Review

Understanding high-density lipoprotein function in disease: Recent advances in proteomics unravel the complexity of its composition and biology

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ABSTRACT

Although the epidemiology of high-density lipoprotein (HDL) cholesterol and cardiovascular risk has been consistent, pharmacologic interventions to increase HDL-cholesterol by delaying HDL catabolism did not translate into reduction in cardiovascular risk. HDL particles are small, protein-rich when compared to other plasma lipoprotein classes. Latest progresses in proteomics technology have dramatically increased our understanding of proteins carried by HDL. In addition to proteins with well-established functions in lipid transport, iron transport proteins, members of the complement pathway, and proteins involved in immune function and acute phase response were repeatedly identified on HDL particles. With the unraveling of the complexity of the HDL proteome, different laboratories have started to monitor its changes in various disease states. In addition, dynamic aspects of HDL subgroups are being discovered. These recent studies clearly illustrate the promise of HDL proteomics for deriving new biomarkers for disease diagnosis and to measure the effectiveness of current and future treatment regimens. This review summarizes recent advances in proteomics and lipidomics helping to understand HDL function in health and disease.

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1. Introduction

Lipids are distributed in the human body by a complex lipid transport system consisting of five main classes of lipoproteins which, in order of molecular size, largest to smallest, are chylomicrons, very low-density lipoproteins, intermediate-density lipoproteins, low-density lipoproteins (LDL), and high-density lipoproteins (HDL) [1]. Plasma levels of LDL-cholesterol are causally involved in the development of atherosclerosis and cardiovascular disease. LDL transfers cholesterol to peripheral tissue and lowering LDL-cholesterol by statins in people with pre-existing cardiovascular disease is effective in decreasing mortality [2,3].

HDL is the smallest of the lipoprotein particles containing the highest proportion of apolipoproteins to lipids. The major HDL associated apolipoproteins A-I (apoA-I) and apoA-II are secreted into plasma by the liver and the intestine, where they are lipidated to form lipid poor, discoidal, nascent HDL [4]. Nascent HDL takes up cholesterol from cell membranes and other lipoproteins. Lecithin-cholesterol acyltransferase (LCAT) converts free cholesterol into cholesteryl-ester thereby remodeling the particle to a spherical shape. Epidemiological studies have clearly shown that levels of HDL-cholesterol are inversely associated with the risk of coronary artery disease (CAD) and its thrombotic complications [5]. Consequently, it was assumed that HDL-cholesterol raising therapies could potentially further reduce cardiovascular mortality. Unexpectedly, pharmacologic interventions to increase HDL-cholesterol by delaying HDL catabolism did not translate into reduction in cardiovascular risk. The inability of HDL-cholesterol raising therapies and new insights into the complexity of HDL composition and function have prompted researchers to redefine the understanding of how HDL might exert its cardioprotective functions [6,7]. Recent studies clearly showed that an inflammation is a critical parameter in modifying the proteome and lipidome of HDL [8–10]. HDL is most widely recognized for its ability to transport cholesterol from the periphery to the liver for excretion during the process of reverse cholesterol transport (RCT). Numerous studies have shown that HDL and several of its apolipoproteins can promote lipid efflux from macrophages and other cells via several mechanisms [11–13] and deliver cholesteryl-esters to the liver in the process of selective uptake [13]. Studies in animals have consistently shown that HDL is protective on several processes involved in atherosclerosis, at least in part by mediating the removal of cholesterol from lipid-laden macrophages. In mice, genetic lowering of plasma HDL decreases the appearance of macrophage-derived cholesterol in the feces [14] and transgenic expression of apoA-I increases HDL and suppresses atherosclerosis in the apo deficient mouse [15–17]. In humans, regressive changes in human atherosclerotic plaques were reported in relatively small studies when reconstituted HDL or apoA-I was provided exogenously [18–21]. However, the mechanisms by which HDL may impact cardiovascular health and disease are complex and remain to be fully understood.

2. Novel insights into potential protective activities of HDL

2.1. HDL mediated cholesterol removal from the vessel wall

Removal of cholesterol from peripheral tissues to the bloodstream via RCT is a process of major biological importance and is believed to be a main reason how HDL might prevent cardiovascular disease. Active export of excess cholesterol to lipopoor apoA-I and lipid-enriched mature HDL is mediated by the ATP-binding cassette transporters ABCA1 and ABCG1/G4 [22] (Fig. 1). In situations of excess cellular cholesterol, the nuclear liver X receptors induce the transcription of ABCA1 and ABCG1 and thus cholesterol efflux. In addition, tethering of HDL to SR-BI and aqueous diffusion facilitates cholesterol efflux [23].

Of particular interest is the recent observation that the lymphatic vessel route is critical for efficient RCT from multiple tissues, including the aortic wall [24] (Fig. 1). This challenges the current view that lymphatic endothelium is a passive exchange barrier for cholesterol transport. Removal of cholesterol by lymphatic vessels is dependent on the uptake and transcytosis of HDL by SR-BI expressed on lymphatic endothelium [24,25]. These results suggest that supporting lymphatic transport function may facilitate cholesterol clearance in therapies aimed at reversing atherosclerosis.

