loss of unsaturated fatty acids in phospholipids and cholesterol esters, and the loss of cholesterol acceptor activity (40).

In addition to decreased lipid acceptor activity, Panzenboeck *et al*. also showed that MPO—or HOCl—modified HDL was more susceptible to uptake and degradation by macrophages, thus turning HDL from a lipid-accepting lipoprotein to a lipid-loading lipoprotein (61).

MPO modification of apoAI is physiologically relevant by demonstrating that: (1) apoAI in plasma and more so in arterial lesions is a selective target of MPO modification that leads to the nitration and chlorination of specific apoAI tyrosine residues; (2) plasma apoAI nitro- and chloro-tyrosine levels are higher in coronary artery disease patients than in control subjects; and (3) apoAI tyrosine chlorination, whether in endogenous plasma or after *in vitro* MPO-mediated modification, is associated with the specific loss of ABCA1-mediated cholesterol acceptor activity, such that apoAI cholesterol acceptor activity was inversely correlated with apoAI nitro—and chloro—tyrosine levels (62-67).

Thus, the levels of plasma apoAI nitro—and chloro—tyrosine may serve as physiological useful and specific markers of MPO activity *in vivo*, even though tyrosine modification does not appear to be responsible

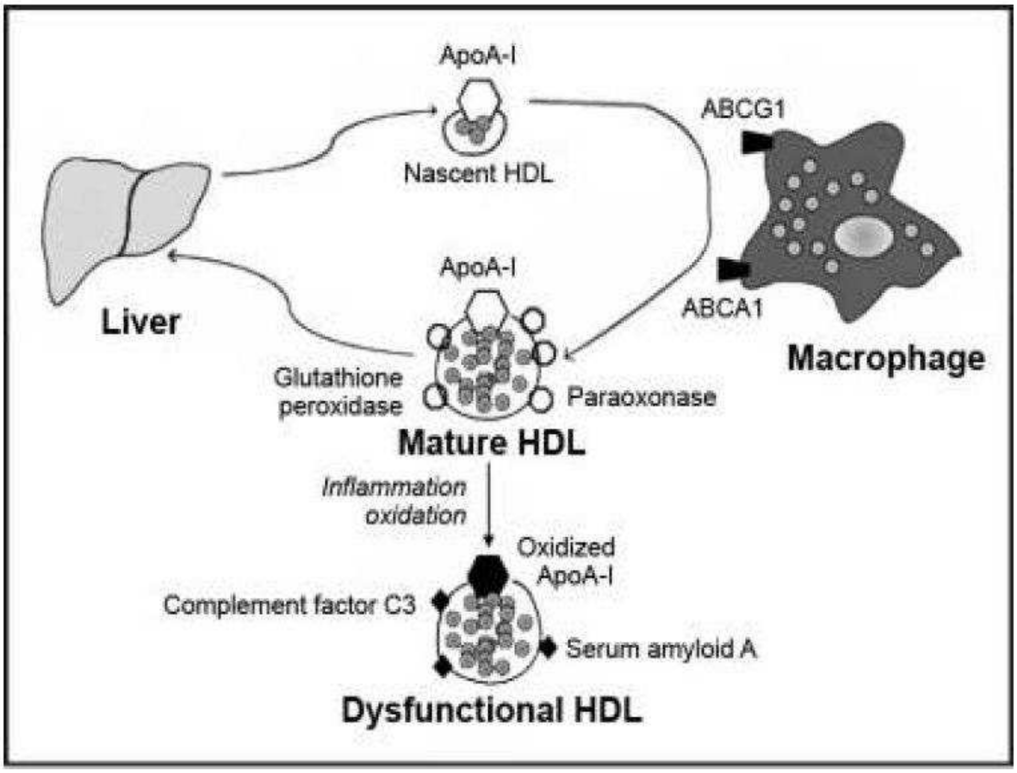

Figure 2. Mature and dysfunctional high – density lipoprotein (Reproduced with permission from Lowenstein CJ, Cameron SJ. Curr Opin Endocrinol Diabetes Obes 2010; 17: 166–70. © Lippincott William & Wilkins).

for turning the "good" HDL-cholesterol "bad." ApoAI activity appears to require both hydrophobic and aromatic residues in the Trp positions. When the 4WF and wild-type apoAI isoforms were treated with increasing oxidant stress from MPO or HOCl, the 4WF isoform is markedly resistant to loss of cholesterol acceptor activity (68). Thus, the 4WF apoAI isoform is MPO resistant and may be a better therapeutic reagent to promote the regression of atherosclerotic plaques, an environment where MPO and MPO-derived oxidants are abundant. If this strategy is successful, then "good" cholesterol can be made "better" (13).

Zheng *et al.*, using mass spectrometry, demonstrated that the level of apoAI chlorotyrosine was a better predictor of cardiovascular disease than the levels of total plasma chloro- or nitro-tyrosine (62). This type of assay is more specific for dysfunctional HDL but is too laborious for routine clinical use. The identification of specific modified residues in apoAI, such as chlorotyrosine and hydroxytryptophan, make it possible to develop novel and scalable immunoassays specific for oxidized/dysfunctional HDL. Through the development of these new assays it will be possible to perform observational and interventional studies to determine the association of dysfunctional HDL with CAD, cerebrovascular events, and mortality (13).

Inflammation is proposed to impair HDL function and RCT. This may be of pathophysiological significance because attenuation of RCT might contribute to atherosclerosis in chronic inflammatory states, including metabolic syndrome and T2DM (69).

Cardiovascular Protective Effects of HDL Beyond RCT

The cardioprotective effects of HDLs have been attributed to several mechanisms, including their involvement in the reverse cholesterol transport pathway. HDLs also have antioxidant, anti-inflammatory amd antithrombotic properties and promote endothelial repair, all of which are likely to contribute to their ability to prevent CHD (8).

The central anti-atherogenic activity of HDL is likely to be its ability to remove cholesterol and oxysterols from macrophage foam cells, smooth muscle cells and endothelial cells in the arterial wall. To some extent, the pleotropic atheroprotective properties of HDL may be related to its ability to promote sterol and oxysterol efflux from arterial wall cells, as well as to detoxify oxidized phospholipids (6).

Atherosclerosis, once regarded simply as excessive accumulation of lipids in the artery wall, is now considered to be a chronic inflammatory disorder that is characterized by the presence of macrophages and other inflammatory cells in the arterial intima (70).There is mounting evidence that HDLs inhibit the inflammation that is associated with atherosclerotic plaque development, including the initial step, where circulating leucocytes become tethered to the endothelial surface and migrate into the artery wall (71).

