REVIEW ARTICLE

The High Density Lipoprotein Cholesterol Hypothesis Revisited

Anna Meiliana[1,2,*], Nurrani Mustika Dewi[2], Andi Wijaya[1,2]

[1]Postgraduate Program in Clinical Pharmacy, Padjadjaran University, Jl. Eijkman No.38, Bandung, Indonesia
[2]Prodia Clinical Laboratory, Jl. Cisangkuy No.2, Bandung, Indonesia

*Corresponding author. E-mail: anna.meiliana@prodia.co.id

Received date: May 17, 2018; Revised date: Jul 20, 2018; Accepted date: Jul 24, 2018

Abstract

BACKGROUND: The strong inverse association of plasma levels of high-density lipoprotein cholesterol (HDL-C) with coronary heart disease (CHD) found in human epidemiological studies led to the development of the 'HDL-C hypothesis', which posits that intervention to raise HDL-C will result in reduced risk of CHD. However, recent evidence has raised doubts about the hypotheses that elevating HDL-C is necessarily therapeutic. Genetic variations that associate with altered HDL-C do not strongly associate with altered cardiovascular disease risk.

CONTENT: HDL-mediated cholesterol efflux from macrophage foam cells or measurements of the flux of cholesterol from macrophages to the liver and feces seem to correlate better with atherosclerotic burden than with HDL-C levels. Thus, it may be time to modify the HDL-C hypothesis to the 'HDL flux hypothesis', where intervention to promote cholesterol efflux and reverse cholesterol transport will reduce CHD risk, regardless of whether it affects plasma HDL-C levels. A deeper understanding of the complex biology of HDL metabolism and its relationship to reverse cholesterol transport and atherothrombotic events is urgently needed. This might lead to biomarkers of HDL flux and functionality that are more informative than simple measurements of HDL-C levels.

SUMMARY: It is now clear from recent clinical trial and genetic studies that some approaches to raising HDL-C levels may have no effect on CHD. This suggests the need to evaluate HDL-C-raising therapies in different clinical populations, as well as therapies targeted toward HDL flux and function rather than simply HDL-C elevation. Perhaps moving from a focus on the HDL-C hypothesis to a focus on the HDL flux hypothesis will permit a biologically based reassessment of the optimal therapeutic approach to targeting HDL for reduction in cardiovascular risk.

KEYWORDS: reverse cholesterol transport, cholesterol efflux capacity, HDL dysfunction, HDL particle size, HDL lipidomics, HDL proteomics

Indones Biomed J. 2018; 10(2): 84-103

Introduction

Classical epidemiology has established the incremental contribution of the high-density lipoprotein cholesterol (HDL-C) measure in the assessment of atherosclerotic cardiovascular disease (CVD) risk, yet, genetic epidemiology does not support a causal relationship between HDL-C and the future risk of myocardial infarction. Therapeutic interventions directed toward cholesterol loading of the HDL particle have been based on epidemiological studies that have established HDL cholesterol as a biomarker of atherosclerotic CVD risk. The reference range of HDL-C is 40-50 mg/dL in men and 50-60 mg/dL in women. However, therapeutic interventions such as niacin, cholesterylester transfer protein (CETP) inhibitors increase HDL-C in patients treated with statins, but have repeatedly failed to reduce CVD events.(1) Clinical trials with pharmacological therapies that increase the cholesterol content of HDL particles have failed to establish this convenient metric as

an effective strategy for the prevention of CVD events. These therapies have included niacin/niacin-laripoprant and CETP inhibitors.(1)

Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides: Impact on Global Health Outcomes (AIM-HIGH) and The Heart Protection Study 2–Treatment of HDL to Reduce the Incidence of Vascular Events (HPS2-THRIVE) trials investigated the effect of niacin and niacin/laripoprant, respectively, in coronary heart disease (CHD) patients with low-density lipoprotein cholesterol (LDL-C) levels at the recommended target <70 mg/dL.(2,3) Elevations in HDL-C were not associated with fewer CVD events. Instead, there was harm from niacin treatment as shown by the increased risk of infections.(4) CETP inhibition with multiple agents (torcetrapib, dalcetrapib, and anacetrapib) has failed to reduce CVD events in high-risk patients treated with high-intensity statin therapy.(5-7) The failure of torcetrapib was ascribed to torcetrapib-induced hypertensive effects via renin-angiotensinaldosterone activation and weak activity of dalcetrapib.(8,9) Early termination of the evacetrapib clinical trial in Assessment of Clinical Effects of Cholesteryl Ester Transfer Protein Inhibition With Evacetrapib in Patients at a High-Risk for Vascular Outcomes (ACCELERATE) (7) was unexpected, not only based on the LDL-C-lowering efficacy of evacetrapib, but also in the increase in very small HDL particles and macrophage cholesterol efflux capacity. (8) Based on event rates observed in statin-treated patients, early termination of ACCELERATE may not have allowed sufficient time to detect a treatment difference in CVD.

HDLs comprise a multitude of discrete subpopulations that differ in composition, metabolism, cellular interactions, and functional properties.(10,11) Expansion of the core cholesterol in HDL particles interferes with apoA-I-mediated cholesterol efflux via ABCA1 and alters the proteome and lipidome, resulting in species that are less effective in antioxidant and anti-inflammatory properties. (12) Of the HDL functional assays, only macrophage cholesterol efflux has been validated in prospective studies. (11,13-16) Efforts to solve the HDL puzzle require high throughput assays that accurately quantify discrete HDL species based on their protein and lipid components. HDL speciation may allow for the identification of specific HDL populations with functional or dysfunctional properties. The macrophage cholesterol efflux assay is an established method that has been used to characterize both functional and dysfunctional HDL.(13,14,17,18) Subsequent steps require evaluation of discrete HDL populations in the prediction of atherosclerosis and cardiovascular events.(19)

HDL-C Hypothesis

The most popular mechanistic hypothesis underlying the HDL-C hypothesis has been the concept of 'reverse cholesterol transport', namely that some component of the HDL system promotes cholesterol efflux from arterial macrophage foam cells and transports it to the liver for biliary excretion, leading to a reduction in lesion size (Figure 1).(20)

However, recent events have raised serious questions about the validity of the HDL-C hypothesis. Two clinical trials involving therapeutic elevations of HDL-C on individuals with low levels of LDL-C lowering were prematurely terminated on the basis of futility. The AIM-HIGH study was conducted with niacin, the most effective HDL-raising drug currently on the market; the Dalcetrapib in Patients with Recent Acute Coronary Syndrome (dal-OUTCOMES) study involved dalcetrapib, a drug in development that partially inhibits the CETP, which transfers cholesterol from HDL to very-low-density lipoprotein (VLDL) or LDL. Although many issues related to trial design are being actively debated, the hypothesis, based on epidemiological studies, that moderate HDL cholesterol raising would cause a marked reduction in events seems to have been refuted. Furthermore, a causal relationship of HDL-C with CHD has been challenged.(20)

Maturation produces diverse HDL particles that vary in size, density, and lipid and protein composition. ApoA-I and apolipoprotein II account for 90% of HDL's protein content, but close to 100 other HDL proteins have been described as HDL-associated.(21) Measuring HDL-C levels takes only the cholesterol mass into account, but cholesterol accounts for no more than 20% of a particular HDL particle, and its level may vary up to 10-fold, depending on a particle's size. (22) Thus, a confounder in interpreting HDL-C as a measure of circulating HDL concentration is the relative balance between large and small HDL particles.(23) HDL particle concentration, that provides information regarding the actual number of HDL particles in the circulation, can be measured by nuclear magnetic resonance (NMR) (24) or ion mobility analysis (25,26). Calibrated ion mobility analyses further demonstrate that precise measurements of small, medium, and large HDL particles can be obtained from plasma.(26) Therefore, evaluating changes in HDL subpopulations in response to therapy might be a better metric than HDL-C levels for predicting risk.(27)

As well as varying in size, HDL particles vary in protein content, as HDL's proteome is very rich. Alterations