The cholesterol taken up by HDL is esterified by LCAT and transported to the liver directly and indirectly via two major pathways (Fig. 1). In humans, CETP transfers HDL-cholesterol esters to apoB-containing lipoproteins, which then are removed by the LDL receptor pathway or the liver-independent transintestinal excretion of cholesterol [26]. RCT thus also involves both formation and catabolism of LDL. Significant proportions of HDL-cholesterol are removed by selective uptake through SR-BI into the liver and steroidogenic organs [27].

2.2. Recent evidence links cholesterol efflux capability with anti-inflammatory activities of HDL

Although it is clear that macrophage foam cell formation and macrophage inflammation are both central processes in atherosclerosis, the detailed mechanisms linking these processes remain incompletely understood. HDL has been shown to have anti-inflammatory and anti-oxidative properties itself [28]. For example, the major proteins of HDL, apoA-I and apoA-II, as well as other proteins such as LCAT, paraoxonase and lipoprotein associated phospholipase A2, that cotransport with HDL in plasma, are known to have antioxidant properties [29,30]. As a consequence, HDL has the capacity to inhibit the oxidative modification of LDL thereby reducing the atherogenic properties of these lipoproteins. HDL also possesses anti-inflammatory properties itself, by inhibiting adhesion molecules expression in endothelial cells and thereby reducing the recruitment of blood monocytes into the artery wall (Fig. 1) [28,31]. An interesting cellular mode of action
for the anti-inflammatory effects of HDL has been proposed to reside in the cholesterol-depleting effects of HDL. Cholesterol efflux via ABCA1 and ABCG1 was shown to regulate the proliferation of hematopoietic stem cells and to suppress the production of monocytes and neutrophils [32,33]. HDL-induced cholesterol efflux from macrophages is associated with a suppressed production of inflammatory cells, cytokines and chemokines in atherosclerotic lesions [33] (Fig. 1). In macrophages, HDL-induced cholesterol efflux decreases foam cell formation and macrophage inflammation, and consequently also the expression of cytokines that instruct the bone marrow to produce monocytes and neutrophils and stimulate monocyte infiltration into atherosclerotic plaques [34]. There is recent evidence that the cholesterol mobilizing activity of HDL acts to suppress inflammatory signaling via Toll-like receptors [35–37]. Since successful signal transduction relies on proper membrane microdomains containing high concentrations of cholesterol and sphingolipids, it is tempting to speculate that perturbation of these microstructures by HDL may explain the observed anti-inflammatory effects in innate immune cells. HDL-induced cholesterol removal disrupts the formation of cholesterol enriched ordered plasma membrane domains (lipid rafts) required for effective Toll-like receptor signaling, at least in part by reducing MyD88-dependent Toll-like receptor trafficking to lipid rafts [35–37]. A more recent study reported that HDL induces the transcriptional regulator ATF3 in macrophages, thereby effectively downregulating the expression of Toll-like receptor induced proinflammatory cytokines in vitro and in vivo [38].

2.3. Endothelial protective effects of HDL

Over the last decade, substantial progress has been made in understanding how HDL exerts direct protective effects on the vascular endothelium. It was initially recognized that HDL can vasodilate precontracted aortic segments [39,40], which was explained by the ability of HDL to activate endothelial nitric oxide synthase (eNOS) and thereby increasing endothelial nitric oxide production. Several studies have provided information about the molecular basis of this reaction involving SR-BI and the lysophospholipid receptor S1P3, leading to the parallel activation of phosphatidylinositol 3-kinase, protein kinase B (Akt) and mitogen-activated protein-kinase signaling [39–41], resulting in phosphorylation of eNOS and increased nitric oxide production (Fig. 1). There is also evidence that the cholesterol mobilizing activity of HDL maintains endothelial function in an ABCG1-dependent manner, preventing active eNOS dimer disruption and cell adhesion molecule expression [37,42]. HDL can inhibit cytokine-induced expression of vascular cell adhesion molecule (VCAM-1), intercellular cell adhesion molecule (ICAM-1), and E-selectin on human endothelial cells [31,43]. These proteins mediate the adhesion of lymphocytes, monocytes, and basophils to the vascular endothelium and promote leukocyte-endothelial cell signal transduction. They are therefore central in the development of atherosclerosis and other inflammatory diseases [44]. Consistently, administration of apoA-I or HDL reduces adhesion molecule expression and monocyte infiltration in small animal models [45,46] and attenuates the secretion of monocyte chemotactic protein 1 from endothelial cells [47].
cells, a chemokine promoting infiltration of blood monocytes into inflammatory sites [47]. Importantly, two recent studies have demonstrated that HDL-mediated eNOS activation is impaired in patients with CAD and chronic heart failure [48,49]. It was observed that post-translational modifications of HDL were associated with a reduced ability to induce eNOS phosphorylation and activation. Post-translational modifications of HDL, such as oxidation by myeloperoxidase, carbamylation, malondialdehyde formation and advanced glycation end products have been shown to decrease antiatherogenic activities of HDL during inflammation [10,48,50,51]. Myeloperoxidase derived reactive chlorinating species target HDL associated plasmalogens generating HDL associated 2-chlorohexadecanal [52]. 2-Chlorohexadecanal was found to directly impair eNOS activity by promoting an intracellular dislocation of eNOS [52]. However, it remains difficult to ascertain a specific post-translational protein or lipid modification as causal, since they occur simultaneously and may have accumulative effects.