The death of macrophages in atherosclerotic lesions by apoptosis or postapoptotic necrosis is thought to contribute to inflammation, necrotic core formation and destabilization of plaques (72-75). Recent study shown that HDL reduces macrophage apoptosis induced by loading with free cholesterol or oxidized LDL (76). Interestingly, the ability of HDL to protect against apoptosis, especially that induced by oxidized LDL, is abolished in ATP Binding Cassette G1 (ABCG1) -/- macrophages. This reflects the specific ability of HDL and ABCG1 to promote efflux of 7-ketocholesterol and related oxysterols modified at the seventh position from macrophages loaded with oxidized LDL. In contrast, ABCA1 and apoA-1 do not promote efflux of 7-ketocholesterol from cells. 7-Ketocholesterol is the major oxysterol found in oxidized LDL and atherosclerotic plaques. Consistent with an *in vivo* role of ABCG1 in promoting efflux of oxysterols, ABCG1- / - mice show accumulation of 7-ketocholesterol in macrophages, as well as reduced levels of 7-ketocholesterol in plasma, when fed a high-cholesterol diet. These observations suggest a specific role of ABCG1 in promoting efflux of toxic oxysterols from plaque macrophages to HDL. As ABCG1 also appears to be highly expressed in arterial endothelium (77), this activity could also potentially protect against oxidized LDL-induced endothelial dysfunction and reduced endothelial nitric oxide synthase (eNOS) activity (78).

ABCA1 and ABCG1 are both induced in phagocytes following ingestion of apoptotic cells (79), and are involved in promoting cholesterol efflux after ingestion of the cholesterol-rich apoptotic cell, thus protecting the phagocyte from free cholesterol-induced cell death (80). The induction of ABCA1 involves both Liver X Receptors (LXRs) and activation of mitogen-activated protein kinase (MAPK) signalling pathways in phagocytes, specifically NFϰβ and p38 MAPK signalling, whilst the induction of ABCG1 is mediated via LXRs (79).

Thus, in atherosclerotic plaques, the activities of ABCA1 and ABCG1 probably help to get rid of cholesterol, oxysterols and oxidized phospholipids that are taken up during ingestion of modified LDL or apoptotic cells. The efficient and healthy clearance of apoptotic cells is likely to be a key event limiting lesion progression, and the failure of this process may lead to directly to postapoptotic necrosis and inflammatory cell death, promoting plaque destabilization (74).

HDL has potent antiinflammatory properties, including the ability to directly bind and sequester lipopolysaccharide (81,82), suppressing activation of Toll-like receptor 4 (TLR4) signaling (83). Recent studies have indicated additional direct cellular effects of HDL leading to suppression of inflammatory responses. By promoting cholesterol efflux, HDL has been proposed to inhibit cellular inflammatory signaling leading to inhibition of Monocyte Chemoattractant Protein-1 (MCP-1) and CD11b expression and monocyte transmigration (84).

These mechanisms (ie, increased sterol synthesis and decreased sterol efflux) likely help to conserve cellular cholesterol required for increased membrane synthesis during cell proliferation. Additional mechanisms may also be involved, such as changes in membrane lipid composition or organization that lead to enhanced growth factor receptor mediated signaling events. These studies strongly suggest that HDL-mediated cholesterol efflux via LXR-regulated ABC transporters plays a key role in down-modulating lymphocyte proliferation and activation. The sterol efflux activities of ABCA1 and ABCG1 modulate macrophage expression of inflammatory cytokines and chemokines as well as lymphocyte proliferative responses. In macrophages, transporter deficiency causes increased signaling via various Toll-like receptors including TLR4. Studies have shown that the traditional roles of HDL and ABC transporters in cholesterol efflux and reverse cholesterol transport are mechanistically linked to antiinflammatory and immunosuppressive functions of HDL. The underlying mechanisms may involve modulation of sterol levels and lipid organization in cell membranes (7).

Several lines of evidence suggest that HDLs have antioxidant properties, which may protect against oxidative damage in atherosclerosis. This beneficial effect has been attributed to enzymes, such as PON1 and platelet-activating factor acetylhydrolase (PAF-AH); also known as lipoprotein-associated phospholipase A2 (Lp-PLA2), present on the HDL surface (85,86).

Oxidative stress, an emerging risk factor for premature atherosclerosis and cardiovascular disease, mediates the formation of proinflammatory, proatherogenic oxidized low-density lipoprotein (oxLDL) in the arterial intima. oxLDL contains multiple products of free radical-induced lipid peroxidation, including lipid hydroperoxides (LOOH) as primary products, secondary LOOH-derived short-chain oxidized phospholipids (oxPL) and oxidized sterols (87). Significantly, products of lipid peroxidation present in oxLDL can induce a proinflammatory phenotype in arterial wall cells, which contributes to both endothelial dysfunction and apoptotic cell death, key elements in the initiation and progression of atherosclerotic lesions (88).

Apolipoprotein A-I (apoA-I), the major HDL apolipoprotein, may play a central role in HDL-mediated antioxidative activity, as Met residues 112 and 148 can reduce LOOH into redox-inactive lipid hydroxides (LOH), thereby terminating chain reactions of lipid peroxidation (89,90).

In addition to apoA-I, apolipoprotein components of HDL, which display antioxidative activity, include apoE, apoJ, apoA-II and apoA-IV. ApoE possesses distinct antioxidative properties (91) and can promote regression of atherosclerosis independently of reduction in plasma cholesterol levels (92-94). The beneficial actions of apoJ may be related to its ability to maintain integrity of membrane and lipoprotein lipids via its hydrophobic-binding domains (95).

The capacity of apoA-II to protect LDL from oxidation is however questionable, given the fact that overexpression of human apoA-II in dyslipidaemic mice accelerates atherosclerosis by altering the HDL proteome, by increasing aortic accumulation of oxLDL and by reducing antioxidative activity of HDL (96,97). Such pro-atherogenic actions of apoA-II may be related to the displacement of antiatherogenic apoA-I and PON1 by apoA-II from HDL particles 97).

Enzymatic components potentially contributing to antioxidative properties of HDL include PON1, PAF-AH or 'lipoprotein-associated phospholipase A2' and LCAT, all of which were proposed to hydrolyse proinflammatory short-chain oxPL (98,99). The distinction between the hydrolytic actions of HDL-associated PAF-AH and of PON1 on LOOH is however difficult as the two activities are typically determined using substrates, such as PAF (for PAF-AH) and paraoxon (for PON1), whose hydrolysis does not parallel the hydrolysis of LOOH (100).

