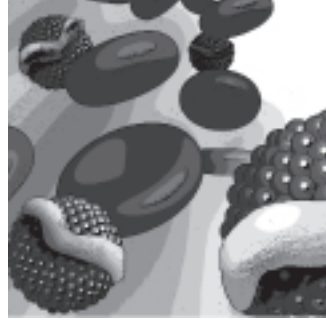

Dysfunctional HDL: the journey from savior to slayer

HDL has many functions such as reverse cholesterol transport, anti-inflammation and antioxidation. Different clinical conditions associated with inflammation, oxidation, advanced glycation and protein carbamylation can alter the functionality of HDL, converting normal HDL into dysfunctional HDL, which is no longer cardioprotective. Dysfunctional HDL leads to the impairment of reverse cholesterol transport and might exhibit proinflammatory, pro-oxidant, prothrombotic and proapoptotic properties, all responsible for the subsequent endothelial dysfunction. This review addresses the current knowledge regarding the loss-of-function and gain-of-dysfunction responsible for the proatherosclerotic properties of HDL in several pathological conditions associated with systemic inflammation.

KEYWORDS: atherosclerosis ■ biomarker ■ dysfunctional HDL ■ HDL ■ lipid disorder

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HDLs, the classic antiatherosclerotic lipoproteins responsible for reverse cholesterol transport (RCT), are spherical particles, with a hydrophobic core of cholesteryl esters and a small amount of triglyceride surrounded by a monolayer of phospholipids (mainly phosphatidylcholine), apolipoproteins (Apo), Apo A-I and Apo A-II, and unesterified cholesterol [1]. HDL is divided into different subclasses, according to its density, size, electrophoretic mobility and Apo composition. By ultracentrifugation, HDL can be separated into two main subfractions, HDL2 (larger and less dense) and the smaller and more dense HDL3 [2]. These fractions can be further divided according to their diameter into HDL2b, HDL2a, HDL3a, HDL3b and HDL3c subclasses, with HDL2b having the largest diameter and HDL3c having the smallest diameter [3].

The first paper reporting that plasma levels of HDL cholesterol (HDL-C) were reduced in patients with coronary heart disease (CHD) dates back to 1951 [4]. In 1977, Gordon *et al.* demonstrated in a landmark paper that low HDL-C is a risk factor for CHD [5]. Later on, large prospective studies of cardiovascular (CV) risk factors reported that reduced plasma levels of HDL-C are associated with increased risk of CHD [6–9]. Furthermore, in the meta-analysis of four large studies that included 38,153 patients treated with statin therapy, HDL-C and Apo A-I levels were strongly associated with a reduced CV risk, even among those achieving very low LDL cholesterol (LDL-C) [10].

However, the observation that patients with normal or even high HDL levels may still present a large proportion of CV events, directed the interest of the scientific community towards the assessment of HDL physicochemical properties by determining the size, shape, antigenicity and charge of HDL particles [11]. Indeed, most of the epidemiological studies and prospective randomized trials evaluated only the quantity of circulating HDL-C, but not the quality and functionality of its subclasses [12]; nowadays it seems to be widely accepted that the functionality of HDL subclasses defines the antiatherogenic quality of HDL [13]. Moreover, research addressing HDL has lately gone far beyond the well-known role in RCT, including its relationship with endothelial dysfunction and insulin resistance. In this respect, the new concept of 'dysfunctional HDL', 'proinflammatory HDL' or 'proatherogenic HDL', which exhibits chameleon-like properties of converting the positive effect of artery protection to a deleterious one, of enhanced atherogenesis, is currently under active investigation [14].

This review addresses the current state of knowledge regarding the loss-of-function and gain-of-dysfunction responsible for the proatherosclerotic properties of HDL in several pathological conditions associated with systemic inflammation.

Search strategy

We searched using electronic databases (MEDLINE [1966–October 2013], EMBASE and

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SCOPUS [1965–October 2013], DARE [1966–October 2013]). In addition, abstracts from national and international CV meetings were searched. Where necessary, the relevant authors were contacted to obtain further data. The main search terms were: "atherosclerosis", "biomarker", "dysfunctional HDL", "functionality", "high-density lipoprotein" and "lipid disorders".