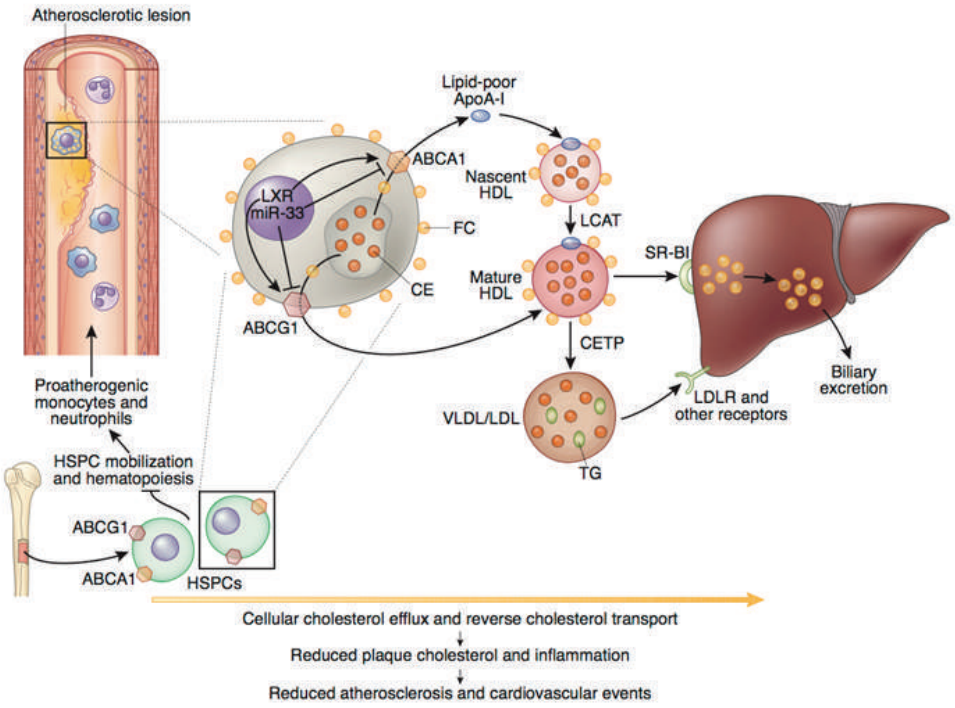

Figure 1. Pathways influencing HDL cholesterol metabolism and flux and potential relationship to atherosclerosis.(20) (Adapted with permission from Springer). HSPC: hematopoietic stem and progenitor cell; LXR: liver X receptor; ABCG1: ATP-binding cassette sub-family G member 1; ABCA1: ATP binding cassette transporter A-1; ApoA-I: apolipoprotein A-I; HDL: high-density lipoprotein; LDL: low-density lipoprotein; VLDL: very low-density lipoprotein; LCAT: lecithin-cholesterol acyltransferase; CETP: cholesterylester transfer protein; SR-BI: scavenger receptor class B type I; LDLR: LDL-receptor; TG: triglyceride.

in protein cargo have been associated with pathological states, such as inflammation (28), poor response to therapy (29), autoimmune disease (30), and diabetes (31). Further improvements in quantitative techniques (32,33) and their application to clinical studies should identify panels of protein biomarkers that may be pharmacologically modifiable to reduce risk. Another important variable is HDL's ability to promote cholesterol efflux from cultured macrophages with samples derived from serum, termed cholesterol efflux capacity (CEC). Cholesterol efflux capacity has been a stronger predictor of prevalent and incident coronary artery disease than HDL-C in multiple clinical trials.(13,14,24) *In vitro* assays of CEC of HDL may include four possible pathways: aqueous diffusion, scavenger receptor class B type I (SR-B1) receptor, and ATP binding cassette transporter A-1 (ABCA1), and ATP-binding cassette sub-family G member 1 (ABCG1) transporters.(27)

Genetic epidemiology does not support a causal relationship between HDL cholesterol and future risk of myocardial infarction. In a Mendelian randomization study that included 20 studies (12,482 myocardial infarction cases and 95,407 controls), single nucleotide polymorphisms in the endothelial lipase gene (LIPG Asn396Ser) had higher HDL-C levels and similar nonlipid and other lipid risk factors for myocardial infarction.(34,35) Neither physiological function nor pathological dysfunction is recorded by the measurement of HDL-C. Novel biomarkers are currently searched and validated in clinical or epidemiological studies toward their potential to aid in drug development and improve risk prediction and monitoring of treatment response. Candidate biomarkers include HDL particle numbers, serum amyloid A, apolipoprotein (apo)C-III, sphingosine-1-phosphate (S1P), certain post-translational modifications of apoA-I, and cholesterol efflux capacity.(36) It is important that future studies take into consideration the role of HDL function in addition to the level when the association of HDL with risk of cardiovascular disease and outcomes. This is especially true in patients with a high burden of oxidative stress and inflammation.(37)

HDL Particle Size, Lipidomics and Proteomics

Despite the spectacular collapse of the HDL-C hypothesis and the paucity of alternative strategies in the clinical space, the field as a whole has experienced a groundbreaking shift in the understanding of the basic biology and metabolism

of HDL. Advances have been made in the areas of HDL functionality as it relates to particle heterogeneity, biogenesis, and variations in lipid and protein cargo. (38) The plasma concentration of HDL particles and their size distribution can be reliably measured using different approaches to identify subspecies with unique functional and compositional profiles. Currently, there is no consensus on the relative value of the different methods and on the number, concentration, functional status, and predictive power of the different HDL subparticles.

HDL particles are highly heterogeneous in structure, composition, metabolism, and function. Distinct HDL subpopulations might, therefore, exert differential effects on atherosclerosis.(39) As a consequence, plasma concentrations of specific HDL subpopulations can be hypothesized to reveal stronger associations with cardiovascular risk as compared to total HDL particle number. NMR measurements allow distinguish between large (size 9.4–14.0 nm), medium (8.3–9.3 nm), and small (7.3–8.2 nm) HDL particles.(24) Among these HDL subpopulations, levels of large HDL frequently display inverse relationships with cardiovascular risk in univariate analyses, whereas concentrations of small HDL particles typically reveal positive correlations with the risk.(40-50) Circulating concentrations of large and small HDL particles as well as those of large HDL particles and total LDL particles as measured by NMR are, however, known to be negatively correlated.(51) Available data reveal that diminished HDL particle number can be superior to reduced HDL-C levels in terms of cardiovascular risk prediction. Measurements of circulating concentrations of HDL particles can, therefore, be useful to improve clinical assessment of cardiovascular risk. This approach can also contribute to the evaluation of novel HDL-targeted therapies, which include CETP inhibitors, peroxisome proliferator-activated receptor (PPAR) activators, and reconstituted HDL.(51)

HDL can be fractionated by different techniques into discrete subclasses according to physicochemical properties and chemical composition. Sequential ultracentrifugation allows separation of HDL into two main subfractions on the basis of density: large, light, lipid-rich HDL2 (d 1.063–1.125 g/mL) and small, dense, protein-rich HDL3 (d 1.125–1.21 g/mL).(10) HDL2 and HDL3 can be further subfractionated on nondenaturing polyacrylamide gradient gel electrophoresis into five distinct subpopulations of decreasing size: HDL2b, HDL2a, HDL3a, HDL3b, and HDL3c (10); equivalent subpopulations of increasing density can be quantitatively isolated using isopycnic density gradient ultracentrifugation (10). Such heterogeneity of HDL particles directly reflects differences in the relative content of proteins and lipids in HDL.(52)

HDLs are a class of heterogeneous lipoproteins; their heterogeneity is attributable to a different content of apolipoproteins, lipids and enzymes and to the remodeling of HDL particles by lipolytic enzymes, lipid transporters and by lipid and apolipoprotein exchange with other circulating lipoproteins and tissues. Different HDL subpopulations carry distinct and specific proteins or lipids, suggesting distinct and characteristic functions. (39) HDL can be classified on the basis of density, resulting in the large buoyant HDL2 and the small dense HDL3, which can be further subfractionated into 5 distinct subpopulations (HDL2b, HDL2a, HDL3a, HDL3b and HDL3). Alternatively, HDLs can be separated on the basis of their electrophoretic mobility in pre-β- particles, α-particles and pre-α-particles. HDLs can also be classified according to their main apolipoprotein content into particles containing only apolipoprotein A-I (apoA-I, LpA-I) or both apoA-I and apoA-II (LpA- I/A-II) (Table 1).(39,53)