The potent antioxidative activity of HDL_3 critically involves transfer of phospholipid hydroperoxides (PLOOH) from oxLDL to HDL_3 in a process dependent on HDL surface lipid rigidity, with concomitant reduction of LDL-derived PLOOH to the corresponding redox-inactive PLOH by Met residues of apoA-I. Indeed, the transfer of LOOH from LDL to HDL can occur directly between lipoprotein phospholipid monolayers, either spontaneously or mediated by lipid transfer proteins (101,102). Concentrations of redoxactive Met residues in apoA-I and of PLOOH suggest a 1 : 1 reaction stoichiometry (89,90,103). Apo A-I therefore constitutes the central element in HDL_3-mediated protection of LDL from free radical – induced oxidation.

HDL particles constitute a major carrier of LOOH in human plasma and have therefore been proposed to function as a 'sink' for oxidized lipids (56) which can accumulate when LOOH-inactivating capacity of HDL is overwhelmed. Consistent with this hypothesis, HDL

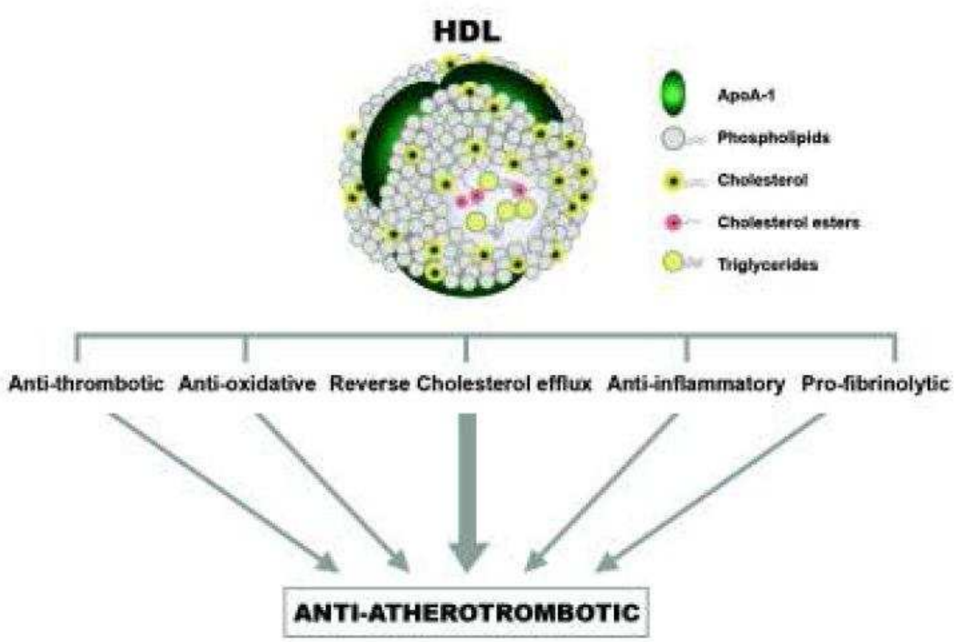

Figure 3. Antiatherothrombotic effects of HDL (Reproduced with permission from Skajaa T, Cormode DP, Falk E, Mulder WJM, Fisher EA, Fayad ZA. Arterioscler Thromb Vasc Biol 2010; 30: 169–76. © American Heart Association).

has been recently shown to represent the major carrier of plasma F2-isoprostanes, stable end-products of lipid peroxidation (104). From the physiological point of view, such 'sink'-like function of HDL may ensure efficient elimination of oxidized lipids from the circulation through the liver (101).

HDL particles are highly heterogeneous in their structure, metabolism and biological functions (98). Among the major HDL subpopulations, small, dense, protein-rich HDL_3 are distinguished by their proteome and lipidome (105,106). Furthermore, HDL_3 exhibit potent capacity to protect LDL from free radical-induced oxidative damage (107) and to inhibit oxLDL-induced apoptosis of endothelial cells (108). Atheroprotective properties of small, dense HDL_3 are consistent with results of clinical and animal studies (109,110).

The most interesting of these identified small dense HDL_3c as a particle subpopulation in which seven proteins occurred predominantly: apoA-I, apoJ, apoL-1, apoF, PON1/3, PLTP and PAF-AH (105,106). The HDL_3c proteome also contained apoA-II, apoD, apoM, SAA 1, 2 and 4, apoC-I, apoC-II and apoE. The unique proteome of HDL_3c has critical functional implications as this particle species exhibited the greatest potency among HDL subpopulations to protect LDL against oxidative stress *in vitro*. Such antioxidative activity was positively correlated with the presence of apoJ, apoM, SAA4, apoD, apoL-1 and PON1/3 in HDL_3c (106).

Replacement of apoA-I by acute phase proteins, primarily SAA, in small, dense HDL particles under conditions of chronic inflammation (111) may represent another mechanism contributing to the impairment of HDL antioxidative activity. Thus, circulating HDL particles, and particularly small, dense, protein-rich HDL_3, may provide potent protection of LDL *in vivo* from oxidative damage by free radicals in the arterial intima, resulting in the inhibition of the generation of proinflammatory oxidized lipids, primarily lipid hydroperoxides (LOOH) but also short-chain oxidized phospholipids (oxPL) (112).

Our understanding of the role of apoA-I/HDL in cardiovascular health and disease has entered a new era recently with the discovery that HDL has direct actions on the vascular endothelium and on mechanisms mediating thrombosis. In particular, the capacity of HDL to modify endothelial NO synthase (eNOS) expression and activity and the production of the atheroprotective signaling molecule nitric oxide (NO). Multiple lines of investigation indicate that NO is atheroprotective and that NO deficiency is critically involved in the pathogenesis of hypercholesterolemia-induced vascular disease. Because eNOS activity is acutely regulated by multiple extracellular stimuli and the NO produced is a labile, cytotoxic messenger molecule with primarily paracrine function (114,115), the intracellular site of NO synthesis has a major influence on the biological impact of the enzyme. In cultured endothelial cells, eNOS is primarily associated with caveolae (115,116). Caveolae are specialized, lipid-ordered plasma membrane microdomains enriched in cholesterol, glycosphingolipids, sphingomyelin, and lipid-anchored membrane proteins, and they contain a variety of signal-transduction molecules. Both N-terminal myristoylation and palmitoylation of eNOS are necessary for optimal targeting to caveolae (117). In addition to co- and posttranslational modifications of resident proteins, membrane cholesterol is essential for normal caveolae function (118). Oxidized LDL (OxLDL) causes depletion of caveolae cholesterol in endothelial cells via the scavenger receptor CD36, leading to eNOS redistribution to an intracellular locale and an attenuated capacity to activate the enzyme (119,120).