■ Looking for optimal biomarkers of lipid disorders

A quarter of a century ago it was reported that the oxidation of LDL-C injured cells in artery walls, and that HDL inhibited LDL-induced cytotoxicity [15]. Therefore, LDL-C and HDL-C became the classical biomarkers of risk assessment and also therapeutic targets in both primary and secondary prevention. However, in the past two decades the new biochemical techniques allowed the quantification of the number of LDL particles (LDL-P), in particular the small, dense LDL, highlighting the fact that low LDL-P is a sensitive indicator of low CV disease (CVD) risk [16]. Moreover, in patients with discordance between LDL-C and LDL-P plasma levels, the CV risk was better indicated by LDL-P, and not by LDL-C values, which could have under- or over-estimated the risk; indeed, in patients with low LDL-C, a high LDL-P level was an indicator of residual risk [17,18]. However, only few studies demonstrated that LDL-P is superior to non-HDL-C so far [17,18].

Whereas traditional serum marker such as LDL-C still represents the main therapeutic targets according to the European Society of Cardiology/European Atherosclerosis Society Guidelines for the Management of Dyslipidaemias [19], recent reports cast increasing doubt on the beneficial role of HDL. Firstly, interventions that increased the concentration of HDL-C in humans have not yet been shown to translate into a reduction in clinical CV events [11]. Indeed, recent clinical trials investigating the effects of raising the level of HDL-C by treatments with CETP inhibitors or with niacin failed to demonstrate any CV benefit [11,20]. Despite the failure in phase 3 of two CETP inhibitors (torcetrapib and dalcetrapib), two potent CETP inhibitors (anacetrapib and evacetrapib), which reported to elevate HDL-C levels and also to reduce LDL-C substantially, are in late stage clinical development [21]. Other HDL-targeted therapies were designed to augment Apo A-I levels either directly (e.g., Apo A-I infusions and upregulators of endogenous Apo A-I production) or indirectly (e.g., through inhibition of CETP or endothelial lipase, or through activation of the high-affinity niacin receptor GPR109A) [22].

Moreover, it has been reported that HDL contains more lipid hydroperoxides than LDL: this observation was related to a greater susceptibility of HDL lipids to *in vivo* oxidation, preferential accumulation of lipid hydroperoxides in HDL and impaired ability of HDL to degrade lipid-oxidation products [23].

HDL particles are also continuously remodeled by plasma factors such as lecithin cholesterol acyl transferase (LCAT), hepatic lipase (HL), endothelial lipase, CETP and PLTP, which clearly impacts on their abnormal properties by changes in size, shape, surface and composition [24]. HDL may acquire oxidized lipids from cells and by exchange with other particles, or HDL lipids may be oxidized *in situ* [25]. Despite the fact that HDL appears to be the major carrier of lipid hydroperoxides, it is not completely clear whether HDL becomes dysfunctional due to excess of oxidative stress [26–28].

In a recent paper, Oravec and colleagues reported the identification of ten HDL subfractions (large HDL 1–3, intermediate HDL 4–7 and small HDL 8–10) using Lipoprint HDL electrophoresis (Quantimetrix, CA, USA) [29]. These authors further proposed that the small HDL subclass exhibits atherogenic properties (= dysfunctional HDL) whereas the large and intermediate HDL subclasses are antiatherogenic. However, to date, neither direct measurements for dysfunctional HDL nor appropriate techniques to isolate specific HDL subpopulations in sufficient amounts are available in order to systematically investigate the lipoprotein functionality in a reproducible manner [20,30]. Therefore, researchers usually use indirect techniques, measuring chlorotyrosine and nitrotyrosine (products of myeloperoxidase [MPO]) levels, dihydrorhodamine, serum amyloid A or LCAT and HL activities among others [11]. Other methods related to HDLs endothelial maintenance and anti-inflammatory activities include the ability of HDL to promote NO production by cultured endothelial cells and the ability of HDL to reduce endothelial cell expression of the proinflammatory VCAM-1 after treatment with an inflammatory cytokine [25,31,32].