The heterogeneity of HDLs is also reflected in their functions, since different subpopulations play distinct roles. HDLs possess several antiatherogenic functions. The best characterized activity of HDL is reverse cholesterol transport, the process by which excess cholesterol is transported from the peripheral tissues to the liver for excretion. Different HDL subpopulations interact with different cellular receptors to remove excess cholesterol from cells. Besides, HDLs, and in particular small dense HDL3, exert antiinflammatory and antioxidant activities. HDLs have a protective effect on vascular endothelium, are antithrombotic and antiinfectious and play a role in the modulation of immune responses and the control of glucose homeostasis.(39,54,55)

The protein constituents of HDL in particular have been heavily dissected in recent years, primarily through liquid chromatography mass spectrometry (LC-MS), revealing several more proteins additional to the classical apolipoproteins, such as apoA-I, apoA-II, apoE and apoC-III, whose biological activities are diverse, but still encompass the role for HDL as antiatherogenic particle. Moreover, LC-MS technology has extended well beyond a tool for identification. Changes in apolipoprotein abundance can now be readily assessed using one or more of a number of strategies including label-free and label-based strategies.(56,57). Quantitative proteomics provides several innovative workflows that are poised to address many of the unknowns of apolipoprotein function and metabolism. Global proteomics studies have demonstrated that the HDL

Table 1. HDL subclasses and major components of the HDL lipidome(52,53) (Adapted with permission from Karger AG, American Society for Biochemistry and Molecular Biology).

Characteristic	HDL-Subclasses
Shape	Discoidal, Spherical
Density (untracentrifugation)	HDL 2, HDL 3
Size (non denaturing gel electrophoresis)	HDL 2a, HDL 2b, HDL 3a, HDL 3b, HDL 3c
Charge (2-dimensional electrophoresis)	Pre-β-article: Pre-β1 (HDL3, LpA-I), Pre-β2 (LpA-I, Pre-β3 (LpA-I), Pre-β4 (LpA-I)
	α-Particles: Very small discoidal α-4 (HDL3, LpA-I), Small spherical α3 (HDL3, LpA-I:A-II), Medium spherical α2 (HDL3, LpA-I:A-II), Large spherical α1 (HDL2, LpA-I)
	Pre-α particles: Pre-α1 (LpA-I), Pre-α2 (LpA-I), Pre-α3 (LpA-I), Pre-α4 (LpA-I)
Composition (immunoaffinity)	LpA-I (prominent components of both HDL2 and HDL3): Large LpA-I, Medium LpA-I, Small LpA-I
	LpA-I: A-II (most found in HDL3)

Lipid Class	HDL Content (% of total HDL Lipid)
Phospholipids	35-50
Phosphatidylcholine	33-45
Lysophosphatidylcholine	0.5-5
Phosphatidylethanolamine	0.5-1.5
Phosphatidylinositol	0.5-1.5
Plasmalogens	0.5-1.5
Phosphatidylserine	0.02-0.04
Phosphatidylglycerol	ND
Phosphatidic acid	ND
Cardiolipin	0.2
Sphingolipids	5-10
Sphingomyelin	5-10
Ceramide	0.05
S1P	0.01-0.02
Sphingosylphosphorylcholine	0.0005
Steroids	5-10
Triacylglycerides	5-12
Cholesteryl ester	30-40

proteome comprises between 90 and 100 proteins. The HDL proteome is differentially distributed across the HDL size fractions giving rise to a heterogeneous population of HDL particles.(58) In addition to proteins consistent with traditionally accepted roles in lipid transport, HDL carries surprising constituents, such as members of the complement pathway, protease inhibitors involved in hemostasis, acute-phase response proteins, immune function mediators, and even metal-binding proteins. This compositional diversity fits well with hundreds of studies demonstrating a wide functional pleiotrophy, including roles in lipid transport, oxidation, inflammation, hemostasis, and immunity.(21) Figure 2A shows the frequency of detection of HDL-associated proteins in some MS-based proteomic studies, meanwhile Figure 2B shows general functional relationships of HDL proteins. Establishing direct relationships between distinct HDL structure and composition on the one hand and specific atheroprotective functions on the other requires further study (Table 1). Such structure-function analysis of HDL particles bears the potential to identify clinically relevant, atheroprotective HDL subpopulations. Furthermore, development of HDL-based therapies specifically designed to target beneficial subspecies of the circulating HDL pool can be facilitated using this approach. HDL lipidomics can equally contribute to the identification of biomarkers of both normal and deficient HDL functionality, which may in turn prove useful as biomarkers of cardiovascular risk. It remains to be shown whether such novel HDL-based lipidomic biomarkers can be superior relative to HDL-C levels.(52)

Lipid-poor and small HDL displayed both high capacity for ABCA1-mediated cholesterol efflux and strong anti-inflammatory action, whereas the larger particles showed stronger antioxidant function. This study provides a novel perspective because it suggests that subspecies of HDL may be involved in different functions, and thus, the right mix of multiple HDL particle sizes may be most desirable to maximize cardioprotective benefits (Figure 3). Most importantly, the study offers an approach to generate these particles *in vivo*.(38)

The HDL mass is equally distributed between lipid and protein cargo. The HDL lipidome is composed mainly of phospholipids, cholesteryl esters, triglycerides, and free cholesterol, for a total of over 200 individual lipid species in normolipidemic subjects. Some lipid components, such as phospholipids, sphingomyelin, and free cholesterol, have already been investigated as modulators of HDL functions, such as sterol efflux, vasodilation, and control of oxidation and inflammation.(52)

Regulation of HDL Metabolism

Understanding of the regulation of HDL metabolism has increased significantly in recent years, although these advances have not, to date, translated into the development of HDL-raising therapies that decrease cardiovascular morbidity and mortality. Given the high economic cost and