HDL maintains the total cholesterol content of caveolae by the provision of cholesterol ester, resulting in preservation of the eNOS signaling module. Based on studies of mimetics of apoA-I, HDL prevents eNOS uncoupling by LDL, which favors O_2 production over NO production. HDL causes membrane-initiated signaling, which stimulates eNOS activity. Binding of HDL to SR-BI via apoA-I causes rapid activation of the nonreceptor tyrosine kinase Src, leading to Phosphoinositide 3-kinase (PI3-kinase) activation and downstream activation of Akt kinase and MAP kinase, which result in enhancement of eNOS activity. Although the apolipoprotein and phospholipid components of HDL are sufficient to activate signaling, there may also be involvement of HDL-associated lysophospholipids SPC, S1P, and LSF acting through the lysophospholipid receptor S1P3. In addition to modulating the acute response, the activation of the PI3-kinase–Akt kinase pathway and MAP kinase by HDL causes an increase in eNOS protein (122).

Multiple proatherogenic factors promote apoptosis in endothelium, and these include OxLDL (123,124), TNF-α (124,125), homocysteine (126), and angiotensin II (126). OxLDL causes a delayed but sustained increase in intracellular $Ca2^+$ in endothelial cells, which results in cell death, and this effect is reversed by HDL and mediated by prevention of the sustained $Ca2^+$ increase. A putative redox motif CXXS residing at amino acids 323 to 326 of SR-BI is required for the promotion of apoptosis and that this is mediated by caspase 8, which is a component of the membrane-related apoptosis pathway. The proapoptotic effects of SR-BI were reversed by HDL and by eNOS, suggesting that under normal conditions, the antiapoptotic actions of NO prevail, whereas with low levels of HDL, SR-BI may promote endothelial cell death (128).

The processes of endothelial cell proliferation and migration are crucial to both neovascularization and to a successful response to vascular injury. Disruptions of endothelial cell monolayer integrity, either by gross denudation related to a vascular intervention or gap formation between cells caused by disturbed shear stress, place the arterial wall at greater risk for vascular disease (129-131). Furthermore, whereas repeated endothelial removal worsens the severity of vascular lesions (132), enhanced reendothelialization blunts lesion formation (133,134). Providing another potential explanation for the cardiovascular protection afforded by HDL.

The molecular mechanisms responsible for the antithrombotic effects of HDL are likely to be multiple (135-137), and they relate to the 3 categories of processes known as Virchow's triad, which contribute to thrombus formation: (1) dysfunction of the cells within the vascular wall, particularly the endothelium; (2) disturbed blood flow; and (3) dysfunction of blood components. Many of the protective activities of HDL in the first 2 categories entail the NO-dependent ability of the lipoprotein to promote endothelial cell health and optimal vasoregulation. In addition to promoting NO production, HDL causes enhanced prostacyclin synthesis, which can modify thrombosis as well as other intravascular events. Prostacyclin acts synergistically with NO to induce Vascular Smooth Muscle (VSM) relaxation, inhibit platelet activation, and diminish the release of growth factors that stimulate the local proliferation of VSM cells. The impact of HDL on prostacyclin production in endothelium occurs by both the provision of arachidonate (140-143) and upregulation of Cox-2 expression (144,145). Recently, it has been shown that HDL_3 induces Cox-2 expression and prostacyclin release via a p38 MAP kinase/CREB-dependent pathway in endothelium that mimics the mechanism that occurs in VSM (146-148).

HDL transports various sphingolipids that are present in plasma in the micromolar range, and at least 4 kinds of HDL sphingolipids (149) may directly or indirectly contribute antithrombotic activity. HDL may promote fibrinolysis by downregulating plasminogen activator inhibitor-I (PAI-I) and by upregulating tissue plasminogen activator (t-PA) (149-151). The oxidation of HDL alters the influence of HDL on fibrinolysis because oxidized HDL_3, but not native HDL_3, promotes PAI-I expression and consequently suppresses fibrinolysis (152).

Platelet aggregation is inversely correlated with HDL levels in humans (153), suggesting that HDL has antiplatelet actions. Mechanistically, although HDL may reduce platelet activation directly (154), HDL may also act indirectly on platelet activation via effects on endothelial cells.

HDL Proteome

The inverse relationship between plasma HDL-C levels and atherosclerotic CVD provides the epidemiological basis for the widely accepted hypothesis that HDL is atheroprotective. Despite intense research, the underlying mechanisms of HDL athero-protection remain incompletely understood. Indeed, recent clinical trials (155,156) indicate the complexity of HDL physiology and the challenges in developing HDL therapies. HDL function, and benefit with a specific therapy, may depend more on the molecular mechanism driving increases in HDL-C than on the absolute level of HDL-C (157). Some interventions that raise HDL-C levels may have no benefit and even promote atherosclerosis (158), while other therapies may reduce CVD without actually changing HDL-C levels (155). Overall, the epidemiological evidence suggests that the majority of mechanisms that result in higher HDL-C levels in vivo will provide athero-protection; the question is how to identify such targets. This requires a shift in mindset toward assessing HDL in terms of its atheroprotective functions rather than just levels of cholesterol and its main apoprotein, APOAI.

Although several measures of HDL particles, composition, and function exist, there are no tractable methods for assessing RCT in humans, and simple, reliable, and reproducible assays of HDL anti-inflammatory functions are lacking. In this setting, recent HDL proteomic studies may provide novel insights into HDL physiology and the potential for development of bioassays of HDL function, although such studies are in their infancy (15,159-162).

Shotgun proteomic analysis identified multiple complement regulatory proteins, protease inhibitors, and acute-phase response proteins in HDL, strongly implicating the lipoprotein in inflammation and the innate immune system (163).

Mass spectrometry and biochemical analyses demonstrated that HDL_3 from subjects with CAD was selectively enriched in apoE, raising the possibility that HDL carries a unique cargo of proteins in humans with clinically significant cardiovascular disease. Collectively, these observations suggest that HDL plays previously unsuspected roles in regulating the complement system and protecting tissue from proteolysis and that the protein cargo of HDL contributes to its antiinflammatory and anti-atherogenic properties (159).