In addition, HDL has been shown to inhibit endothelial cell apoptosis induced by modified lipoproteins, tumor necrosis factor alpha, angiotensin II and oxidative stress, [53,54]. Several HDL-associated mediators with anti-apoptotic activity have been identified. Purified apoA-I, the major protein constituent of HDL, or HDL associated clusterin are suggested to attenuate apoptotic intracellular signaling [55,56]. The impact of the lipid moiety of HDL on its anti-apoptotic potential is less studied. Lysosphingolipids, in particular sphingosine-1-phosphate, have been shown to protect endothelial cells from apoptosis [57].

2.4. The failure of recent HDL-cholesterol raising drugs

Given that several prospective observational studies have shown a strong inverse relationship between HDL-cholesterol and cardiovascular risk, HDL-cholesterol raising therapies were expected to reduce cardiovascular mortality. This assumption was supported by evidence from preclinical and (limited) clinical studies showing that HDL is able to promote the regression of atherosclerosis when the levels of functional particles are increased from endogenous or exogenous sources [58]. Unexpectedly, drugs increasing HDL-cholesterol by delaying HDL catabolism, such as inhibitors of cholesteryl-ester transfer protein (CETP) or niacin have failed to significantly reduce the risk of major cardiovascular events, especially when combined with statins [59]. Moreover, genetic analysis did not show a causal association between genetically raised plasma HDL-cholesterol levels and risk of myocardial infarction [60]. Those findings prompt the suggestion that the composition and function of HDL may be more important to disease outcome than the quantity of HDL-cholesterol itself. Direct measures of HDL composition and function are needed rather than relying on surrogate markers such as the concentration of HDL-cholesterol for assessing cardiovascular disease risk [6]. In line with that assumption are the recent observations that the inverse relationship of HDL-cholesterol with cardiovascular mortality is markedly weakened in patients with CAD and that HDL-cholesterol concentration is not an appropriate biomarker in the secondary prevention setting [61]. Therefore, the usefulness of considering HDL-cholesterol for cardiovascular risk stratification seems limited in such patients and more informative markers of HDL-functionality are needed.

Because of these controversial data, the suitability of HDL-cholesterol as a therapeutic target has been questioned [6]. Risk stratified atherogenic lipoprotein burden (low-density lipoprotein cholesterol and non-HDL-cholesterol) should remain the primary and secondary targets of therapy in patients at cardiovascular risk [6], as described by established guidelines. Plausible reasons for the disappointing results of recent HDL-cholesterol raising therapies are:

(i) Steady-state levels of HDL-cholesterol provide no information on the rate of cholesterol-flux from vascular macrophages to the liver, which is influenced by many factors beyond the mass of HDL-cholesterol [10,62–64]. Moreover, steady-state levels of HDL-cholesterol provide no information on metrics of anti-inflammatory, anti-oxidant, anti-thrombotic and endothelial function promoting activities of HDL despite increasing evidence supporting the clinical significance of these pleiotropic functions [10,62–64].

(ii) Increasing the catabolism of HDL-cholesterol (e.g. by augmenting hepatic expression of SR-BI) has been shown to be a positive regulator of cholesterol efflux from macrophages, despite dramatically reduced HDL-cholesterol levels [65]. CETP inhibitors or niacin slow down HDL catabolism, therefore cholesterol efflux from macrophages might be unaltered or even decreased. Moreover, recent studies reported that the addition of niacin to statin therapy did not improve metrics of HDL functionality despite significant increases in HDL-cholesterol [66]. This finding is in line with the observation that niacin and CETP inhibitors raise HDL-cholesterol with little effect on HDL particle number [67]. In addition, a recent multiethnic study suggested that HDL particle number correlated better with HDL function than HDL-cholesterol [68].

(iii) Levels of HDL-cholesterol in the circulation do not reflect the pathobiology of a diseased artery wall. There is mounting evidence that HDL isolated from human aortic tissues is markedly distinct from those of circulating HDL. Specifically, HDL in human aorta was found to be extensively crosslinked [69] and rendered dysfunctional by myeloperoxidase-mediated carboxylation of lysine residues and oxidation of methionine and tryptophan residues [50,70,71].

(iv) There is strong evidence that inflammation profoundly changes HDL composition, structure and function in the bloodstream [8,10,63]. The functional heterogeneity inherent to plasma HDL is driven in large part by its compositional diversity [8–10]. Phospholipid depletion and enrichment with pro-inflammatory proteins like serum amyloid A proteins (SAA) or apoC-III generate dysfunctional or even pro-atherogenic forms of HDL [56,72–76].

Taken together, it is becoming increasingly apparent that direct measures of HDL composition and metrics of functionality are needed.