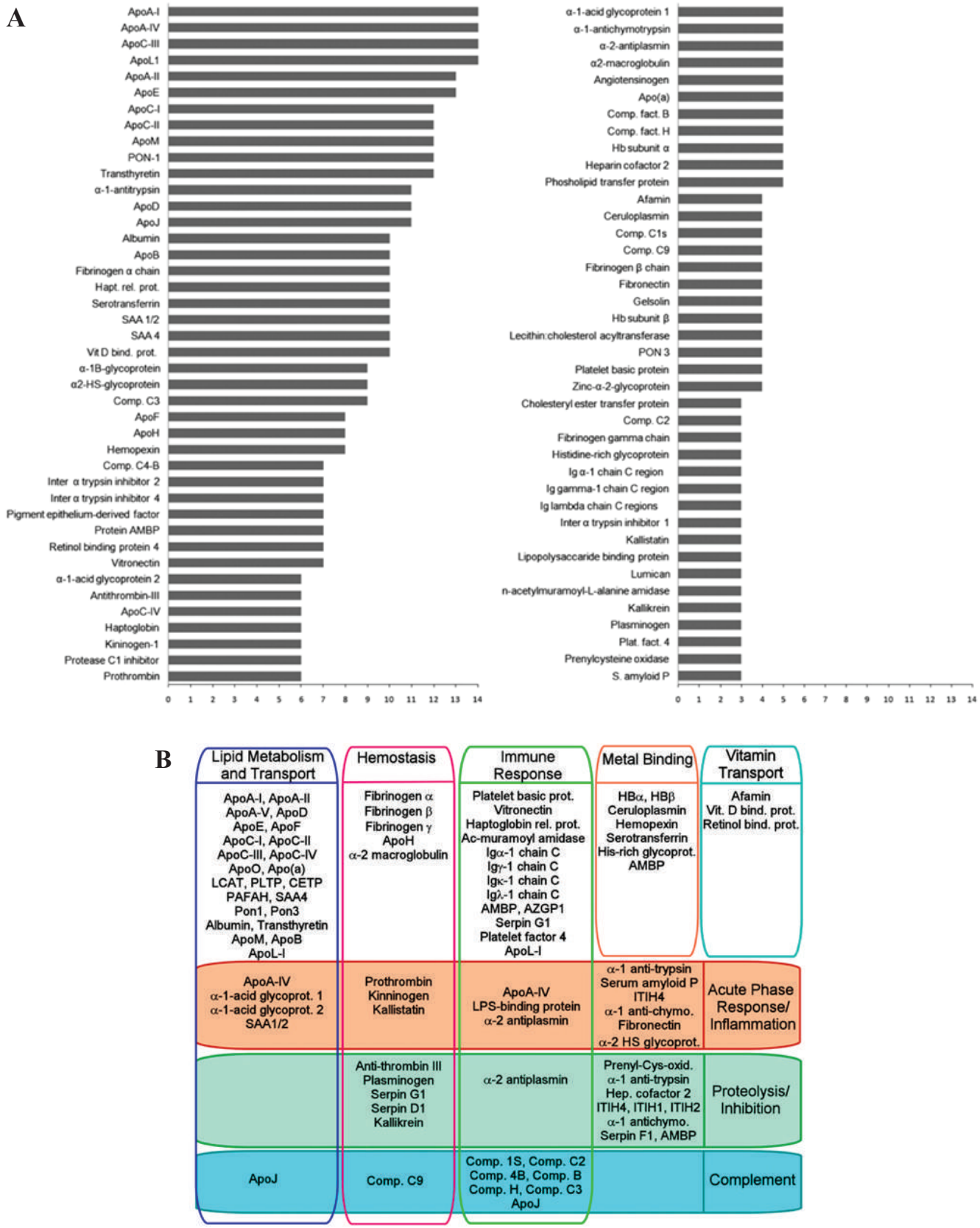

Figure 2. Relationship of HDL proteins. A: General functional relationships of the HDL proteins; B: Frequency of detection of HDL-associated proteins in MS-based proteomic studies.(21) (Adapted with permission from American Society for Biochemistry and Molecular Biology).

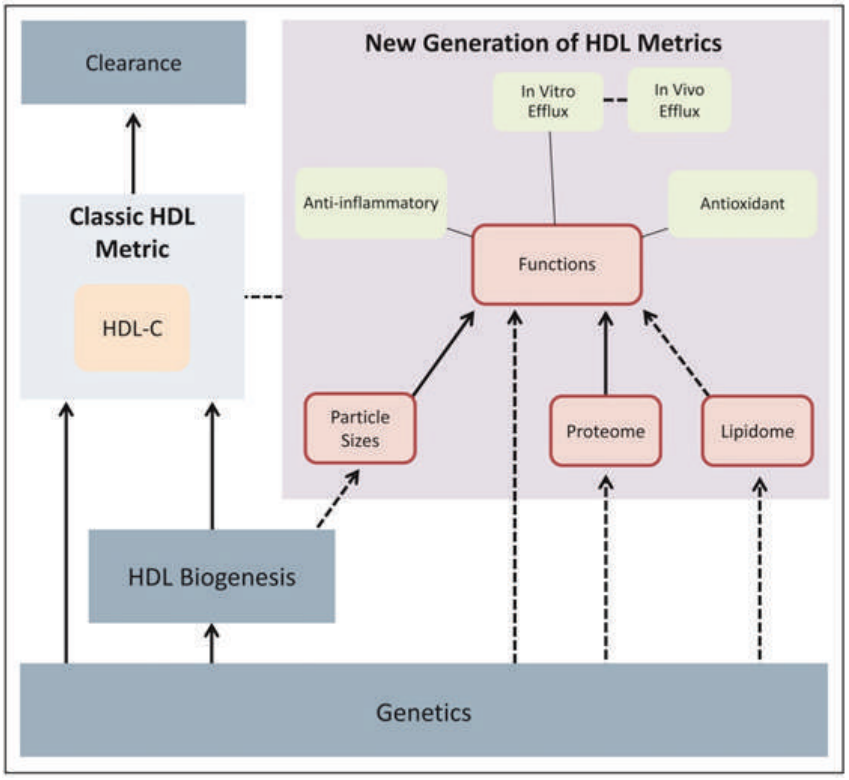

Figure 3. Overview of the regulation and interaction of traditional and novel high-density lipoprotein (HDL) measures.(38) (Adapted with permission from American Heart Association).

increasing burden of CVD worldwide, there is a compelling need to identify strategies and to develop therapeutic agents that can begin to resolve these issues.(59) The heterogeneity is a consequence of the continual remodeling and interconversion of HDL subpopulations by multiple plasma factors. Evidence that the remodeling of HDLs may impact on their cardioprotective properties is beginning to emerge. This serves to highlight the importance of understanding not only how the remodeling and interconversion of HDL subpopulations is regulated but also how these processes are affected by agents that increase HDL levels.(59)

The 4 main apolipoproteins in human HDLs, in order of decreasing abundance, are apoA-I, apoA-II, apoA-IV, and apoE (Figure 4). ApoA-I is synthesized in the liver and intestine. Hepatic apoA-I is initially generated as a preproprotein that is cleaved intracellularly by a signal peptidase.(60) The resulting propeptide is secreted before cleavage by bone morphogenic protein-1 in a process that is facilitated by procollagen C-proteinase enhancer-2.(61,62) In vitro studies have found that ≤45% of apoA-I is lipidated before it is secreted from hepatocytes.(63-65) The initial lipidation of apoA-I occurs in the endoplasmic reticulum and is independent of the ABCA1.(63) Additional lipidation of apoA-I takes place in the golgi and at the plasma membrane in processes that are dependent on a dimeric form of ABCA1.(66,67)

Most types of cells in the body do not express the capability of catabolizing cholesterol, so cholesterol efflux is essential for homeostasis. For instance, macrophages possess four pathways for exporting free (unesterified) cholesterol to extracellular HDL. The passive processes include simple diffusion via the aqueous phase and facilitated diffusion mediated by SR-BI. Active pathways are mediated by ABCA1 and ABCG1, which are membrane lipid translocases. The efflux of cellular phospholipid and free cholesterol to apoA-I promoted by ABCA1 is essential for HDL biogenesis.(68)

Recently, S1P, a lipid mediator that acts via G-protein-coupled receptors, has featured prominently in HDL biology. The ability of HDL to protect the endothelium (69), myocardial ischemic injury and vasodilate (70) depends on the S1P cargo. HDL-bound apolipoprotein M binds, carries, and promotes receptor activation in a physiologically relevant manner.(71)

In addition, HDL-bound S1P seems to be distinct from albumin-bound S1P in the inhibition of endothelial inflammatory processes (72), barrier function (73) and lymphopoiesis (74), suggesting that chaperone- bound S1P acts as a biased agonist to evoke specific biological processes. These observations suggest a major function of S1P in the cardiovascular protection mediated by HDL.(75,76)