Using shotgun proteomics, Vaisar *et al*. identified 48 proteins in HDL, isolated by ultracentrifugation from healthy controls and/or CAD subjects (159). They included 22 of 23 known HDL proteins with well-characterized roles in lipid metabolism, which validates our experimental approach. Importantly, they found 13 proteins not previously known to reside in HDL. Surprisingly, acute-phase response proteins (23 of 48), whose plasma concentrations are altered markedly by acute inflammation (158), outnumbered proteins implicated in lipid metabolism. Several proteins not previously known to reside in HDL, including complement factors C4A/C4B and C9 as well as the complement regulatory protein vitronectin, were identified. Vitronectin is an extracellular matrix protein, which raises the possibility that certain HDL components can be derived from non-cellular sources or cells distinct from those that synthesize apoA-I in the liver and intestine. The detection of multiple proteins with roles in complement activation, together with pioneering studies of HDL protein complexes that kill protozoa (164), is consistent with the suggestion that HDL serves as a platform for the assembly of proteins involved in the innate immune response. Given that proteolysis of structural proteins in atherosclerotic lesions is thought to play a critical role in plaque rupture, the major cause of myocardial infarction and sudden death in subjects with CAD (165), it is noteworthy that a family of proteins in HDL that contain serine proteinase inhibitor domains. Serine protease inhibitors, termed serpins, are key regulators of numerous biological pathways involved in inflammation, coagulation, angiogenesis, and matrix degradation. Moreover, they also detected the thiol proteinase inhibitor kininogen-1 and haptoglobin-related protein, which contains a crippled catalytic triad residue that may allow it to act as a decoy substrate to prevent proteolysis. These observations suggest that HDL plays a previously unsuspected role in preventing plaque rupture, perhaps by protecting vascular lesions from promiscuous proteolysis.

These observations support the proposal that alterations in HDL's protein cargo might boost the formation of inflammatory particles (138,169), raising the possibility that quantifying those proteins could provide insights into atherogenesis. Importantly, the CAD subjects were newly diagnosed (though at least 3 months past any acute CAD event) and not on medications that alter lipid levels. Using statistical approaches based on spectral counting [the number of MS/MS spectra unique to a protein (166,167)] and random permutation analysis (159,167), they found five proteins that were apparently enriched in HDL isolated from the CAD subjects. These proteins related to lipid metabolism (apoE, apoC-IV, and apoA-IV), oxidative stress (paraxonase-1), and the immune system

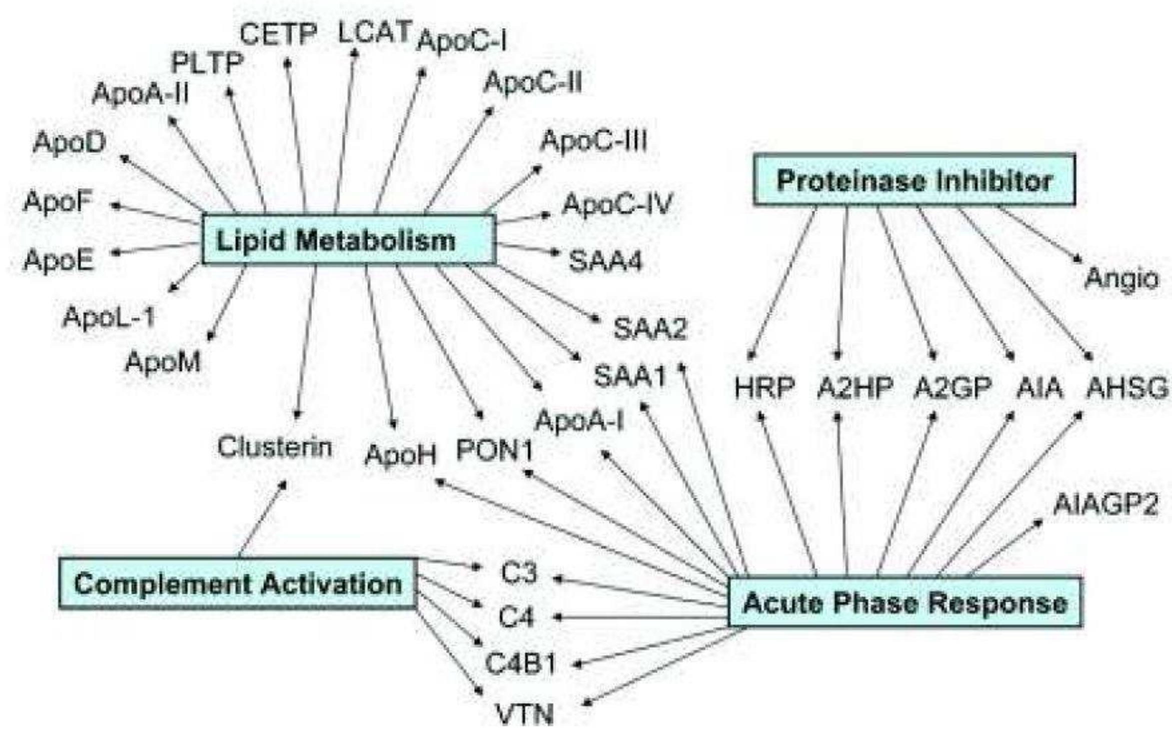

Figure 4. Global view of biological processes and molecular functions of HDL proteins (Reproduced with permission from Heinecke JW. J Lipid Res 2009: S167–S71. © American Society for Biochemistry and Molecular Biology, Inc.).

(complement factor C3). ApoA-IV, located in the apoA-I/apoC-III/apoA-IV gene cluster, inhibits atherosclerosis in mouse models of hypercholesterolemia (168). ApoE, apoC-IV, and C3 are expressed by macrophages (169,170). C3 is needed to assemble the membrane attack complex of the complement system, suggesting a link between HDL, macrophages, and innate immunity.

Elevated levels of apoE in HDL_3 isolated from two independent groups of subjects with established CAD raises the possibility that alteration in HDL's protein content serves as a marker, and perhaps a mediator, of CAD.

Proteomics studies have extended the list of identified apolipoproteins and associated proteins present in HDL and its subclasses. These proteins appear to cluster around specific functions related to lipid metabolism, inflammation, the immune system, hormone-binding, hemostasis, and antioxidant properties (171).

In moving forward, the challenge will be to develop relatively simple HDL biomarkers that can be measured before and after a clinical intervention, then correlated with clinical outcomes or atherosclerosis imaging, and ultimately used in CVD risk prediction. Examples could be an immunoassay for the content of specific proteins in HDL or its subfractions; e.g., the proportion of HDL or subclasses containing APOE, LCAT, or $Lp\text{-}PLA_2$, specific complement proteins, antiproteases, or oxidized lipids could turn out to have predictive value. Application of a broad spectrum of assays that address HDL functionality as well as composition is likely to provide the greatest insight into the relationship between HDL and atherosclerosis and the effects of novel therapies (14).