Many individuals are diagnosed with hypoalphalipoproteinemia or hyperalphalipoproteinemia, as genetically-induced proinflammatory conditions. The defects in the gene encoding for *ABCA1* were demonstrated

to be one of the genetic causes for familial hypoalphalipoproteinemia [33].

The hypothesis that impaired HDL function caused by Apo A-I deficiency increases the risk of atherosclerosis has led to the characterization of mutations in the Apo A-I gene and the establishment of several genotype–phenotype associations [15]. One of the most investigated mutations, Apo A-I (Milano), has been associated with reduced levels of plasma HDL; however, with no increase in CVD, further raising doubts on the protective role of the quantity HDL [34]. These effects might be associated with greater recombinant HDL (Milano) anti-inflammatory and plaque-stabilizing properties, as recently suggested in a study using the recombinant Apo [35]. In this elegant *in vivo* experimental study, performed on Apo A-I-null mice infected with adeno-associated virus expressing either wild type Apo A-I or Apo A-I (Milano), no enhancement of macrophage RCT could be demonstrated in the case of the latter [35]. Accordingly, the authors speculated that an eventual cardioprotective ability of the mutant apoprotein it is likely attributable to an increased antioxidant activity. In a subsequent experimental study in rabbits, the same group provided further insights on the Apo I (Milano)-related vascular protection, demonstrating anti-inflammatory and plaque-stabilizing properties of the recombinant Apo-I (Milano) [35]. Indeed, in animals treated with recombinant Apo-I Milano, a significant decrease in plaque macrophage density, COX-2, MCP-1, caspase-3 antigen levels and MMP-2 activity; also, in oxLDL loaded macrophages the expression of COX-2 and MCP-1 were significantly reduced in the presence of Apo A-I (Milano) protein [35].

Mechanisms of proatherogenic properties of dysfunctional HDL

■ Decrease in RCT

Normally, HDL is implicated in RCT, removing cholesterol from peripheral tissues including vessels and delivering it to the liver for bile excretion [36]. Four different mechanisms are known as mediators for the efflux of cholesterol to HDLs:

- The efflux of cholesterol to Apo A-I from cells expressing *ABCA1*
- The efflux of cholesterol to HDLs from cells expressing *ABCG1*
- The bidirectional exchange of cholesterol between HDLs and cell membranes expressing scavenger receptor-B1 (SR-B1)
- Passive, aqueous diffusion of cholesterol from cell membranes to HDLs [20]

It is well known that the antiatherogenic activity of HDL is related to its ability to promote sterol and oxysterols efflux from arterial wall cells, as well as to detoxify oxidized phospholipids [37]. Furthermore, it has been demonstrated that there is no association between cholesterol efflux capacities and the anti-inflammatory property of HDL particles [38]. The major protein in HDL, ApoA-I may be reduced owing to decreased synthesis, accelerated HDL catabolism and Apo A-I replacement by serum amyloid A [39]. Apo A-I is responsible for RCT through the macrophage ABCA1, which is indispensable for the production of circulating HDL by formation of nascent HDL particles [40,41]. Recently, it has been reported that both the function and the distribution of Apo A-I in human aorta are quite distinct from those found in plasma. The lipoprotein is markedly enriched within the atherosclerotic plaque, extensively oxidatively cross-linked and functionally impaired. Indeed, the impaired Apo A-I located in the plaque showed an important decrease in both cholesterol efflux and LCAT activity as compared with circulating Apo A-I; the lack of an inadequate RCT might be responsible for the loss of its antiatherogenic properties and the perpetuation of the inflammation in the vascular walls [42].

Two independent study groups reported that MPO selectively interacts with Apo A-I leading to the loss of its function [43,44]. Specifically, it was demonstrated that MPO modification of Apo A-I *in vitro* leads to loss of Apo A-I cholesterol acceptor activity [43,45].

