# Dysfunctional high-density lipoproteins in coronary heart disease: implications for diagnostics and therapy

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**Low plasma levels of high-density lipoprotein (HDL) cholesterol are associated with increased risks of coronary heart disease. HDL mediates cholesterol efflux from macrophages for reverse transport to the liver and elicits many anti-inflammatory and anti-oxidative activities which are potentially anti-atherogenic. Nevertheless, HDL has not been successfully targeted by drugs for prevention or treatment of cardiovascular diseases. One potential reason is the targeting of HDL cholesterol which does not capture the structural and functional complexity of HDL particles. Hundreds of lipid species and dozens of proteins as well as several microRNAs have been identified in HDL. This physiological heterogeneity is further increased in pathologic conditions due to additional quantitative and qualitative molecular changes of HDL components which have been associated with both loss of physiological function and gain of pathologic dysfunction. This structural and functional complexity of HDL has prevented clear assignments of molecules to the functions of normal HDL and dysfunctions of pathologic HDL. Systematic analyses of structure-function relationships of HDL-associated molecules and their modifications are needed to test the different components and functions of HDL for their relative contribution in the pathogenesis of atherosclerosis. The derived biomarkers and targets may eventually help to exploit HDL for treatment and diagnostics of cardiovascular diseases. (Translational Research 2016;173:30–57)**

**Abbreviations:** ABCA1 = ATP-binding cassette transporter A1; ACS = acute coronary syndrome; ADCY9 = adenylate cyclase 9; apo = apolipoprotein; CAD = coronary artery disease; CETP = cholesteryl ester transfer protein; CHD = coronary heart disease; CKD = chronic kidney disease; eNOS = endothelial nitric oxide synthase; ERK = extracellular signal-regulated kinase; HDL = high-density lipoproteins; HOCl = hypochlorous acid; IL = interleukin; IVUS = intravascular ultrasound; LCAT = lecithin:cholesterol acyltransferase; LDL = low-density lipoproteins; LOX-1 = lectin-like oxidized LDL receptor-1; miRNA = microRNA; MMP-9 = matrix metalloproteinase 9; PON1 = paraoxonase 1; rHDL = reconstituted HDL; S1P = sphingosine-1-phosphate; SAA = serum amyloid A; SDMA = symmetric dimethylarginine; SR-BI = scavenger receptor class B type I; TLR = toll-like receptor; TNF-$\alpha$ = tumor necrosis factor $\alpha$; VCAM-1 = vascular cell adhesion molecule 1

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## INTRODUCTION

According to epidemiologic, pathophysiological, genetic, and clinical evidence, dyslipidemias play an important pathogenic role in the development of atherosclerosis. Notably, low-density lipoprotein (LDL)–cholesterol lowering by the use of statins has become one of the most successful developments in preventive medicine because they help to reduce coronary heart disease (CHD) event rates by up to 50% in the highest dosage.[1] Besides intensified LDL–cholesterol lowering, increasing of high-density lipoprotein (HDL)-cholesterol has been another interesting target for cardiovascular risk reduction for a long time. Many clinical and epidemiologic studies as well as meta-analyses thereof have shown the inverse relationship of HDL-cholesterol plasma levels with the risk of CHD.[2] Furthermore, the development of atherosclerotic lesions could be decreased or even reverted in several animal models by transgenic overexpression or exogenous application of apolipoprotein A-I (apoA-I), that is the most abundant protein of HDL.[3]

For LDL-cholesterol and high blood pressure, this type of epidemiologic and biological evidence has been successfully translated into drugs that lower CHD risk. To date, however, it has been proven difficult to successfully reduce CHD risk with drugs increasing HDL-cholesterol such as fibrates, niacin, or inhibitors of cholesteryl ester transfer protein (CETP).[4,5] Moreover, in several inborn errors of human HDL metabolism and genetic mouse models with altered HDL metabolism, the changes in HDL-cholesterol levels were not associated with the opposite changes in cardiovascular risk and atherosclerotic plaque load, respectively, expected from epidemiologic studies.[3,6] Because of these controversial data, the causal role and hence suitability as a therapeutic target of HDL has been increasingly questioned.

However, previous intervention and genetic studies targeted LDL- and HDL-cholesterol, that is the cholesterol measured by clinical laboratories in LDL and HDL, respectively. By contrast to the pro-atherogenic and hence disease-causing cholesterol in LDL (that is LDL-cholesterol), which after internalization turns macrophages of the arterial intima into pro-inflammatory foam cells, the cholesterol in HDL (that is HDL-cholesterol) neither exerts nor reflects any of the potentially anti-atherogenic activities of HDL. By contrast to LDL-cholesterol, HDL-cholesterol is only a nonfunctional surrogate marker for estimating HDL particle number and size without deciphering the heterogeneous composition and hence functionality of HDL.[5] In a prototypic HDL particle, 2–5 molecules of apoA-I and about 100 molecules of phosphatidylcholine form an amphipathic shell in which several molecules of un-esterified cholesterol are imbedded and which surround a core of completely water-insoluble cholesterol esters and, albeit less, triglycerides. Already molar differences in the content of the major protein and lipid constituents of HDL, that is apoA-I, phosphatidylcholine, sphingomyelin, cholesterol, and cholesteryl esters, cause considerable heterogeneity of HDL in shape, size, and charge.[5] Some of these model particles have been artificially reconstituted for experimental but also therapeutic purposes.[7] This macro-heterogeneity is further increased by the presence or absence of quantitatively minor proteins or lipids, some of which may contribute to the pleiotropic functions of HDL. Previous proteomic and lipidomic studies revealed a much greater structural complexity: HDL particles carry >80 different proteins and hundreds of lipid species.[8] Most recently, even microRNAs (miRNAs) were found to be transported by HDL.[9,10] Many of these molecules are not passive cargo but biologically active and contribute to the pleiotropic and potentially anti-atherogenic properties of HDL. This micro-heterogeneity is further increased in HDL of patients with various inflammatory diseases, including CHD, by the loss or structural modification of typical HDL constituents or by the acquisition of atypical constituents.[5,11,12] Of note, many physiological as well as pathologic components and modifications are present at concentrations which are several orders of magnitude lower than the concentration of HDL-cholesterol or even HDL particles and hence reflected by measurements of neither HDL-cholesterol, nor apoA-I, nor HDL-particle concentrations.[5] In the search for biomarkers that reflect the functionality of HDL better than these high-throughput markers, bioassays have been developed for clinical studies as well as for discovery of functional biomarkers by proteomics and lipidomics.

## HDL AND PROTECTION AGAINST CARDIOVASCULAR DISEASE

HDL particles exert many beneficial actions that may help protect against cardiovascular disease.[5,13]

The classical anti-atherogenic role of HDL in cardiovascular disease is its potential to drive cholesterol export from macrophage foam cells and subsequent transport toward the liver for excretion into bile and feces, that is, reverse cholesterol transport.[14,15] HDL elicits the first step, cholesterol efflux from macrophages, by several mechanisms and different subclasses. The lipid-containing, buoyant, and $\alpha$-migrating HDL facilitates aqueous diffusion of plasma membrane residing cholesterol by tethering to scavenger receptor class B type I (SR-BI). $\alpha$-Migrating