Subfractions of high-density lipoprotein (HDL) and dysfunctional HDL in chronic kidney disease patients

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Abstract
A number of studies have shown that chronic kidney disease (CKD) is associated with increased risk for cardiovascular disease (CVD). Chronic kidney disease is characterized by significant disturbances in lipoprotein metabolism, including differences in quantitative and qualitative content of high-density lipoprotein (HDL) particles. Recent studies have revealed that serum HDL cholesterol levels do not predict CVD in CKD patients; thus CKD-induced modifications in high-density lipoprotein (HDL) may be responsible for the increase in CV risk in CKD patients. Various methods are available to separate several subclasses of HDL and confirm their atheroprotective properties. However, under pathological conditions associated with inflammation and oxidation, HDL can progressively lose normal biological activities and be converted into dysfunctional HDL. In this review, we highlight the current state of knowledge on subfractions of HDL and HDL dysfunction in CKD.

Key words: high-density lipoprotein (HDL), subfractions, dysfunctional HDL, chronic kidney disease.

Introduction
Chronic kidney disease (CKD) affects approximately 16% of the general population, and this figure is projected to rise [1]. Chronic kidney disease is accompanied by very high cardiovascular mortality due to accelerated atherosclerosis [2]. In addition, declining kidney function has been identified as a strong cardiovascular (CV) risk factor [3, 4]. Cardiovascular damage starts early in the development of renal disease, and even mild kidney dysfunction may be an independent predictor for cardiovascular disease (CVD) or stroke [5, 6]. Moreover, some studies have demonstrated that in CKD patients left ventricular diastolic dysfunction occurs frequently and is associated with coronary artery disease and higher mortality [7]. Chronic kidney disease is an inflammatory state characterized by quantitative and qualitative alterations of the plasma lipids. It involves all lipoprotein classes and shows considerable variations depending on the stage of CKD. The causes of disturbances in lipoprotein metabolism are complex and depend on the rate of decline of the glomerular filtration rate (GFR) [8]. The lipid profile of CKD patients is typified by lower serum concentration of high-density lipoproteins (HDL), higher serum concentrations of triglycerides (TG), apolipoprotein B...
Recent studies have shown that serum HDL cholesterol levels are associated with cardiovascular and anti-proteinuric benefits in CKD patients [12–15]. The low-density lipoprotein (LDL) cholesterol level is not usually raised, and it may even be decreased. This heterogeneity in patients with CKD results from differences in quantitative and qualitative content of lipids, apolipoproteins (apos), lipid transfer proteins and enzymes, which directly affect their biological activity and metabolism [11]. High serum LDL cholesterol levels are associated with CV risk in the general population as well as in the CKD population. It is well known that statins exert a beneficial effect on the kidney. Lipid-lowering agents are associated with cardiovascular and anti-proteinuric benefits in CKD patients [12–15].

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High-density lipoprotein subfractions

High-density lipoproteins (HDL) are spherical micelles, with a density of 1.063–1.210 g/ml and a small diameter (7.5–10 nm). All the components of HDL are convertible; thus traditional methods such as X-ray crystallography or nuclear magnetic resonance imaging are useless in identification [16]. The functional heterogeneity of HDL makes their comprehensive characterization quite challenging for investigators, who are searching for more practical and helpful laboratory methods [17]. High-density lipoproteins are composed of cholesteryl esters and triglycerides, which fill the hydrophobic core, and apolipoproteins, phospholipids and unesterified cholesterol forming the outer layer [18]. The main apolipoproteins associated with HDL are apolipoprotein A-I (apoA-I) and apolipoprotein A-II (apoA-II), supported by minor apolipoproteins (apoE, apoC-I, apoC-II, apoC-III, apoC-IV, apoC-V). High-density lipoproteins particles are heterogeneous lipoproteins carrying a large variety of enzymes, globulins, microRNAs, complement components and acute phase reactants [19]. The enzymes responsible for an antioxidant effect are paraoxonase-1 (PON1), platelet-activating factor acetyl hydrolase (PAF-AH), glutathione selenoperoxidase (GSPx), phospholipid transfer protein (PLTP) and lecithin-cholesterol acyltransferase (LCAT) [20, 21].