HDL and β-Cell Function in T2DM

Impaired glucose-stimulated insulin secretion is characteristic among individuals with T2DM (172), and both β-cell dysfunction and loss of β-cell mass are known to be important in the pathogenesis of T2DM (173). Changes in lipoprotein particles are evident in both metabolic syndrome and T2DM (174), and are likely to play an important role in the development of atherosclerosis. Recent data, however, suggest that changes in HLD and LDL could also influence β-cell function and mass, implying a role for lipoprotein particles in the pathogenesis of T2DM. Patients with diabetes exhibit an increase in small, dense pro-atherogenic LDL particles (174). Importantly, the diabetic dyslipidaema frequently precedes T2DM by years, suggesting that disturbances in lipid metabolism may be an early event in the development of this disease (175,176). In agreement with this, low HDL is an independent risk factor for the development of T2DM (175). Furthermore, bezafibrate treatment, which raises HDL-cholesterol levels by 16% and decreases triglyceride levels by 24%, reduces the incidence and delays the onset of T2DM in coronary artery disease patients with impaired fasting glucose (177).

The most common abnormality detected in HDL lipid composition in T2DM is an enrichment of triglycerides in the HDL core and a depletion in cholesterol ester (178). However, other alterations include increased SAA concentration, accumulation of glycated ApoA1 and ApoA2, changes in phospholipid composition and decreased levels of HDL-associated enzymes including PON1 and LCAT (178). As a result of these changes in composition, ApoA1 or HDL isolated from T2DM patients has decreased cholesterol efflux capacity (179,180) and defective antioxidative activity (181). Furthermore, HDL3 isolated from people with metabolic syndrome has defective antiapoptotic activity (182). Therefore, in addition to decreased absolute levels of HDL, patients with T2DM also have functionally defective HDL particles. Over the last 5 years, there have been a number of studies that point to the role of β-cell cholesterol homeostasis on insulin secretion (183).

Cholesterol can regulate signal transduction through membrane microdomains and gene expression through cholesterol-activated transcription factors (184). For example, intracellular cholesterol regulates glucose metabolism and gene expression in adipocytes (185). Glucose Stimulated Insulin Secretion (GSIS) is a complex process involving a cascade of regulatory factors. Misregulation of cholesterol could result in disruption of any one pathway and lead to partial or a near complete loss of secretory function. One potential mechanism for cholesterol regulation of GSIS that involves modification of neuronal NO synthase (nNOS) and GK activity through cholesterol-rich membrane microdomains on the insulin granules (186).

As HDLs are the predominant acceptors of cell cholesterol, it follows that they may be important for maintaining normal β-cell function and insulin secretion. Indeed, it has recently been reported that infusing supraphysiological (80 mg/kg) doses of discoidal reconstituted HDLs (rHDL) containing Apo A-I complexed with phosphatidylcholine, (A-I)rHDL, into humans with T2DM increases plasma insulin levels, reduces plasma glucose levels, and increases myocyte glucose uptake (187). The ABCA1, which exports cholesterol from β-cell membranes to lipid-free/lipid-poor apo A-I, is also important for maintaining normal insulin secretion and glucose tolerance in mice (188). Moreover, HDLs have been reported to inhibit β-cell apoptosis and promote β-cell survival (189–191).

Recent study showed that apoA-I and apoA-II, in either the lipid-free form or as a constituent of discoidal rHDLs, as well as HDLs from human plasma, markedly increase insulin secretion from Min6 cells and primary islets under basal and high-glucose conditions without depleting the insulin or cholesterol content of the cells. These effects, which were calcium dependent and involved expression of ABCA1, ABCG1, and SR-B1, indicate that HDLs enhance β-cell insulin secretory function (192).

Thus, abnormal cellular cholesterol handling in islets may contribute to β-cell dysfunction in T2DM. β-cell deficiency for the ABCA1, which mediates the efflux of cellular cholesterol, leads to altered intracellular cholesterol homeostasis and impaired insulin secretion in mice (193).

In addition to its effects on β-cell function, HDL has been shown to directly and positively influence β-cell survival. HDL has been shown to decrease caspase-3 cleavage (189) and downregulate the death receptor TNF receptor superfamily member 6, also known as Fas (191). In addition, inducible nitric oxide synthase (iNOS), which is known to mediate the proapoptotic effects of glucose and cytokines is downregulated by HDL treatment (191).

In addition, the antioxidative activity of HDL is decreased in T2DM (181), which could limit its ability to prevent oxLDL-induced β-cell death in T2DM. These data suggest that diabetic dyslipidaemia could potentially contribute to the loss of β-cell mass in T2DM. The insulin resistance associated with metabolic syndrome and low HDL may progress to frank diabetes. Is it possible that apoprotein-stimulated insulin secretion is attenuated during this progression? First, the stimulation of insulin secretion is apoprotein concentration dependent over a physiologically relevant range. So with low HDL *in vivo*, especially if this HDL is dysfunctional, as is likely the case in diabetes and the metabolic syndrome, the optimal cofactors for insulin secretion and compensatory function of the β-cell may be limited. Furthermore, in the prediabetic and diabetic state, many proteins may become glycated or modified by carbonyls derived from carbohydrate oxidation, such as glycolaldehyde, glyoxal, and methylglyoxal. ApoA-I modified by these carbonyls is still able to mediate ABCA1-dependent cholesterol efflux (194). However, these carbohydrate-derived aldehydes have the capacity to influence ABCA1 function and particularly stabilization so that the functioning of this interacting couple of proteins may well be impaired in diabetes or prediabetes (195).

Therapeutic normalization of both the quantity and quality of HDL particles in patients with T2DM may provide a unique approach to promote β-cell survival and function and to decrease islet inflammation. HDL could thus represent a new therapeutic target in T2DM (196).

HDL and Aging Process

HDL plasma concentrations decline with age in prospective studies. Decline in HDL concentration and function may occur secondary because of hormonal changes, inflammatory processes, and diabetes mellitus. Beyond these effects specific aging processes may be involved. Replicative aging, the telomere-driven loss of divisional capacity, is a species-specific aging mechanism that may decrease HDL concentration and function. Vice versa, HDL may modulate the aging process, not only by its well-known antiatherogenic effects, eg, its ability to remove cellular lipids and by antiatherogenic pleiotropic effects on cell survival, but possibly also by direct interfering with aging signaling or survival factor KLOTHO.

Aging is a complex process that (on the cellular level) includes cell cycle arrest, morphology remodeling with functional decline, chromatin silencing with profound gene expression changes, and changes in metabolism. Different triggers may act together in a cooperative fashion and use overlapping signaling pathways to induce and propagate the aging process (198). Replicative aging results from the progressive shortening of telomeres (composed of conserved nucleotide sequences, TTAGGG in vertebrates) attributable to incomplete end replication during cell divisions. It may protect against increased cancer risk but may also contribute to atherosclerosis in later life, particularly at "atherosclerosis-prone areas" with high replication rates (199).