2.5. HDL-mimetic infusion therapies

The ideal HDL-directed therapy would increase the amount of functional HDL available to antagonize lipid deposition, inflammation, oxidation, and potential for thrombosis in the arterial wall. Some clinical studies have suggested protective effects of HDL infusions on coronary plaque burden when compared with baseline [18–21]. However, interpretation is limited by the small sample sizes of those studies [18–21]. More recently, a well powered prospective, randomized trial on infusion of the HDL-mimetic agent CER-001 in coronary atherosclerosis patients was conducted at centres in the USA, the Netherlands, Canada, and France [77]. Intravascular ultrasonography and quantitative coronary angiography were performed at baseline and two to five weeks after the last study infusion of the HDL-mimetic agent CER-001. Disappointingly, when compared with placebo, infusions of CER-001 did not result in a significant reduction in coronary atherosclerosis. The failure of the study to achieve its primary efficacy parameter is
likely to be multifactorial. Some possible reasons for not achieving primary efficacy parameters are: (i) the duration of the study could have been too short and potentially favorable effects of infusion of the HDL-mimetic agent CER-001 in the long term is observed. (ii) The dosage could have been too low and/or not frequent enough. (iii) As described above, the infused apoA-I might have been rendered dysfunctional by myeloperoxidase-mediated carboxylation of lysine residues and oxidation of methionine and tryptophan residues [50,70,71]. (iv) Moreover, a large fraction of the phosphatidylcholine moiety of administered reconstituted HDL is rapidly hydrolyzed by secretory phospholipases and LCAT in the blood stream [78,79]. As a consequence, lysophosphatidylcholine levels dramatically increase [78,79]. This raises the possibility that the potent anti-inflammatory activity of administered reconstituted HDL may be counteracted by formed lysosphospholipids. Despite these negative results, the development of novel drugs designed to modulate the serum levels and functionality of HDL particles should continue, assuming the considerable amount of basic scientific and clinical investigation demonstrating protective activities of HDL in several animal models [6].

3. Recent advances in linking HDL composition to function

3.1. The toolbox of HDL-proteomics

Recent advances of mass spectrometry technology have dramatically increased our understanding of the proteomic diversity of HDL. The Davidson Lab maintains an up to date list of all published HDL proteins which even includes a ranking system based on frequency observed (http://homepages.uc.edu/~davidswm/HDLproteome.html). Depending on the method of HDL isolation, upwards of 89 proteins have been identified repeatedly in independent studies and the list continues to grow [8]. Among those proteins are members of the complement pathway, immune function, protease inhibitors involved in hemostasis, acute phase response proteins, and even metal-binding proteins in addition to proteins consistent with traditionally accepted roles in lipid transport [8,80]. This compositional diversity fits well with studies demonstrating a wide functional pleiotropy, including roles in lipid transport, oxidation, inflammation, hemostasis and immunity.

Identification of the HDL-proteome typically involves tryptic digest of isolated particles, liquid chromatography coupled to tandem mass spectrometry (LC–MS²) analysis of the resulting peptides and public protein database search for matching peptides and their fragment masses to proteins. While the number of proteins identified on HDL particles is astonishing from a biology point of view, state of the art proteomic methods are capable of identifying and quantifying thousands of proteins.

Label free quantification is often the method of choice to determine changes in protein abundance between different groups of samples in the discovery phase [74–76,81–84]. The label free quantification technique applied in all these studies is called spectral counting. It relies on the fact that mass spectrometers have been rendered dysfunctional by myeloperoxidase-mediated carbamoylation of lysine residues and oxidation of methionine and tryptophan residues [50,70,71]. Moreover, a large fraction of the phosphatidylcholine moiety of administered reconstituted HDL is rapidly hydrolyzed by secretory phospholipases and LCAT in the blood stream [78,79]. As a consequence, lysophosphatidylcholine levels dramatically increase [78,79]. This raises the possibility that the potent anti-inflammatory activity of administered reconstituted HDL may be counteracted by formed lysosphospholipids. Despite these negative results, the development of novel drugs designed to modulate the serum levels and functionality of HDL particles should continue, assuming the considerable amount of basic scientific and clinical investigation demonstrating protective activities of HDL in several animal models [6].

First attempts to span the gap to a more routine clinical setting by multiplexed quantification of a subset of HDL proteins by multiple reaction monitoring mass spectrometry (MRM–MS) have been made [87,88]. In MRM, the abundance of one (or more) fragment(s) of each precursor of interest is measured under optimized conditions. This allows quantification of the precursors with high sensitivity, high selectivity and a very high dynamic range. However, it is a strictly targeted approach and any changes not covered by the precursor list would remain unnoticed. Currently, MRM transitions for 32 HDL proteins have been described [88].

More recently, several research groups have started to assess whether HDL proteomic composition is altered in CAD [56,80,84]

(i.e. isobaric Tags for Relative and Absolute Quantitation) become attractive options [85,86]. iTRAQ uses isobaric (i.e. identical mass/charge ratio) tags, that can only be distinguished on MS² level. Individual samples are labeled with individual iTRAQ tags (up to 8-plex), pooled and analyzed by LC–MS². Since the tags are isobaric, the same precursor ions of all mixed samples appear as one signal in the full MS scan. However, as soon as the precursor ions get fragmented, each of the iTRAQ tags shows a distinct fragment, termed the reporter ion. It is therefore possible to calculate the relative abundance of the precursors in the individual samples by comparing the intensities of the individual reporter ions. While the preparation of iTRAQ samples is more time consuming, there are several advantages of the iTRAQ approach. First, the dynamic range is improved compared to the spectral counting approach. Also, the significance threshold is independent of the analyte’s abundance. Finally, by measuring several samples as one pool, the instrument time is reduced. In the two published iTRAQ studies 44 out of the 89 HDL proteins listed by the Davidson Lab were found to be dysregulated between experimental and control groups. [85,86].