■ Proinflammatory properties

Normally, HDL has anti-inflammatory properties through at least two mechanisms [11]. First, normal HDL prevents oxidation of LDL-C, decreasing the ability of oxidized LDL to attract monocytes into arterial tissue and thus foam cell generation [46]. Second, normal HDL mediates RCT by removing cholesterol from the artery walls and has several antioxidant enzymes that work to maintain an anti-inflammatory state [46]. It has also been reported that HDL can also prevent inflammatory responses by acting directly on monocytes. HDL and Apo A-I exhibit an anti-inflammatory effect on human monocytes by inhibiting activation of CD11b. Apo A-I acts through ABCA1, whereas HDL may act through several different receptors [47]. Since

monocyte activation is a central event in both triggering and maintaining vascular inflammation the observation that both HDL and Apo A-I can prevent/reverse this process might have therapeutic implications. Indeed, as these authors suggested, Apo A-I mimetic peptides may provide an effective therapeutic alternative in atherosclerosis as well as in other conditions associated with vessel walls inflammation [47].

More than a decade ago, Van Lenten *et al.* found that during an acute-phase response in animals or in humans following surgery, HDL can become proinflammatory [48]. During systemic inflammation antioxidant enzymes can be inactivated and HDL can accumulate oxidized lipids and proteins that render it proinflammatory [15]. *In vivo* experiments have demonstrated that inflammation impaired RCT at multiple levels [49]. Dysfunctional HDL also induces the release of proinflammatory cytokines, responsible for the recruitment of monocytes into vascular walls, which ultimately leads to macrophage accumulation [50]. Moreover, inflammatory cytokines downregulated the expression of *ABCA1* and *ABCG1* in mouse macrophages, thus decreasing cholesterol efflux and promoting atherosclerosis [51].

Another mechanism for the appearance of dysfunctional HDL, which lost its ability to promote cholesterol efflux, is that MPO preferentially associates with HDL, causing oxidative damage to Apo A-I [52]. This heme protein, which is expressed by macrophages in human atherosclerotic lesions, generates an array of reactive oxygen and nitrogen species *in vitro* [53].

■ Pro-oxidant properties

Apo A-I, Apo A-II, Apo A-IV and Apo E have antioxidant properties *in vitro*, but their role as *in vivo* antioxidants is less investigated [20]. A number of factors related to atherosclerosis might regulate the levels of these proteins in HDL, including their production by the liver, their expression in macrophages, and remodeling of HDL particles [54]. Normally, the antioxidant activity of HDL particles is facilitated, in part, by the transport of PON1, platelet-activating factor acetylhydrolase (PAF-AH), and LCAT, key enzymes that can hydrolyze diverse molecular species of oxidized lipids and thereby inhibit LDL oxidation [55–57]. An antioxidant mechanism of HDL may result from its ability to accept phospholipid-containing hydroperoxides and other lipid peroxidation products from oxidized LDL [58]. In addition, HDL functions as an antioxidant enzyme by hydrolyzing oxidized phospholipids, such as F2-isoprostanes, formed during the oxidative modification of LDL [59]. In addition, some studies have demontrated that the antioxidant activity of HDL3 is superior to that of HDL2 [60]. Indeed, a recent study of phospholipid and sphingolipid profiling has revealed that all the protective biological properties of HDL subpopulations were predominantly associated with small, dense, protein-rich HDL3. In particular, an enrichment of phosphatidylserine of this subpopulation that positively correlated with all metrics of HDL functionality has been reported [61]. The enrichment of HDL with MPO, triglycerides, group IIA secretory phospholipase A2, ceruloplasmin, serum amyloid A and haptoglobin–hemoglobin complex reduces its antioxidant capacity and even turns it into pro-oxidant HDL [39].

■ Prothrombotic activity

Apparently, HDL has been shown to have beneficial effects in atherosclerosis by providing an antithrombotic function by reducing platelet activation [11]. The inhibition of platelet aggregation has been observed with both the infusion of rats with Apo A-I (Milano) and the administration of rHDL to humans, further supporting the concept that HDL inhibits platelet activation *in vivo* [62]. One potential mechanism contributing to antithrombotic effects of HDL is increased endothelial prostacyclin synthesis. Prostacyclin acts synergistically with NO to induce vascular smooth muscle relaxation, inhibit platelet activation and diminish the release of growth factors, which further promotes an inflammatory plaque phenotype [63]. In a recent study performed in atherosclerosis-prone Apo E-knockout mice treated with aldosterone, the PlGF released from aldosterone-treated Apo $E^{-/-}$ vessels caused the increase of factors, which promote monocyte chemotaxis, and elicited early atherosclerosis in regions of turbulent blood flow; the signaling effect that was inhibited by blocking monocyte PlGF receptors [63].