The KLOTHO gene, identified by insertional mutagenesis in mice, is a suppressor of the expression of multiple aging phenotypes, including atherosclerosis (200). Antiaging properties of the shed transmembrane form of KLOTHO protein are mostly explained by its hormonal effect on insulin signaling: KLOTHO moderately inhibits insulin/IGF1 signaling, which is the only proven evolutionary conserved mechanism for life span extension in men.

HDL cholesterol levels are inversely associated with the detrimental effect of a dysfunctional KLOTHO protein in humans. HDL cholesterol levels in the high normal range seem to be completely protective against KLOTHO dysfunction (201,202). The underlying mechanism of this relationship is unknown. It is obvious, however, that HDL and KLOTHO modulate similar signaling pathways. Both molecules induce NO synthesis, counteract apoptosis, induce angiogenesis, and counteract insulin signaling in cell culture models (200,203,204). Several mechanisms may be involved to explain a functional interrelationship between KLOTHO and HDL. HDL may compensate for KLOTHO dysfunction by its ability to remove cellular lipids. One recently proposed model is that the main antiaging effect of KLOTHO is its ability to prevent cellular lipid overload (and lipoapoptosis) by reducing insulin-stimulated availability of the lipogenic substrate glucose (205). Future studies are required to find strategies to mimic the positive (antiaging) effects of HDL and to prevent the negative effects of aging on HDL (197).

HDL and Hematopoietic Stem Cell Proliferation

Yvan-Charvet *et al*. present an entirely new role for HDL in regulating stem cell proliferation in the bone marrow (206). A relationship between cellular cholesterol content, HDL, and cells of the myelomonocytic lineage opens up the possibility that disorders characterized by the proliferation of immature white blood cells could be treated by targeting cholesterol transport in these cells. Many cell surface receptors assemble in membrane "rafts" that contain a high content of cholesterol and glycolipids. These rafts enhance receptor oligomerization and signaling in many cell types (207). In hematopoietic progenitor stem cells, growth factor receptors are organized in lipid rafts to promote receptor signaling and consequently, cell proliferation and migration (208). Because cholesterol overload can cause havoc in cells, its concentration is regulated by several mechanisms. Excess cholesterol is removed by ATP binding cassette (ABC) transporters in the plasma membrane, which move cholesterol to extracellular HDL particles at the cell surface (6).

As hematopoietic growth factor receptors in stem cells organize in membrane rafts, the availability of cholesterol for raft formation modulates receptor function. Membrane cholesterol can come either from lipoprotein uptake or local synthesis in the cell and is balanced by elimination of cholesterol from the cell. This balance depends on ABCA1 and ABCG1 transporters that mobilize cholesterol from the membrane, and on HDL particles that act as cholesterol acceptors. Lack of ABCA1 and ABCG1 results in increased raft formation, whereas addition of HDL reduces rafts. Increased raft formation helps to cluster receptors for IL-3 and GM-CSF, leading to increased responsiveness to these hematopoietic growth factors and to increased proliferation of myeloid precursor cells (209,210).

In atherosclerosis, leukocytosis is observed (211), but the reason has been unclear. The results of Yvan-Charvet *et al*. suggest that individuals with hypercholesterolemia may develop leukocytosis because of increased proliferation of myeloid progenitors in the bone marrow. Again, the cholesterol efflux pathway could be an interesting target for therapy (212).

HDL, Apo-A1 Mimetics and LXR Agonist

Emerging evidence suggests that HDL function is not always accurately predicted by HDL cholesterol levels. The functions of HDL include reverse cholesterol transport and modulation of inflammation. These functions appear to have evolved as part of the innate immune system. HDL may be thought of as a shuttle. The size of the shuttle can be estimated by HDL cholesterol levels. The shuttle's cargo can change dramatically from one that efficiently promotes reverse cholesterol transport and is anti-inflammatory to one that is less effective in promoting reverse cholesterol transport and is also proinflammatory without any change in the size of the shuttle (i.e., these changes in HDL cargo can occur without any change in HDL cholesterol levels). Understanding these issues may lead to improved use of HDL as a biomarker and may also lead to new therapeutic targets and therapies (213).

Measuring HDL cholesterol levels may not accurately predict the composition, functionality, and anti-inflammatory properties of HDL. HDL in diseases associated with a chronic acute-phase response has been found to be dysfunctional and proinflammatory. Currently, there are no tests widely available for measuring the composition, functionality, and inflammatory properties of HDL in clinical practice. However, it appears that the composition, functionality, and inflammatory properties of HDL are directly related to the presence or absence of conditions that are known to induce a chronic acute-phase response. These conditions are clinically recognizable (e.g., diabetes, visceral obesity, CHD, rheumatic diseases, chronic inflammatory gastrointestinal conditions, chronic renal disease, and chronic infections) (213).

Simply increasing the amount of circulating HDL-cholesterol does not reduce the risk of coronary heart disease (CHD) events, CHD deaths, or total deaths (214). Singh *et al*. (215) suggested that HDL could be a therapeutic target by modifying its lipid and protein cargo to improve its antiinflammatory properties. One method that has been reported to modify the lipid and protein cargo of HDL involves treatment with apolipoprotein mimetic peptides (216).

HDL mimetics have been constructed from a number of peptides and proteins with varying structures, all of which bind lipids found in HDL. HDL mimetics containing a peptide or protein have been constructed with as few as 4 and as many as 243 amino acid residues. Some HDL mimetics have been constructed with lipid but without a peptide or protein component. Some HDL mimetics promote cholesterol efflux, some have been shown to have a remarkable ability to bind oxidized lipids compared to human apoA-I. Many of these peptides have been shown to have antiinflammatory properties. Based on studies in a number of animal models and in early human clinical trials, HDL mimetics appear to have promise as diagnostic and therapeutic agents (217).

Use of apo A-I mimetic peptides could potentially overcome some of the limitations associated with use of the intact apo. Studies to establish the most efficacious peptides, optimal dosing regimens, and routes of administration are needed. Use of apo A-I mimetic peptides shows great promise as a therapeutic modality for HDL replacement and enhancing HDL function in treatment of patients with CHD (218).