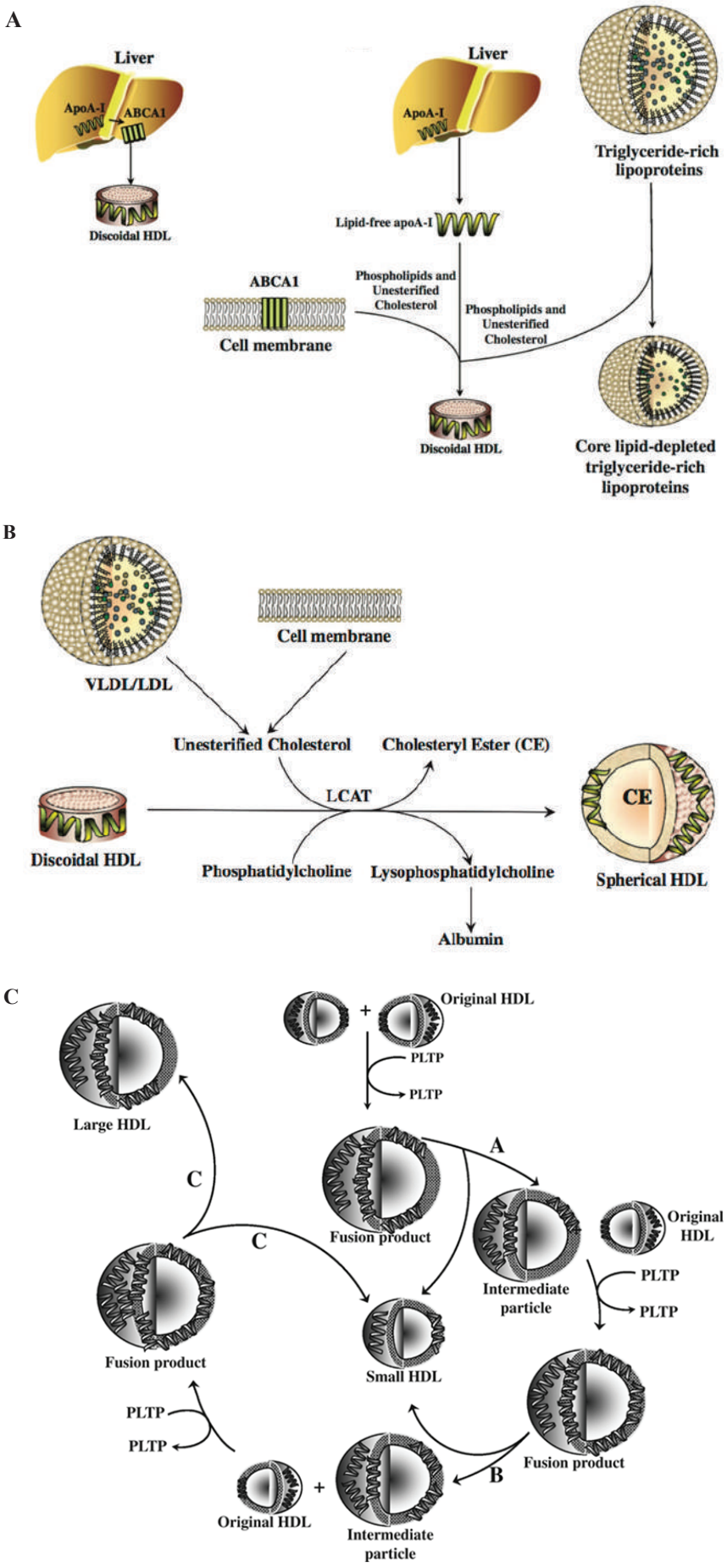

Figure 4. The biogenesis of many kind of lipoproteins. A: Biogenesis of apolipoprotein A-I (apoA-I)–containing discoidal high-density lipoproteins (HDLs); B: Biogenesis of spherical high-density lipoproteins (HDLs); C: Remodeling of apolipoprotein E (apoE)–containing high-density lipoproteins (HDLs) by phospholipid transfer protein (PLTP).(59) (Adapted with permission from American Heart Association).

There are 3 different transmembrane transporters along the apical membrane of the hepatocyte that actively promotes this process: the heterodimer ABCG5/ABCG8 (which facilitates cholesterol efflux), ABCB11 and the phospholipid pump, ABCB4.(78) Moreover, another transporter, ATP8B1, is also necessary for correct secretion of bile. ATP8B1 moves phosphatidylserine in the opposite direction to the transport of phosphatidylcholine by ABCB4 to maintain the asymmetry of phospholipids required for proper membrane function.(78,79) Because cholesterol cannot be degraded in the cell, reverse cholesterol transport (RCT) is an essential process to ensure that cholesterol levels are balanced within the body. Work over the past years has identified miRNAs as important regulators of HDL-C metabolism. miRNAs control most of the steps of RCT including HDL biogenesis, cellular cholesterol efflux, hepatic HDL-C uptake, and bile acid synthesis and secretion.(80) In addition to their role in regulating HDL-C metabolism, HDL-enriched miRNAs regulate gene expression in recipient cells, thus providing an exciting novel mechanism that could explain a part of the antiatherogenic effect of HDL (Figure 5).(81,82)

Recent studies have highlighted the close partnership between activation of ecto-F1-ATPase by HDL or their major protein, apoA-I, and P2Y receptor signaling. For instance, on hepatocytes the HDL-apoA-I/ecto-F1-ATPase/P2Y13 sequence contributes to HDL uptake and would be atheroprotective.(83-88) On adipocytes, HDL-apoA-I, ecto-F1-ATPase and P2Y signaling are all involved in lipid metabolism.(89-91) On endothelial cells, ecto- F1-ATPase is also activated by HDL-apoA-I and is potentially coupled to P2Y1 or P2Y12 receptors, promoting cell survival and HDL transcytosis.(92,93) In addition, HDL-apoA-I receptor signalling and P2Y receptor signaling share other common features on endothelium protection, such as the regulation of NO production and of pro-inflammatory cell adhesion molecules.(94-96) In many instances, these roles seem to rely on the enzymatic ATP hydrolase activity of ecto-F1-ATPase, which generates extracellular ADP. Thus, it is tempting to propose a common framework that would involve the sequential activation of ecto-F1-ATPase by HDL- apoA-I, modulation of the extracellular ATP/ADP ratio and of downstream activation/inactivation of P2Y-mediated signalling pathways.(97)

As described, the metabolism of the HDL particle is a multistep process involving several apolipoproteins, enzymes, and transporters; therefore, genetic variation in genes regulating each of these steps will greatly affect HDL-C concentrations.(98) Mendelian disorders of high and low HDL-C levels have provided clues about the biology of HDL.(99) Candidate gene studies have identified Mendelian causes of low HDL-C levels, including mutations in ABCA1, APOA1, and lecithin-cholesterol acyltransferase (LCAT). Conversely, mutations in CETP and endothelial lipase (LIPG) result in high HDL-C levels. (100) Our microbiota has been linked to intestinal health, immune function, bioactivation of nutrients and vitamins, and recently, complex disease phenotypes, such as obesity and insulin resistance.(101) Interestingly, recent studies showed that intestinal microbial processing of dietary choline to trimethylamine, which is further metabolized to trimethylamineoxide by flavin monoxygenases in human and rodent livers, was significantly correlated with

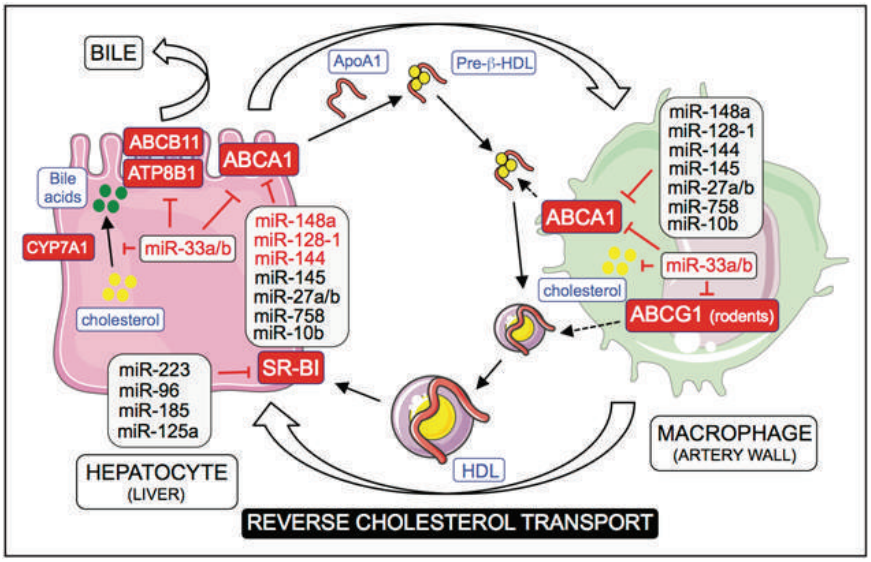

Figure 5. Micro-RNA (miRNA) regulation of high-density lipoprotein (HDL) metabolism and reverse cholesterol transport.(82) (Adapted with permission from American Heart Association).