It is also known that normal HDL contains PAF-AH and paraoxonase, which could prevent or destroy the formation of LDL-derived oxidized phospholipids [64]. The oxidation of HDL alters the influence of HDL on fibrinolysis because oxidized HDL3, but not native HDL3, promotes plasminogen activator inhibitor-I expression and consequently suppresses fibrinolysis [30]. Recently, it was observed that the difference in the proteome of HDL from patients with

CHD, in particular reduced HDL-associated clusterin and increased HDL-associated Apo C-III, plays an important role for altered activation of endothelial anti- and pro-apoptotic signaling pathways and HDL–proteome remodeling [65]. Beyond their well-known prothrombotic effects, platelets have proatherogenic properties, by increasing the proatherogenic properties of Apo B-containing lipoproteins and decreasing the antiatherogenic properties of HDLs [66]. Indeed, incubation of LDL from mice expressing secretory phospholipase A2 with activated platelets increased the content of lysophophatidylcholine content, rendering these particles dysfunctional. The deleterious effects of activated platelets were blocked either by a platelet inhibitor or by a sPLA2 inhibitor. Therefore, activated platelets induce oxidative modifications of native HDL and transform it into dysfunctional HDL with possible prothrombotic activity [66].

■ Inducing endothelial dysfunction

Normally, HDL has been suggested to protect endothelial cells by preventing LDL oxidation and its adverse endothelial effects [67]. HDL promotes the production of the atheroprotective signaling molecule NO and the endothelial repair mechanisms via the upregulation of endothelial NO synthase (eNOS) expression [54]. The mechanisms are involved in the maintenance of the lipid environment in caveolae, where eNOS is colocalized with partner signaling molecules, and direct eNOS stimulation takes place by activating the kinase cascade via the high-affinity HDL receptor SR-BI [30,58]. The impaired capacity of circulating HDL to stimulate endothelial NO production is related to oxidized LDL receptor-dependent PKCβII activation rather than to a major impairment in SR-BI binding or cholesterol efflux [58].

■ Proapoptotic function

It is already known that HDL prevents cell death by reducing apoptosis and necrosis [68,69]. Several mechanisms have been proposed for the endothelial antiapoptotic effects of HDL, depending on the trigger of apoptosis [68,69]. TNF-α-induced endothelial cell apoptosis is inhibited by HDL, and is associated with attenuated induction of CPP32-like protease (caspase 3), a component of all primary apoptotic pathways [63]. Growth factor deprivation activates the mitochondrial pathway of apoptosis in endothelial cells, which can be suppressed by HDL [70]. Indeed, HDL attenuated the dissipation of mitochondrial potential, oxygen-derived free radical generation, cytochrome C release to the cytoplasm, and activation of caspases 3 and 9 [71]. Recently, it has been reported that patients with CHD have a HDL subclass enriched in a novel isoform of Apo C-I able to induce human aortic smooth muscle cell apoptosis *in vitro* [72]. Individuals with this apoptotic HDL phenotype have higher Apo C-I and HDL-C levels, consistent with an inhibitory effect of Apo C-I on CETP activity [72].

Dysfunctional HDL in pathology

■ Dysfunctional HDL & CHD

The first studies on the impaired functionality of HDL concerned patients with CHD or diabetic subjects [11]. Dysfunctional HDL from patients with CHD limits the anti-inflammatory and endothelial repair properties of normal HDL due to the activation of lectin-like oxidized LDL receptor-1 with a subsequent failure in endothelial NO production [58]. Recent analysis also reported an impaired eNOS-activating capacity of HDL in patients with CHD, demonstrating that patients who had developed a strong inflammatory response after ST-elevation myocardial infarction had HDLs that were defective in stimulating endothelial eNOS and NO production [73]. Furthermore, HDL from CHD subjects also contained markedly elevated levels of chlorotyrosine and nitrotyrosine, two characteristic products of MPO, indicating that oxidative damage might generate dysfunctional HDL [54]. Also, in CHD patients, HDL promotes LDL-induced endothelial MCP-1 expression and monocyte adhesion [74].