High-density lipoproteins are extremely complex and have been classified according to size, density, electrophoretic mobility and apo composition. Different subfractions of HDL particles can be distinguished using various techniques. By density gradient ultracentrifugation, the earliest laboratory method, two main subfractions, HDL2 (larger and less dense) and HDL3 (smaller and more dense), can be separated [22]. It is still controversial which of the two has greater anti-atherogenic potency. Maeda et al. [23] found that subjects with a higher concentration of HDL2 were less likely to develop atherosclerosis. Also Kasko et al. [24] observed an increase of the small HDL3 subclass in individuals with newly diagnosed lower extremity artery disease (LEAD) without diabetes mellitus and without hyperlipidemic therapy, which suggests that HDL3 is a potentially proatherogenic subclass. Conversely, the analysis of the Framingham Offspring Study (FOS) revealed that HDL-3 was protective and associated with decreased CV risk, while there was no significant association between HDL2 and CV risk. Using gradient gel electrophoresis, HDL particles can be further divided into HDL2b, HDL2a, HDL3a, HDL3b and HDL3c subclasses [25] (HDL2b has the largest diameter and HDL3c the smallest). In addition, HDLs have been classified according to both electrophoretic mobility and size using two-dimensional gel electrophoresis, which allows more than 10 subspecies to be distinguished [26].

2D electrophoresis, the most informative method, separates lipid-poor pre-β-HDL and cholesterol ester-containing α-HDL. Moreover, pre-β1-HDL (very small, rich in apo-A-I), pre-β2-HDL, pre-β3-HDL (large), α4-HDL (very small, discoid, containing apoA-I, phospholipids and free cholesterol), small spherical α3-HDL (containing apoA-I, apoA-II, phospholipids, free cholesterol, cholesteryl ester and triglyceride, medium size), spherical α2-HDL, which contains the same constituents as α3-HDL, and large, spherical α1-HDL, with the same components as α3- and α2-HDL except the absence of apoA-II and pre-α (pre-α1, pre-α2 and pre-α3, with similar size as α-particles, but without apoA-II) can be identified [27]. Asztalos et al. [28] reported a significant association between lower serum concentrations of α1 and α2 and higher α3-HDL level and elevated risk for new CVD events.

The division of HDL into separate lipoprotein particles can be achieved using anti-apolipoprotein immunoaffinity chromatography. Immunoseparation divides HDLs into a fraction containing only apoA-I (LpAI) and a fraction containing both apolipoproteins (LpAI:All), which are the two most abundant HDL particles. High density lipoproteins containing only apoE (LpE) and apoA-I (LpE:All) are minor lipoprotein particles but also important [29, 30].

Another electrophoretic approach to study HDL heterogeneity is the LipoPrint system (Quantimetrix), which uses a non-denaturing polyacrylamide gel to separate subfractions from pre-stained serum or plasma lipids. The method is approved by the Food and Drug Administration (FDA) as a diagnostic technique for lipoprotein subfraction inves-
tigation in the USA [31]. High-density lipoproteins subclasses are divided into ten bands (HDL1-10), which form three groups: large (HDL1-3), intermediate (HDL4-7) and small (HDL8-10) particles. Oravec et al. [32] proposed that small HDL subfractions (HDL8-10) might have impaired functionality and that there is a correlation between higher concentrations of the small HDL subpopulation and atherogenic lipid profile.