CVD.(102,103) Furthermore, previous study found that trimethylamineoxide suppressed RCT via an intestinal microbiota-dependent mechanism *in vivo*.(104) These findings suggest a new concept that specific combinations of intestinal microbiota and host genetics may provide cardiometabolic regulation.(105)showed that intestinal microbial processing of dietary choline to trimethylamine, which is further metabolized to trimethylamineoxide by flavin monoxygenases in human and rodent livers, was significantly correlated with CVD.(102,103) Furthermore, previous study found that trimethylamineoxide suppressed RCT via an intestinal microbiota-dependent mechanism *in vivo*.(104) These findings suggest a new concept that specific combinations of intestinal microbiota and host genetics may provide cardiometabolic regulation.(105)

Functional and Dysfuctional HDL

HDL-C has direct effects on numerous cell types that influence CVD and metabolic health. These include endothelial cells, vascular smooth-muscle cells, leukocytes, platelets, adipocytes, skeletal muscle myocytes, and pancreatic cells. The effects of HDL or apoA-I, its major apolipoprotein, occur through the modulation of intracellular calcium, oxygen-derived free-radical production, numerous kinases, and enzymes, including endothelial nitric-oxide synthase (eNOS). ApoA-I and HDL also influence gene expression, particularly genes encoding mediators of inflammation in vascular cells.(106) There are several well-documented HDL functions such as RCT, inhibition of inflammation, or inhibition of platelet activation that may account for the atheroprotective effects of this lipoprotein. Mechanistically, these functions are carried out by a direct interaction of HDL particle or its components with receptors localized on the cell surface followed by generation of intracellular signals. Several HDL-associated receptor ligands such as apoA-I or S1P have been identified in addition to HDL holoparticles, which interact with surface receptors such as ABCA1; S1P receptor types 1, 2, and 3 (S1P1, S1P2, and S1P3); or SR-BI and activate intracellular signaling cascades encompassing kinases, phospholipases, trimeric and small G-proteins, and cytoskeletal proteins such as actin or junctional protein such as connexin. In addition, depletion of plasma cell cholesterol mediated by ABCA1, ABCG1, or SR-BI was demonstrated to indirectly inhibit signaling over proinflammatory or proliferation-stimulating receptors such as Toll-like or growth factor receptors.(107)

Several well-documented functions of HDLs and apoA-I have the potential to protect against cardiovascular disease. The most extensively studied of these relates to the ability of HDLs to promote efflux of cholesterol from macrophages in the artery wall.(108) HDLs also inhibit vascular inflammation (109,110) and has antioxidant (109) and antithrombotic properties (95). They enhance endothelial function (111), promote endothelial repair (112,114), increase agiogenesis (114), suppress the production and mobilization of monocytes and neutrophils from bone marrow (115), and have recently been reported to have antidiabetic properties (55,116). It has been hypothesized that paraoxonase/arylesterase 1 (PON1) located on HDL possesses the capacity to hydrolyze lipid hydroperoxides and is largely responsible for the antioxidant effect of HDL. Transgenic animal evidence and clinical epidemiology strongly support an antiatherogenic role for PON1.

Direct *in vitro* evidence for the PON1 antioxidant hypothesis has proved controversial, and other HDL components have been proposed to account for the antioxidant capacity of HDL, such as apoAI and apoM. These and other HDL components may interact with PON1 to produce its antioxidant effects. The environment provided for this interaction by HDL may be critical.(117) The increasing evidence that HDL not only augments hypoxia-mediated angiogenesis but also inhibits inflammatory driven neovascularization. This suggests that the regulation of angiogenesis by HDL is dependent on the pathophysiological context. One previous example of this was demonstrated in a study using the apoA-I mimetic peptide, D-4F. This peptide significantly increased the vascular expression and activity of haeme oxygenase-1 (HO-1).(118) HO-1 is induced by hypoxia to facilitate angiogenesis in response to ischaemia, but conversely also inhibits leucocyte infiltration by suppressing cytokine expression and consequently reduces inflammation-mediated neovascularization.(119) Studies recently confirmed these observations by directly comparing the effects of apoA-I/rHDL on angiogenesis in both hypoxic/ischaemic and inflammatory conditions. Mechanistically, the key to the conditional regulation of angiogenesis by HDL may be vascular endothelial growth factor (VEGF). VEGF uniquely contains both HIF-1α and NF-κB response elements in its promoter region and is therefore stimulated under both hypoxic and inflammatory conditions. HDL stabilizes hypoxia-inducible factor (HIF)-1α in hypoxia via modulation of its post-translation regulation, and in inflammation has striking inhibitory effects on each stage of the NF-κB activation pathway.(120)

The antithrombotic properties of native HDL are also related to the suppression of the coagulation cascade and stimulation of clot fibrinolysis. Furthermore, HDL stimulates the endothelial production of nitric oxide and prostacyclin, which are potent inhibitors of platelet activation. Thus, HDL's antithrombotic actions are multiple and therefore, raising HDL may be an important therapeutic strategy to reduce the risk of arterial and venous thrombosis.(121) There is now convincing evidence that HDL modulates glucose metabolism in multiple tissues. These actions have deepened our understanding of the pathophysiology of a variety of disease states associated with low or dysfunctional HDL. While there are still many unanswered questions relating to the underlying mechanisms and key HDL component(s) responsible for the metabolic effects, this opens up the possibility of targeting glucose metabolism with HDL therapeutics currently in development. Future preclinical investigations and clinical trials will determine the relevance of HDL-mediated modulation of glucose metabolism to both glycemic control as well as tissue glucose supply to vital organs including the heart and the brain, especially under ischemic conditions.(122)

HDLs and the main HDL apolipoprotein, apolipoprotein (apo)A-I, increase insulin synthesis and secretion in pancreatic β-cells. HDL apolipoproteins increase insulin synthesis and secretion in pancreatic β-cells by a mechanism similar to that of the intestinally derived, endogenous incretins, glucose-dependent insulinotropic peptide (GLP-1) and gastric inhibitory polypeptide (GIP).(123) Systemic and vascular inflammation has been proposed to convert HDL to a dysfunctional form that has impaired anti-atherogenic effects. A loss of anti-inflammatory and antioxidative proteins, perhaps in combination with a gain of proinflammatory proteins, might be another important component in rendering HDL dysfunctional. The proinflammatory enzyme myeloperoxidase induces both oxidative modification and nitrosylation of specific residues on plasma and arterial apoA-I to render HDL dysfunctional, which results in impaired ABCA1 macrophage transport, the activation of inflammatory pathways, and an increased risk of CAD.(16) Figure 6 shows the acute-phase of HDL.

The development of monoclonal antibodies that identify specific forms of dysfunctional apoA-I is a promising area of investigation for monitoring pathophysiological

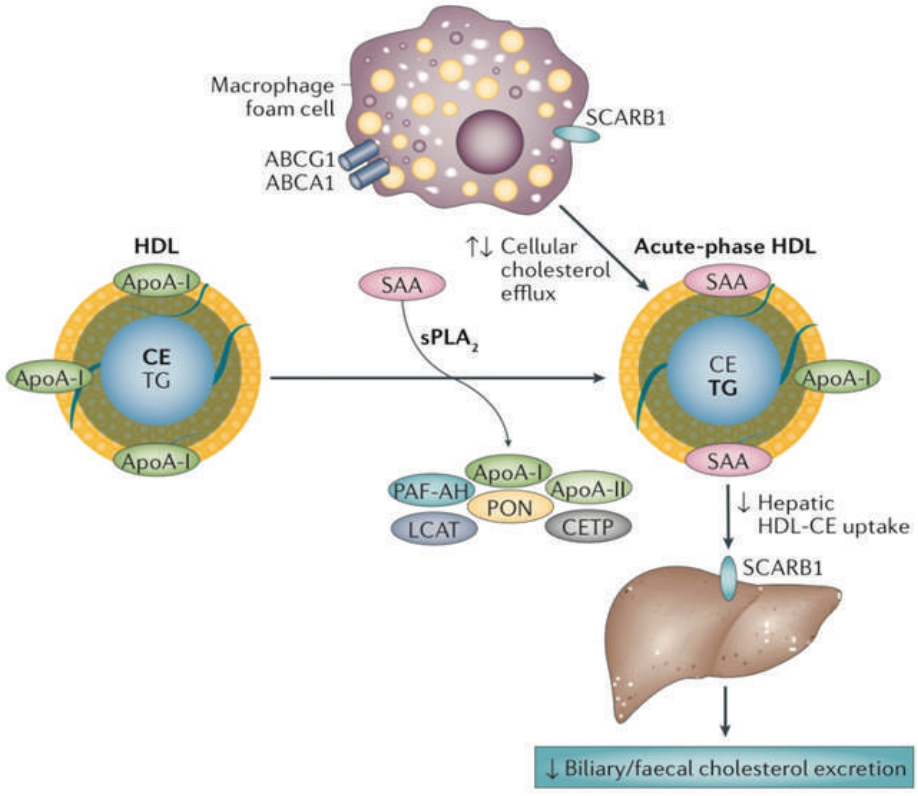

Figure 6. Acute-phase HDL. HDL undergoes substantial modification during an acute-phase response.(16) (Adapted with permission from Springer).