ApoA-I mimetic peptides show remarkable capacity to support cholesterol efflux, share the antiinflammatory properties of HDL, and reduce development of athero-sclerosis in animal models (110,219-222). ApoA-I mimetic peptides cost a fraction of the cost of reconstituted HDL; they are safe and well tolerated (223), and approaches for oral delivery are being developed (220,224). Perhaps the best advantage of these peptides is the ability to modify their structure to better understand the mechanisms of atheroprotective action of HDL, with a view to further improving the atheroprotective capacity of the peptides (225).

Dysfunctional HDL has been identified in animal models and humans with chronic inflammatory diseases including atherosclerosis. The loss of antiinflammatory function correlated with a loss of function in reverse cholesterol transport. In animal models and perhaps in humans, dysfunctional HDL can be improved by apoA-I mimetic peptides that bind oxidized lipids with high affinity.

ApoA-I peptide mimetic removed seeding molecules from human LDL, rendering the LDL resistant to oxidation by human artery wall cells. The apoA-I-associated seeding molecules included hydroperoxyoctadecadienoic acid (HPODE) and hydroperoxyeicosatetraenoic acid (HPETE) (12).

It is now generally accepted that HDL and its apolipoproteins are the major initial acceptors of excess cellular cholesterol. Members of the ABC transporter family have been identified as key, and potentially complementary, cellular participants in export of cholesterol to these acceptors. ABCA1 mediates cholesterol efflux most efficiently to lipid-poor apoAI, whereas ABCG1 promotes cholesterol export to lipidated particles such as HDL. In addition, the SR-BI receptor pathway can also promote cholesterol export to HDL particles (226).

The liver X receptors, LXRs, are nuclear receptors that act as cholesterol sensors in controlling transcription of genes involved in cholesterol homeostasis and lipid metabolism. LXR agonists have been demonstrated to delay atherosclerosis in mice (227) by stimulating reverse cholesterol transport from macrophages. Additional studies have revealed that expression of LXR in macrophages is a prerequisite for the atheroprotective effect of LXR (228). Because ABCG1 and ABCA1 gene expression is stimulated by LXR agonists in human macrophages (229), upregulation of macrophage ABCG1 and ABCA1 by LXR agonists may constitute an effective pharmacological approach to attenuate foam cell formation, and thereby to prevent arterial lipid accumulation and lesion progression in dyslipidemic patients.

Coffee and green tea are a major source of polyphenols (231), a group of compounds that has received substantial interest in recent years. The term polyphenol represents a wide variety of compounds derived from plants, and polyphenols are present in many components of the human diet (232).

Polyphenol-rich foods have previously been associated with decreased risk of cardiovascular disease in multiple studies (233). Interestingly, certain polyphenols, such as resveratrol and anthocyanins (both found in red wine among other sources), have been shown to increase macrophage cholesterol efflux *ex vivo* (234-236).

Uto-Kondo *et al*., describe a careful series of experiments that tested the hypothesis that polyphenols found in coffee or green tea may promote macrophage cholesterol efflux (237). The authors focused their study on caffeic and ferulic acids, phenolic acids (a subclass of polyphenols) known to be present in coffee and green tea and increased in plasma by coffee consumption (238). Importantly, both compounds increased HDL-mediated cholesterol efflux *in vitro* in a dose-dependent fashion via enhanced expression of the ABCG1 (and SR-

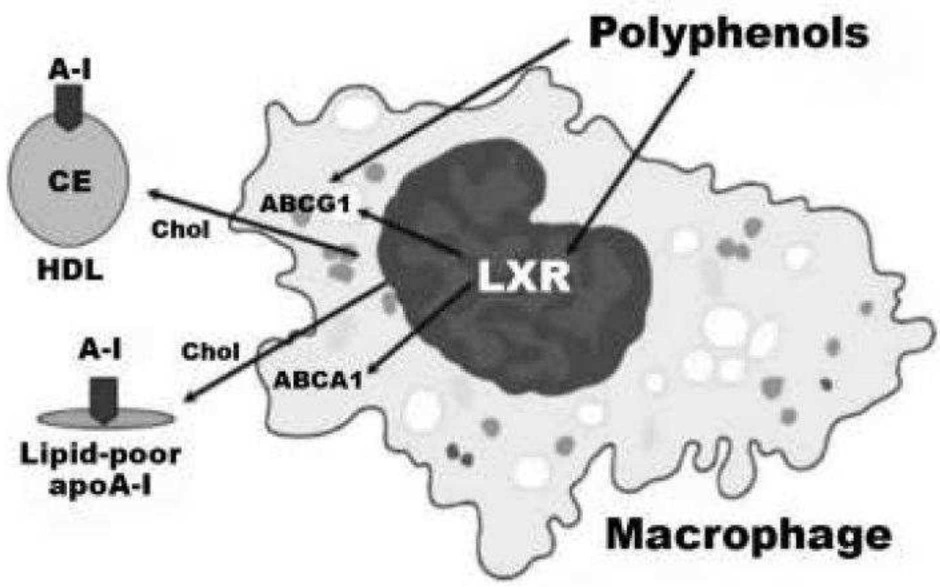

Figure 5. Postulated mechanisms for effects of polyphenols on macrophages cholesterol efflux (Reproduced with permission from Burke MF, Khera AV, Rader DJ. Polyphenols and cholesterol efflux. Circ Res 2010; 106: 627–9. © American Heart Association).

BI) transporters. Ferulic acid, but not coffee itself, was additionally shown to modestly enhance macrophage RCT *in vivo* in mice.

Uto-Kondo *et al*. provide a useful methodologic framework for studies that explore the association between various compounds and cholesterol efflux. Their work adds to a growing body of evidence that suggests a role for polyphenols in cellular cholesterol efflux. If confirmed, this conceptual approach to enhancement of macrophage RCT flux could prove valuable in the prevention and treatment of cardiovascular disease in humans (230).

Conclusion

HDL has many other activities aside from its role in RCT that can also contribute to its protective mechanism. These include the removal or detoxification of oxidized sterols and phospholipids, anti-inflammatory activity, antioxidant activity, antithrombotic activity, and its protective and healing activities on endothelial cells.

Proteomic analysis indicate that HDL carries a population of proteins that function in lipid metabolism, proteinase inhibition, complement activation, and the acute-phase response. Raising the possibility that these proteins contribute to the innate immune system and the cardioprotective properties of HDL.

Synthetic high-density lipoprotein (HDL) and apolipoprotein (apo) A-I mimetic peptides emulate many of the atheroprotective biological functions attributed to HDL and can modify atherosclerotic disease processes. Administration of these agents as HDL replacement or modifying therapy has tremendous potential of providing new treatments for cardiovascular disease.

We will ultimately determine how to take optimal advantage of the potent cardiovascular protective properties of HDL for prophylactic and therapeutic benefit.

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