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3.2. HDL-proteome remodeling in disease

Fig. 2. HDL protein hotspots changed in disease. The three diseases with most reported changes are shown. All abbreviations areUniProt gene names. Proteins which were reported to be significantly changed (p < 0.05) in at least three studies are listed. CAD, changes in coronary artery disease vs healthy subject HDL [80,84]; ESRD, changes in end stage renal disease vs healthy subject HDL [74,76,85]; PS/RA, changes in psoriasis/rheumatoid arthritis vs healthy subject HDL [75,86]; all shown in red; total, occurrence in all reported diseases shown in blue. Color intensity and numbers refer to number of studies reporting significant changes in protein abundance.
or chronic inflammatory diseases like chronic kidney disease [74,76], rheumatoid arthritis [86], type 2 diabetes [89], psoriasis [75,90] and aging [83]. All studies reported multiple alterations in the protein composition of HDL. Fig. 2 shows a heat map of HDL proteins found to be changed in disease vs. control settings by either label free or stable isotope labeling techniques visualizing hot spots for HDL proteome changes in disease. Proteins involved in lipid metabolism, transport and inflammation were mainly affected. Especially proteins of the acute phase response and the complement system were enriched on diseased HDL particles. In affected. Especially proteins of the acute phase response and the complement system were enriched on diseased HDL particles. In contrast, some apolipoproteins were found to be enriched while others were depleted (Table 1).

3.2.1. ApoA-I and apoA-II

Recent proteomic studies have shown a decrease in HDL associated apoA-I in chronic kidney disease, psoriasis and rheumatoid arthritis [74–76,86]. Traditionally, the loss of HDL function has been attributed to replacement of apoA-I by other proteins, notably acute-phase proteins such as SAA, which in severe inflammation can represent more than 50% of HDL proteins [91]. ApoA-I, the major protein of HDL, is probably among the most intensively studied of all proteins. It functions in HDL assembly, in the removal of excess cholesterol from cells, and in the RCT. It is a cofactor for LCAT [92], the enzyme responsible for cholesterol esterification in HDL, and it stabilizes certain antioxidant enzymes, such as serum paraoxonase (PON1), that are carried on HDL [93]. Impaired LCAT activity might be responsible for altered cholesterol hemostasis of acute-phase HDL compared with native HDL. In contrast, apoA-II, the second most abundant protein of HDL, has no known function, and its presence on HDL has been reported to have no influence on cholesterol efflux from cells, nor is it a cofactor for any known enzyme [94]. ApoA-II enriched HDL particles are more triglyceride enriched and resistant to reductions in size and apoA-I content, suggesting inhibition of hepatic lipase by apoA-II [95]. A loss of apoA-II was observed in chronic kidney disease and patients suffering from rheumatoid arthritis, whereas increased apoA-II levels were observed in HDL from psoriatic subjects [74–76,86,96].

3.2.2. SAA proteins

SAA proteins represent the hottest spot for HDL proteome changes in diseases (Fig. 2). HDL derived from patients suffering from chronic kidney disease, psoriasis, rheumatoid arthritis and acute coronary syndrome was shown to be enriched in SAA [74–76,80,83–86]. Enrichment of HDL with SAA is linked to decreased apoA-I contents in line with the observation that SAA replaces apoA-I on HDL under inflammatory conditions [97,98]. Triggered by inflammation the concentrations of SAA may increase during the acute-phase reaction to levels 1000-fold [99]. The SAA family of proteins is encoded by multiple genes that display allelic variation and a high degree of homology in mammals. It has to be noted, that the UniProt entry of SAA was only recently divided into the 2 individual entries for SAA1 and SAA2. Moreover, due to their high degree of identity (93%), they are usually not distinguished in proteomic studies in contrast to SAA4. SAA1/SAA2 increases the

| Table 1 Common proteomic features of dysfunctional HDL |
|-------------------------------------------------------|
| **Proteins** | **Known functions** | **Alteration** | **Reference** |
| ApoA-I | Major protein of HDL | ESRD, RA | [74–76,86] |
| | Lipid binding and transport | | |
| | Cofactor of LCAT | | |
| | Anti-oxidative properties | | |
| ApoA-II | 2nd major protein of HDL | ESRD, RA | [74–76,86,89] |
| | Inhibitor of hepatic lipase | | |
| SAA1/SAA2 | Major acute phase protein | ESRD, RA | [74–76,80,83–86] |
| | Pro-inflammatory properties | | |
| Complement C3 | Cleaved into C3a & C3b | CAD, RA, ACS | [75,80,83,84,86] |
| | C3a: mediator of local inflammation | | |
| | C3b: opsonizing factor | | |
| Hemoglobin, haptoglobin | Oxygen transport | ESRD, RA | [75,80,83,84,86] |
| | Hemoglobin binding | | |
| ApoA-IV | Modulates LPL | ESRD, RA | [76,80,85] |
| | Activator of LCAT | | |
| ApoC-II | Activates LPL | | |
| | Inhibitor of LCAT | ESRD, RA | [74,85] |
| ApoC-III | Inhibits LPL and HL | ESRD, RA | [56,76,85] |
| | Delays TGRL catabolism | | |
| ApoM | Lipid transport | ESRD, RA | [75,76,89] |
| | Binds S1P and fatty acids | | |
| Clusterin | ATP-independent chaperone | CAD, RA | [56,80,121] |
| | Inhibits apoptosis | | |
| PON1 activity | Hydrolyzes lactones, aromatic carboxylic acid esters and organophosphates | ESRD, RA | [48,49,83,86,89,96] |
| | β2-glycoprotein 1 | | |
| | Lp-PLA2 activity | | |
| | Hydrolyzes PAF | ESRD, RA | [75,76,96] |
| | Ca2+-independent PLA2 | | |