processes within the arterial wall and plasma, and for the evaluation of HDL/apoA-I therapies that are directed at mitigating the proatherogenic effects of dysfunctional HDL. As further research expands our knowledge in this arena, testing of HDL function could allow for better clinical risk stratification, optimization of individual clinical treatments, and the evaluation of novel therapeutics.(16)

The Changing Face of HDL and The Best Way to Measure It

It is now well established from large-scale epidemiologic studies that increased plasma levels of HDL-C and LDL-C are associated with decreased and increased cardiovascular risk, respectively.(124) Consequently, HDL-C and LDL-C are routinely used as serum biomarkers for assessing an individual's cardiovascular disease (CVD) risk.(125) Recent studies have questioned whether HDL-C is only a biomarker and is not mechanistically linked to cardiovascular protection.(126) While a central focus has been placed on the role of HDL in the RCT process, our appreciation for the other cardioprotective properties of HDL continues to expand with further investigation into the structure and function of HDL and its specific subfractions. Development of novel assays is empowering the research community to assess different aspects of HDL function, which at some point may evolve into new diagnostic tests.(127)

The generation of different HDL subfractions throughout the RCT pathway is demonstrative of the size, compositional, and functional diversity of HDL. In addition to altering the size of the HDL subfractions, integration of cholesteryl esters and triglycerides fundamentally impacts the cardioprotective properties of HDL.(53,128) Further, the makeup of HDL subfractions is dynamic, as different lipolytic enzymes, lipid transporters, and apolipoprotein exchange mechanisms (with adjacent circulating lipoproteins and tissues) contribute to the formation and remodeling of HDL subfractions.(65) This also illustrates how simply measuring plasma HDL-C concentrations may not fully capture the impact of HDL on cholesterol flux between tissues, or its larger effect on CVD.

A common methodology for assessing the antiatherogenic functionality of HDL is measuring CEC, or the ability of HDL to initiate the RCT pathway by accepting cholesterol from lipid-laden macrophages.(34,129) The methodology for determining CEC, first validated in a large clinical trial by Khera, *et al*., involves quantification of total cholesterol efflux from macrophages with apoB-depleted serum, and demonstrated CEC as a strong inverse predictor of coronary artery disease status independent of HDL-C concentration.(34) CEC quantified with macrophages *in vitro* is a much better predictor of prevalent CVD than is HDL-C.(34,130) In 2 different cohorts, cholesterol efflux capacity strongly and negatively associated with CVD status.(34) This relationship remained highly significant after correction for HDL-C. HDL-C accounted for only 34% of the variance in efflux capacity, indicating that HDL-C is not a major determinant of cholesterol efflux capacity. (34,130)

Two recent studies provided strong evidence that impaired CEC is also a strong predictor of incident CVD. In the Dallas Heart Study, impaired efflux capacity was the strongest predictor of future CVD events in a large cohort of multiethnic healthy subjects.(13) Similar results were reported in the European Prospective Investigation of Cancer (EPIC)-Norfolk study.(14) In both studies, impaired CEC remained a strong predictor of future events after adjusting for other risk factors, including HDL-C and LDL-C, suggesting that this metric provides clinically valuable information that is independent of traditional lipid risk factors.(131) Given the disappointment so far in developing new HDL-C-targeted drugs for treatment of CVD, there has been great interest in determining whether another HDL metric may better capture its potential antiatherogenic effects. A functional assay assessing HDL-promoted cholesterol efflux has garnered interest, and may be a convenient way to assess HDL function in RCT. In this section, we also discuss other potential functions of HDL and novel qualitative and quantitative assays. HDL is known to bind over 80 different types of proteins and transports more than 100 different species of lipids, so it likely has other functions outside RCT.(132,133) Finally, the function of HDL is highly associated with its com-osition, so we also discuss methodologies for identifying HDL-associated proteins, lipids, and physical properties.

There is also good evidence that the concentration of HDL, HDL particle number (HDL-P), provides clinically useful information that is distinct from HDL-C. Two methods, NMR spectroscopy (134) and ion mobility (IM) (135), have been used to quantify HDL-P. However, these methods give different estimates of HDL-P concentration and size.(134,135) In future studies, it will be critical for investigators to validate the quantification of HDL-P by NMR and ion mobility (IM). At present, NMR and IM are the only available methods for ascertaining HDL-P. (10) In some recent studies, HDL-P concentration has emerged as a predictor of CVD risk that may be superior

to that of HDL-C in both population studies (136,137) and randomized, clinical trials of lipid-modifying therapies. (46,138,139) In the Multi-Ethnic Study of Atherosclerosis (MESA), low HDL-P predicted higher risk of elevated carotid intima-medial thickness regardless of whether the baseline HDL-C level was high (≥55 mg/dL) or low (<42 mg/dL).(137) In the Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER), HDL-P was a better marker of residual risk in statin-treated patients than chemically measured HDL-C, apoA-I, or average HDL size.(139)

Evaluation of antioxidant effects of HDL has encompassed several functional methods that evaluate the efficiency of HDL particles to protect LDL against oxidative modification. LDL lipid peroxidation by free radicals the presence of HDL proceeds in a 2-step process that involves a slow rate of conjugated diene accumulation ascribed to the presence of antioxidants including those in HDL and a second rapid phase that is principally dependent on the antioxidative functionality of HDL. (15) An alternative method for evaluating the antioxidant effects of HDL is the cell-free assay. The cell-free assay or the HDL-oxidized 1-palmitoyl- 2-arachidonoyl-sn-glycero-3-phosphorylcholine (OxPAPC) assay measures the ability of plasma HDL to reduce formation of oxidized phospholipids.(140-142) The addition of the fluorochrome 2',7'-dichlorofluorescein produces a fluorescent signal that depends on the concentration of OxPAPC *in vitro*.

Some candidate biomarkers associated with metrics that reflect HDL functions have recently emerged. They fall into three categories: size and quantitative features such as HDL particles size and number (51,143), markers of HDL properties such as HDL-associated sphingosine-1-phosphate that contributes to the cytoprotective activity of HDL and endothelial nitric oxide production (144-148) and markers of hepatobiliary RCT such as CEC, HDL turnover and hepatic HDL uptake (13,14,149) Among HDL cellular partners, the ecto-F1-ATPase is a receptor for apoA-I that contributes to several HDL atheroprotective properties, including cellular uptake of HDL and endothelial cell protection.(97) Thus, indicators of ecto-F1-ATPase activity might have the potential to cover several categories of HDL-related biomarkers.(150)

Circulating inhibitory factor 1 (IF1) is strongly and independently associated with mortality in coronary artery disease patients but, in the same population, HDL-C is not. (151) According to our hypotheses, the IF1 level assesses metabolic and vascular HDL protective properties by reflecting the ecto-F1-ATPase/P2Y pathway activation by apoA-I, both in hepatic reverse cholesterol transport and vascular endothelium protection. In addition, serum IF1 might also reflect myocardial function, which can explain its correlation with heart rate and left ventricular ejection fraction (LVEF). F1 thus joins other emerging biomarkers that have proven to be better than HDL-C level for evaluating cardiovascular risk and determining pharmacotherapy, such as HDL CEC or HDL particles number. IF1 can be used in a panel of biomarkers to better stratify cardiovascular risk and to set treatment.