In addition, a novel, gas-phase differential electrophoretic macromolecular mobility based method – ion mobility – has been designed to separate HDL2b from smaller HDL. This method revealed that large HDL2b subspecies inversely correlate with coronary artery disease (CAD) in the prospective Malmo Diet and Cancer Study [33]. The classification and quantitation of HDL particles may also be performed using nuclear magnetic resonance (NMR) spectroscopy [34]. Due to the specific physical structure and natural magnetic distinctness of HDLs, NMR spectroscopy does not require physical fractionation. Although the NMR method currently accounts for 26 different-size HDL subfractions, the measurement precision is limited, and only three HDLs are reported (large, medium and small) [35].

Because of various physical methods used in measurement of lipoproteins, an integrated nomenclature has recently been proposed: very large, large, medium, small and very small HDL particles, including discoid pre-β-HDL [35].

Dysfunctional high-density lipoprotein

Clinical and experimental evidence suggests that the association between individual HDL subclasses and coronary heart disease risk is complex. For years, many authors have suggested that larger HDL particles are more atheroprotective, but recent studies have demonstrated conflicting data [27]. The situation is also complicated in patients with CKD or end-stage renal disease (ESRD). In the study of Abakowska et al. [36], in patients with ESRD, HDL2b (very large) particles were reduced and HDL3c (very small) particles were much more prevalent. On the other hand, Soto-Miranda et al. [37] observed a shift in HDL size distribution towards large particles in patients with proteinuria. Recent studies have shown that levels of the HDL3 subfraction decreased along with increasing CKD severity [38]. Diverse laboratory methods confirmed different HDL subfractions as being anti-atherogenic or associated with decreased CV risk. Nevertheless, HDL particles are not always vasoprotective, and pathological conditions such as inflammation, oxidative stress or diabetes can impair their functionality [39–41]. The vasoprotective properties of HDL have been exerted by reverse cholesterol transport (RCT). High density lipoproteins shuttle excess cholesterol and phospholipids from peripheral cells and macrophages to the liver, where they are excreted by the biliary route [42]. Chronic kidney disease reduces expression of ABCA1 (ATP-binding cassette transporter), which promotes the efflux of cholesterol from macrophages to apoA-I. The major protein in HDL, apoA-1, is decreased in patients with kidney disease because of diminished gene expression in the liver and deficiency of one of the constituent enzymes of HDL – lecithin cholesterol acyltransferase (LCAT) [43]. The LCAT plays a major role in the formation of HDL by esterification of free cholesterol on the surface of HDL and its sequestration in the core of the molecule. Thus, LCAT deficiency, due to CKD-mediated down-regulation in the liver, hinders the uptake and maturation of HDL [44]. The study by Yamamoto et al. indicated that patients with CKD demonstrate abnormal HDL capacity to mediate cholesterol efflux, and it was significantly decreased compared with HDL from healthy subjects [45]. Moreover, Holzer et al. [46] observed that the efflux capacity of HDL in patients with ESRD was reduced compared with HDL from controls without renal disease. Patients with stages 3–4 CKD showed a progressive reduction in cholesterol efflux capacity of HDL [47]. There are numerous observations demonstrating anti-inflammatory, antithrombotic and antioxidant activities of HDL particles [48]. High-density lipoproteins from healthy subjects stops LDL oxidation and inhibits the expression of vascular cell adhesion protein 1 (VCAM-1), intercellular adhesion molecule (ICAM-1) and E-selectin in endothelial cells, which are responsible for infiltration of vascular walls by monocytes and macrophages, leading to atherosclerosis [49, 50]. However, anti-inflammatory activity may also be affected by systemic conditions, and under pathological conditions, such as inflammation or oxidative stress, HDL is referred to as dysfunctional HDL [51]. After post-transitional changes, which impair the anti-atherogenic function, dysfunctional HDL can even promote the production of inflammatory cytokines. Weichhart et al. [52] reported that HDL from healthy subjects inhibited the output of inflammatory cytokines (IL-10, IL-12, TNF-α), while HDL from ESRD patients promoted the production of inflammatory cytokines.