The table summarizes identified alterations of the protein composition/activity of HDL in inflammatory diseases identified in at least 3 independent studies. ACS, acute coronary syndrome; apo, apolipoprotein; CAD, coronary artery disease; ESRD, end-stage renal disease; HD, hemodialysis; HL, hepatic lipase; LCAT, lecithin-cholesterol acyltransferase; LPL, lipoprotein lipase; Lp-PLA2, lipoprotein associated phospholipase A2; PAF, platelet activating factor; PON1, paraoxonase 1; RA, rheumatoid arthritis; SAA, serum amyloid A, S1P, sphingoosine-1-phosphate; TGRL, triglyceride rich lipoprotein; TR2, Toll-like receptor 2.
binding of HDL to vascular proteoglycans and the retention in the arterial intima thereby boosting the exposition to oxidative modifications [100]. SAA1/SAA2 was shown to have chemotactic properties promoting migration, adhesion, and tissue infiltration of monocytes and polymorphonuclear leukocytes [101]. The substitution of apoA-I and apoA-II by SAA1/SAA2 in uremic HDL negatively correlated with the phospholipid content of HDL and resulted in an impaired cholesterol acceptor capability of HDL [76]. Of particular interest, SAA1/SAA2 was identified as being responsible for the pro-inflammatory effects of HDL isolated from chronic kidney disease patients, affecting cytokine production and adhesion molecule expression on monocytes and myeloid dendritic cells [74]. In contrast to SAA1 and SAA2, SAA4 is reported to be expressed constitutively. Under non-inflammatory conditions it accounts for more than 90% of the total SAA content of HDL [102]. However its function remains unknown. Despite the proposed constitutive expression, it was reported to be increased in chronic kidney disease and rheumatoid arthritis [76,85,86] and decreased in psoriatic subjects in one study [75].

3.2.3. Complement component 3, hemoglobin and haptoglobin
Increased levels of HDL associated complement C3 and HDL associated hemoglobin and haptoglobin were identified in patients with CAD, psoriasis and rheumatoid arthritis [75,80,83,84,86]. Complement C3 is produced by human monocyte-derived macrophages and contributes to innate immunity [109] and has been suggested to contribute to vascular disease [104]. Concordantly, the complement inhibitor clusterin is downregulated in disease (see below). It is thought that haptoglobin is an acute-phase protein because haptoglobin sequesters the iron within hemoglobin, preventing iron-utilizing bacteria from benefiting from hemolysis. Both, hemoglobin and haptoglobin were shown to alter HDL functionality rendering HDL pro-inflammatory [105].

3.2.4. ApoA-IV
ApoA-IV was reported to be up regulated in HDL from hemodialysis and stable CAD patients [76,80,85]. In humans and the majority of animal species, apoA-IV is synthesized primarily by the intestine and to a lesser extent by the liver. It is present in plasma, the lymph chylomicrons, and the cerebrospinal fluid [106]. Hydrolysis of the triglycerides of chylomicrons by lipoprotein lipase in plasma causes dissociation of apoA-IV and its redistribution in HDL [106]. Although its primary function in human lipid metabolism has not been established, ApoA-IV has been found to modulate apoC-II [107] and to activate LCAT [108]. LCAT esterifies free cholesterol with phosphatidylcholine as an acyl-donor and is therefore required for efficient uptake of free cholesterol by HDL.

3.2.5. ApoC-II
ApoC-II was reported as up-regulated exclusively but consistently in HDL of hemodialysis patients [74,76,85]. It is a co-factor of lipoprotein lipase, thereby regulating triacylglycerol content of HDL particles and missense mutations of apoC-II lead to hyperlipoproteinemia 1B [109]. The phenotype is indistinguishable from lipoprotein lipase deficiency. Furthermore, apoC-II was reported to inhibit LCAT activity [110].