■ Dysfunctional HDL & smoking

Taking into account the processes that may impair the functionality of HDL (especially inflammation and oxidative stress), the researchers have also started thinking whether dysfunctional HDLs might only be present in patients with risk factors of CVD.

Cigarette smoking can alter the critical enzymes of lipid transport; by lowering LCAT activity and altering CETP and HL activities, smoking interferes with both HDL metabolism and distribution of its subfractions [75]. HDL is susceptible to oxidative modifications by cigarette smoking that render the lipoprotein dysfunctional. In a recent study, physiological levels of nicotine synergized with ox-LDL to increase human macrophage expression of CD36, an effect that was prevented by CD36 small interfering RNA. It is known that CD36 mediates

ox-LDL uptake, thus promoting foam cells formation in the vascular wall and accelerated atherosclerosis [76].

Another recent study provided further insights into the mechanism by which smokers have lower HDL-C and Apo A-I plasma concentrations as compared with nonsmokers. They reported that cigarette smoking-related environmental contaminant benzo(a)pyrene promoted hypoalphalipoproteinemia partly through the inhibition of apolipoprotein A-I gene by aryl hydrocarbon receptor (AhR) [77]. Collectively, cigarette smoking has a negative impact on both HDL quantity and function, which can explain, in part, the increased risk of CVD in smokers [75].

■ Dysfunctional HDL & obesity

Obesity is associated with insulin resistance, hyperinsulinemia and adipokine abnormalities. In obesity, hypertrophied adipocytes secrete several inflammatory cytokines (such as adipokines, TNF-α and IL-1β) that have been correlated with HDL levels [78,79]. The same results have been recently observed for obese and overweight children, where trends across the groups indicated that serum amyloid-A increased in serum and HDL 2 and 3 as BMI increased, as did HDL 2-CETP and HDL 2-LCAT activities. On the basis of the results, the authors suggested that overweight and obese children are exposed to an inflammatory milieu that impacts the antiatherogenic properties of HDL and that could increase CVD risk [80].

■ Dysfunctional HDL & diabetes mellitus

Hyperglycemia and advanced glycation may contribute to dysfunctional HDL by decreasing its antioxidative capacity [3]. Advanced glycation has deleterious effects on anti-inflammatory function of HDL; a study showed that glycated lipid-free Apo A-I infusion did not decrease adhesion molecule expression following vascular injury, as opposed to normal lipid-free Apo A-I [81]. Dysfunctional HDL from diabetic subjects inhibits eNOS activity with subsequent reduction of NO production in endothelial cells [82,83], reduces the capacity for cholesterol efflux and inhibits its antioxidant function [84–86]. In Type 2 diabetic subjects, the activity of PON1 was shown to be reduced by approximately 40%, which was accompanied by a loss of ability to prevent LDL oxidation, and also correlated with the degree of metabolic control [87]. There is also a recognized association between HDL and insulin sensitivity, with low HDL levels and dysfunctional HDL associated with insulin resistance [88].

■ Dysfunctional HDL & obstructive sleep apnea

Intermittent hypoxia due to repeated episodes of brief oxygen desaturation in the blood, followed by reoxygenation, is the main mechanism by which obstructive sleep apnea promotes atherosclerosis [89]. Moreover, patients with obstructive sleep apnea develop systemic inflammation with increased levels of circulating TNF-α and IL-6, along with other cytokines and adhesion molecules with known proatherogenic properties [90].

■ Dysfunctional HDL & chronic kidney disease

It is still remains unclear why patients with chronic kidney disease (CKD) have elevated CV risk [89–92]. Recently, the HDL from the blood of CKD patients has been shown to be dysfunctional, promoting endothelial dysfunction via reduced NO bioavailability and endothelial cell activation, thus generating the conditions for the development of atherosclerotic disease [91]. A recent study also proved that dysfunctional HDL from patients with CKD promotes superoxide production and raises blood pressure via Toll-like receptor-2 activation, leading to impaired endothelial repair and increased proinflammatory activation [92–94].