Due to the activities of HDL-associated enzymes (lipoprotein-associated phospholipase A2 (Lp-PLA2), LCAT, paraoxonase (PON), platelet-activating factor acetylhydrolase (PAF-AH), glutathione peroxidase (GPX) and apoprotein components (apoprotein A-I and A-II)), HDL plays an antioxidant role, including prevention of LDL oxidation [53–55]. Many studies have indicated that apoA-I (most among all apos) mediates antioxidant activ-
ities by removing oxidized phospholipids from LDL and from endothelium [56]. Systemic oxidative stress, which is very common in CKD patients, has been shown to reduce antioxidant and anti-inflammatory effects of HDL and even to convert it into a pro-oxidant and pro-inflammatory agent [57, 58]. During chronic inflammation, the concentration of leukocyte myeloperoxidase (MPO) is increased. Zheng et al. [59] reported that MPO altered the function of HDL, especially apoA-I. Moradi et al. [60], who analyzed diminished antioxidant activity of HDL, noted reductions of apo-AI (41%), LCAT (60%), GPX (50%) and PON (30%) levels in ESRD patients compared to healthy subjects. These results were accompanied by a 127% reduction in HDL antioxidant activity. Even in moderate CKD, PON activity is diminished and correlates with non-fatal myocardial infarction, stroke and death [61]. Moreover, apoA-I oxidation decreased its interaction with ABCA1 and inhibited cholesterol efflux from macrophages [62]. Apparently, HDL exerts an antithrombotic effect through reducing platelet aggregation and thrombus formation. HDL was found to inhibit secretion of thromboxane A, which is mediated via scavenger receptor type B1 and/or the apolipoprotein E receptor apoER2/LRP8 [63]. In addition, HDL has a different mechanism contributing to antithrombotic effects. Due to the delivery of arachidonic acid to artery wall cells, which plays a pivotal role in prostacyclin (PGI2) synthesis, HDL improves its antithrombotic value. Prostacyclin has been shown to regulate the release of growth factor and to inhibit platelet activation [64]. High-density lipoproteins modified by an inflammatory response is unable to reduce thrombus formation. High-density lipoproteins is also known to stimulate fibrinolysis by reducing the production of plasminogen activator inhibitor-1 (PAI-1), which then increases the synthesis of tissue plasminogen activator (tPA) and the output of plasmin. High-density lipoproteins of CKD patients has shown impairment in profibrinolytic function [65]. Several studies have reported that HDL mediates endothelial cell proliferation, migration and adhesion molecule formation [66, 67]. Interestingly, these functions of HDL in CKD patients become profoundly depressed. Speer et al. [68] observed significantly decreased stimulation of endothelial cell proliferation and impaired ability to promote endothelial cell survival and repair in children and adults with CKD.

To sum up, these studies clearly indicate that HDL particles can lose their normal biological activities and acquire impaired properties as a result of perturbations in metabolism and composition. These alterations in HDL structure are characteristic for CKD and other pathological conditions associated with inflammation, infection or oxidative stress [69]. Chronic kidney disease, especially in advanced stages, affects the ability of HDL to accept free cholesterol and phospholipids from peripheral tissues, to control inflammation and oxidation, and to support the endothelium.

In conclusion, available data obtained using various laboratory techniques employed to describe plasma HDL support the heterogeneity of HDL particles. Further analysis of HDL particles’ composition may explain biological functions of HDL subclasses, including those with altered properties. Alterations in HDL subpopulations in CKD patients not only predict CV risk but also accelerate the development of atherosclerosis. Even though the findings of recent studies have widened our view of HDL subclasses, there is still a lack of research performed on patients with CKD.

Most clinical studies concentrate on the quantity of HDL, and not on the quality and functionality of its subpopulations. Available data confirm that HDLs of CKD patients become deprived of anti-inflammatory, antioxidant and vasoprotective properties. However, it is still unknown whether size, density or functional alteration causes their dysfunctionality. Further clinical investigations are required to evaluate which subclasses are atheroprotective and which tend to become dysfunctional in patients with CKD.

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Conflict of interest

The authors declare no conflict of interest.

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