3.2.6. ApoC-III
Enrichment with apoC-III in HDL of patients with chronic kidney disease and CAD is another very robust observation in proteomic studies from different laboratories [56,76,85]. ApoC-III acts as a pro-inflammatory mediator and an endogenous ligand of Toll-like receptor 2, which has been implicated in the aggravation of atherosclerosis [111]. Previous experimental studies have shown that apoC-III, an inhibitor of lipoprotein lipase and hepatic lipase, may be a crucial link between renal dyslipidemia and increased atherosclerosis [112]. Strikingly, separating HDL according to apoC-III identified two types of HDL with opposing associations with risk of CAD. It was found that apoC-III-containing HDL was associated with increased cardiovascular risk, whereas the apoC-III-lacking fraction was associated with cardioprotection [113]. The proatherogenic effects of apoC-III, as a component of VLDL and LDL, may therefore extend to HDL. Interestingly, subjects with chronic kidney disease exhibit catabolic defects in apoC-III resulting in increased apoC-III plasma levels [114] resulting in high HDL associated apoC-III levels observed in chronic kidney disease patients. A recent study showed that HDL from CAD patients is enriched in apoC-III thereby stimulating endothelial apoptotic pathways [56]. The apoC-III levels of uremic HDL positively correlated with HDL-associated triglycerides and negatively with the phospholipid content of HDL. In addition, these compositional alterations were associated with an impaired cholesterol acceptor capability of HDL [76].

3.2.7. ApoM
A loss of apoM was shown by different research groups in type 2 diabetes mellitus [89], chronic kidney disease [76] and psoriasis [75]. Being a member of the lipocalin protein family, apoM is able to sequester and transport low molecular weight compounds. Myristic acid, palmitic acid and stearic acid, retinol, all-trans-retinoic acid and 9-cis-retinoic acid have been reported to bind to apoM [115]. Moreover, it was recently shown that apoM mediates the enrichment of sphingosine-1-phosphate content in HDL [116], which promotes arterial vasodilation by stimulating endothelial nitric oxide production and inhibiting monocyte recruitment into the intima [39,117]. ApoM also plays a key role in cholesterol efflux to HDL, at least in animal models [118]. Interestingly, transgenic overexpression of apoM in mice leads to reduced atherosclerotic lesions [119].

3.2.8. Clusterin
Two research groups observed a loss off clusterin in CAD patients [56,80]. Clusterin, also known as apol, has been implicated in a variety of activities including programmed cell death, regulation of complement mediated cell lysis and acts as a complement inhibitor [120]. Clusterin concentrations in HDL negatively associated with insulin resistance and anti-apoptotic activity of HDL, implicating a protective role of HDL associated clusterin [56,121].

3.2.9. Paraoxonase 1
The activity of PON1, an important HDL associated enzyme, was found to be decreased in HDL of patients with CAD [49], type 2 diabetes mellitus [89], chronic kidney disease [122–124], rheumatoid arthritis [86] and psoriasis [96]. PON1 has been implicated in antioxidant and anti-inflammatory functions [125,126], and its activity has been related to cardiovascular risk [127]. A recent study demonstrates that PON1 may interact with apoA-I and LCAT to inhibit LDL oxidation, with the combination preventing LCAT inactivation [29]. The decreased PON1 activity showed associations with reduced anti-oxidative activities of HDL in various disease conditions. One study suggested that low HDL associated PON1 activity is involved in the inhibition of nitric oxide production in endothelial cells [49], however this was not observed in a subsequent study [48].

3.2.10. Lipoprotein associated phospholipase A2
The HDL-associated enzyme lipoprotein associated phospholipase A2 (Lp-PLA2) is significantly increased in psoriasis patients and patients suffering from end-stage renal disease [75,76,96]. Lp-PLA2 has recently been associated with an enhanced risk of CAD [128,129]. These findings led to the hypothesis that Lp-PLA2...
activity and/or mass levels could be used as biomarkers of cardiovascular disease. In circulation, Lp-PLA2 normally travels mainly with LDL, but about 20% of Lp-PLA2 is associated with HDL. Interestingly, Lp-PLA2 content of HDL negatively correlated with phospholipid levels of HDL, suggesting that Lp-PLA2 hydrolyzes short-chain phospholipids or oxidized-phospholipids in HDL. In line with this observation, it was found that HDL associated Lp-PLA2 activity, and lyso-phosphatidylcholine content significantly correlated with HDL-associated Lp-PLA2 mass in end-stage renal disease patients [76].

3.3. Proteomics of HDL subclasses

The majority of the HDL proteomics studies so far have been carried out assuming a homogenous HDL population. However, it has long been known that some HDL particles contain apoA-II while others do not [130]. Moreover, even within the HDL population, size differences do exist. Therefore, numerous attempts have been made to define subclasses of spherical HDL. The probably most common definition uses five subclasses of HDL. These are HDL2b, HDL2a, HDL3a, HDL3b, and HDL3c, in order of decreasing diameter and increasing density [4]. More recently a novel nomenclature based on the size of the particles was suggested: very large HDL particles (VL-HDL), large HDL particles (L-HDL), medium HDL particles (M-HDL), small HDL particles (S-HDL), and very small HDL particles (VS-HDL) [131]. To our knowledge, only a single proteomics publication so far compares healthy to diseased state on an HDL subclass level. Gordon et al. investigated 12 size exclusion fractions from lean, obese and type 2 diabetic subjects [89]. They found that apoE was reduced almost fivefold in type 2 diabetes compared to lean, healthy subjects. Moreover, the distribution maximum of apoE was shifted towards smaller particles in the type 2 diabetes group. Also, less apoA-II and PON1 were found in the type 2 diabetes group, with a more pronounced effect in larger HDL subclasses.