■ Dysfunctional HDL & autoimmune diseases

Navab *et al.* have developed a novel assay that determines the functional properties of HDL by evaluating the ability of HDL to prevent lipid oxidation [95,96]. A study using this assay in patients with CHD and normal lipid profiles revealed that 25 of 26 patients had proinflammatory HDL [97]. Another study found that 44.7% of women with systemic lupus erythematosus, and 20.1% of women with rheumatoid arthritis had proinflammatory HDL despite normal HDL-C levels [98]. Also, HDL from mice with many features of human scleroderma and from patients with scleroderma was reported to be proinflammatory [99].

Dysfunctional HDL & miRNA

Circulating miRNAs are relatively stable in plasma and represent a new class of disease biomarkers [100]. In the last decade, miRNAs

have emerged as novel regulators of vascular inflammation by suppressing several genes and by forming a complex regulatory network [101]. miRNAs are expressed in the CV system, and have a significant role in the pathogenesis of neointimal lesion formation, atherosclerosis and CHD [102]. miRNAs regulate lipid metabolism, and miR-33a/b is critically involved in regulating cholesterol and fatty acid homeostasis, thereby controlling atherogenesis [103]. Recently, it was found that HDLs have an important role as carriers of miRNAs to a range of cell types where they subsequently regulate gene expression, and that the miRNA cargo of HDLs is altered in people with CVD [104]. Despite the fact that HDL-bound miRNAs were found to be only slightly altered in patients with coronary artery disease, concentrations of most miRNAs were substantially higher in HDL as compared with LDL and patient-derived HDL transiently reduced miRNA expression particularly when incubated with smooth muscle and peripheral blood mononuclear cells [105].

In connection to the hypothesis that different subfractions of HDL, including dysfunctional HDL, carry specific miRNAs, dysfunctional HDL-miRNAs could become novel important biomarkers of CVD. Moreover, targeting HDL-miRNA might be a very promising diagnostic method, and the basis for the development of new drugs [11].

Despite the fact that HDL-bound miRNAs were found to be only slightly altered in patients with coronary artery disease, concentrations of most miRNAs were substantially higher in HDL as compared with LDL and patient-derived HDL transiently reduced miRNA expression particularly when incubated with smooth muscle and peripheral blood mononuclear cells.

Conclusion

Patients with low or normal levels of HDL-C who are at great risk of a CV event may benefit from determination of HDL function. Developing reliable and reproducible laboratory tests for assessing HDL function will allow early evaluation of dysfunctional HDL and will guide future HDL-targeted therapies. It is also crucial to select the patients/conditions at the highest risk of dysfunctional HDL formation, as well as individuals which might still benefit from HDL increasing, and therefore reducing their CVD residual risk [106–113].

Future perspective

Although many questions about the functionality of HDL subpopulations have been resolved, much still remains unknown and the gaps in knowledge are considerable. There is an urgent need for improved assessment of CV risk by means of biological markers that can identify the properties or composition and provide a thorough characterization of dysfunctional HDL.

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The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Executive summary

- Different clinical conditions associated with inflammation, oxidation, advanced glycation and protein carbamylation can alter the functionality of HDL, converting normal HDL into dysfunctional HDL which is no longer cardioprotective.
- It is a large need to find an easy and possibly direct diagnostic method to measure dysfunctional HDL, as well as the optimal method for HDL subfractions/subpopulations analysis.
- In connection to the hypothesis that different subfractions of HDL (including dysfunctional HDL) could be novel, important biomarkers of cardiovascular disease. In addition, targeting HDL-miRNA might be very promising as a new diagnostic method and a starting point for new drug development.
- There is an urgent need for improved assessment of cardiovascular risk by means of biological markers that can identify the properties or composition and provide a thorough characterization of dysfunctional HDL.
- It is crucial to select the patients/conditions at the highest risk of dysfunctional HDL formation.
- It is important to carefully select the patients which might still benefit from HDL increasing to reduce cardiovascular disease residual risk.
- Some of the existing pharmacological agents already used, such as statins and fibrates, present various mechanisms of protection from the deleterious effects of chronic inflammation on HDL functionality.
- A need for novel therapeutic approach has emerged in order to prevent or restrain the transformation of native HDL into dysfunctional HDL.

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