3.4. The HDL lipidome

HDL particles are the smallest and densest of all lipoproteins. The lipid fraction of HDL amounts for roughly 50% of the total weight [9]. The amphipathic shell is formed by a single layer consisting mainly of phospholipids and free cholesterol, whereas the core consists of neutral lipids such as cholesteryl-esters and triacylglycerols. Phospholipidome predominates, accounting for 40–60% of total lipids, followed by cholesteryl-esters (30–40%), triacylglycerols (5–12%) and free cholesterol (5–10%) [9]. Lipidomic approaches have provided novel insights into the HDL lipidome and over 160 individual molecular lipids species were identified in HDL isolated from healthy subjects [9].

HDL subclasses differ in the ratio of shell to core lipids, as well as in the composition within the amphipathic and neutral lipids. While the shells of large HDL particles tend to be enriched with sphingomyelins and ceramides, small particles have more phosphatidylserine, phosphatidic acid and phosphatidylcholine present on their surface. Moreover, the very small particles are enriched in lyso-phosphatidylcholine and depleted of free cholesterol [132]. Also the side chain distribution is shifted towards more unsaturated moieties in smaller particles [9]. These changes do not only influence the physical properties such as surface charge and membrane fluidity of the particles, but also their biological functions. The smaller HDL particles have a higher capability to stimulate cholesterol efflux from macrophages and their anti-oxidative potential to protect LDL and pro-thrombotic compounds such as thromboxane B2 and p38-MAPK from oxidation is higher. Also, anti-inflammatory and anti-apoptotic protective functions are more pronounced in the small and very small subpopulations [132]. On the other hand, a recent study observed that decreased large HDL particles isolated from type 2 diabetes inversely correlated with vascular stiffness assessed by pulse wave velocity [89]. Yet another study found a significant shift in average HDL particle size only for type 2 diabetes combined with dyslipidemia, while the normolipidemic type 2 diabetes group showed the same average HDL particle size as the healthy control group [133].

It is therefore becoming increasingly apparent that HDL-cholesterol alone is not a suitable marker for CAD risk assessment, while HDL particle number and especially subpopulation distribution might be better suited to fulfill this task [7,63].

3.5. HDL composition and function: novel biomarker with implications for vascular wall biology

HDL in inflammatory disease states is enriched in proteins that play critical roles in macrophage biology, lipid metabolism, and the inflammatory response (Table 1). Several of these proteins are produced by cholesterol-loaded macrophages, suggesting that a fraction of the proteins that are overproduced under inflammatory conditions might be derived from macrophages in atherosclerotic lesions. Alternatively, inflammation may enhance their production by the liver and promote their association with circulating HDL. Significant increases in SAA, apoA-IV, apoC-III and complement C3 levels and loss of atheroprotective apoA-I, apoM and clusterin indicate a significant shift to an inflammatory profile and may provide clues to the overall metabolic processes that underlie these diseases. HDL associated enzymatic activities in disease states and antioxidative activities of HDL are altered in disease states. Inflammation is associated with low HDL associated paraoxonase activity whereas Lp-PLA2 activity is increased in chronic kidney disease and psoriasis (Table 1). Notably, proteomic analysis did not consistently provide evidence for a reduction in PON1 mass, suggesting that inflammation alters enzymatic activity. Previous studies demonstrated that HDL associated PON1 is rapidly inactivated by neutrophil derived reactive species [50,134]. In particular, leukocyte released myeloperoxidase has emerged as an enzymatic catalyst that promotes HDL oxidation and carbamylation in vivo [50,71,135–137]. In addition to HDL proteomics, HDL lipidomics is expected to significantly add to the identification of mediators and biomarkers of normal and dysfunctional HDL. The HDL phospholipidome exhibits significant correlations with multiple metrics of HDL function [132]. HDL isolated from subjects with chronic kidney disease and psoriasis showed decreased levels of phospholipids that strongly associated with a reduced ability to promote RCT [75,76,96]. HDL-triaclyglycerol content negatively correlated with cholesterol efflux potential of uremic HDL [76]. However, more detailed and quantitative analyses of alterations in the lipid moiety of HDL are warranted and will contribute to an improved understanding of impaired HDL particle formation, maturation and function.

4. Conclusion

Several studies have clearly shown that the composition of HDL can change in a variety of disease states and these alterations affect metrics of HDL functionality. These recent findings suggest that distinct disease states harbor a characteristic disease-specific HDL proteome. It remains to be seen whether these changes in HDL composition contribute to the disease etiology or if these changes are secondary to other processes occurring during disease progression. However, there is still a substantial need for validation and extension of these recent observations. HDL proteomic analysis is yet in its early years, and general conclusions about the HDL proteome as a biomarker for disease are limited by the small number of published studies. Moreover, differences in
sampling and isolation procedures may result in some variation in HDL composition and function. Future attempts will be targeted towards improving isolation procedures, differentiating between HDL subclasses as well as protein isoforms. These recent studies clearly illustrate the promise of HDL proteomics for deriving new biomarkers for disease diagnosis and to measure the effectiveness of current and future treatment regimens.

Conflict of interest
The authors declare that there are no conflicts of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.

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