The challenge is to develop laboratory assays that quantify the various HDL functions that may improve CVD risk assessment and augment the evaluation of HDL-modifying therapies. Efforts to develop reproducible, cost-effective, validated assays that measure the potentially protective functions of HDL are now recognized as a major challenge for the cardiovascular field. Currently, there is no consensus concerning the HDL functions that should be targeted, nor are there standardized assays to measure HDL function as a tool to improve either CVD risk assessment or the assessment of therapeutic interventions. Another challenge is to validate measurements of HDL particles to be able to standardize assays of function with HDL quantification.(15)

The Development Of Future HDL-Targeted Therapies

In population studies, HDL-C is inversely related to the risk of myocardial infarction and death.(152-155) Of note, in patients fully treated according to current guidelines with intense statin therapy and LDL-C at target levels, HDL-C remains predictive of outcome for major adverse cardiovascular events.(6) Unfortunately, it has been proven difficult to reduce coronary risk with drugs increasing HDL-C, such as brates, niacin, or inhibitors of CETP, beyond that achieved with statin therapy alone. (2,5,156,157) Moreover, in several inborn errors of human HDL metabolism and genetic mouse models with altered HDL metabolism, the changes in HDL-C levels were not associated with accompanying changes in cardiovascular risk or atherosclerotic plaque load, respectively, as has been expected from epidemiological studies.(35,158,159) Thus, the pathogenic role and, suitability of HDL as a therapeutic target has increasingly been questioned. In fact, it has been argued that low HDL-C may only represent a marker for proatherogenic risk factors, rather than HDL being a mediator protecting against atherogenesis (Figure 7).(160)

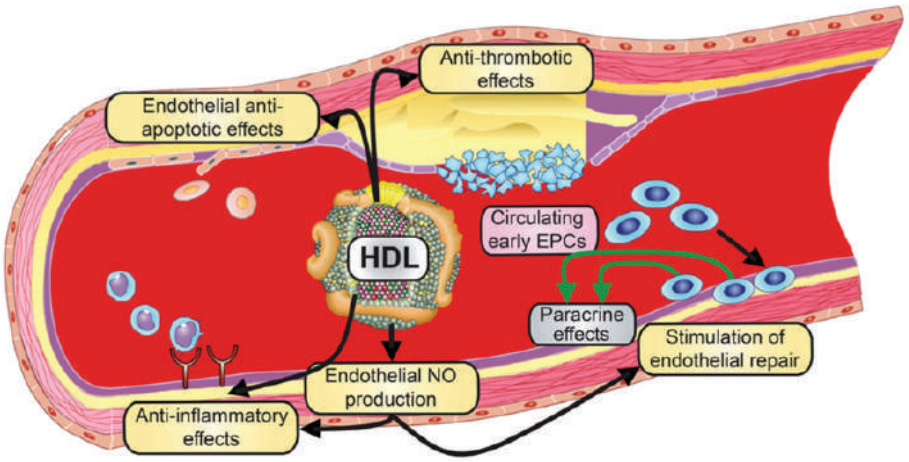

Figure 7. Proposed direct vascular protective and potentially antiatherogenic effects of normal high-density lipoprotein (HDL). (160) (Adapted with permission from American Heart Association).

One argument to explain the HDL controversy is that HDL-C concentration is a poor measure for targeted intervention. HDL-C concentration is considered a surrogate for the efficiency of cholesterol efflux from tissues. However, given that macrophage-derived cholesterol represents only a minor proportion of the cholesterol transported by HDL particles, this may be an inadequate measure. Moreover, HDL-C concentration is a static measurement, and does not take into account the dynamics of the HDL particle population and HDL functionality, which might differ depending on the metabolic status of individuals. For instance, patients with type 2 diabetes display a higher catabolic rate of HDL-ApoA-I (148) and HDL from coronary patients does not have endothelial anti-inflammatory effects (161), illustrating the need to identify more precisely the patient subgroups that should benefit from personalized HDL therapies.(162) Therefore, it will be important to demonstrate that novel drugs not only increase HDL-C plasma levels but also improve HDL function in patients at high cardiovascular risk.(163)

Although RCT was first postulated to be a major contributor to the causative association between low plasma HDL-C and ischaemic heart disease, numerous other plausible contributing mechanisms have been uncovered. Animal models have been an important vehicle for these discoveries, with more recent investigations providing a clinical context for several antiatherothrombotic actions of HDL. These include antiplatelet (164,165), antioxidative (166), anti-inflammatory (167), anti-apoptotic (168,169) and vasodilatory (111,170,171) activities, as well as effects on glucose metabolism (55,172-174). These mechanisms have the potential to act at multiple stages throughout the development of atherothrombosis. Via these mechanisms, HDL-targeted therapies could both prevent the formation and reduce the progression of plaques by slowing the accumulation of cholesterol in the artery wall and also by stabilizing inflamed plaques that are vulnerable to rupture. (175)

Several strategies to therapeutically target the metabolism, particle structure and function of HDL are emerging. These include ApoA1-mimetic peptides, agonists of the liver X receptor (LXR), agonists of the farnesoid X receptor (FXR), inhibitors of LIPG, antagonists of microRNAs (miRNAs) and antisense oligonucleotides (ASOs) targeted to genes that are implicated in HDL metabolism, including the CETP and ApoC3 genes.(175)

HDL functions reflect the physiological role of the lipoprotein better than HDL-C quantity, the intake of olive oil phenolic compounds resulted in an improvement in CEC, HDL antioxidant defenses, HDL size distribution, and other characteristics related to HDL quality. Olive oil phenolic compounds bound to HDLs, or surrounding the lipoprotein, improve their oxidative/inflammatory status which may justify an increase in HDL functionality. Modifications in HDL composition because of the consumption of virgin olive oil (VOO) might also explain these changes. However, large-scale, randomized controlled trials with VOO-rich dietary interventions are required to definitively confirm the protective role of olive oil phenolic compounds in HDL biological functions.(176)

Nevertheless, HDL has not yet been successfully exploited for therapy. One potential reason for this shortfall is the structural and functional complexity of HDL particles, which carry more than 80 different proteins and more

than 200 lipid species as well as several microRNAs and other potentially bioactive molecules. This physiological heterogeneity is further increased in several inflammatory conditions that increase cardiovascular risk, including coronary artery disease itself but also diabetes mellitus, chronic kidney disease, and rheumatic diseases. The quantitative and qualitative modifications of the proteome and lipidome, as well as the resulting loss of functions or gain of dysfunctions, are not recovered by the biomarker HDL-C. As yet the relative importance of the many physiological and pathological activities of normal and dysfunctional HDL, respectively, for the pathogenesis of atherosclerosis is unknown. The answer to this question, as well as detailed knowledge of structure-function-relationships of HDL-associated molecules, is a prerequisite to exploit HDL for the development of anti-atherogenic drugs as well as of diagnostic biomarkers for the identification, personalized treatment stratification, and monitoring of patients at increased cardiovascular risk.(177)

Conclusion

The clinical utility of HDLs has been scrutinized upon the publication of Mendelian randomization studies showing no effect of HDL-C modifying variants on CVD outcome. The failures of randomized controlled HDL-C-directed intervention trials have further fueled this skepticism. This general criticism originates from oversimplification that has equated 'HDL-C' with 'HDL' and misconceived both as the 'good cholesterol'. HDL particles are heterogeneous and carry hundreds of different lipids, proteins, and microRNAs. Many of them but not cholesterol, that is, HDL-C, contributes to the multiple protective functions of HDLs that probably evolved to manage potentially life-threatening crises. Quantification of HDL particle numbers, distinct proteins or lipids, and modifications thereof as well as bioassays of HDL functionality are currently explored toward their diagnostic performance in risk prediction and monitoring of treatment response. Any successful clinical exploitation of HDLs will depend on the identification of the most relevant (dys)functions and their structural correlates. Stringent or prioritized structure-(dys)function relationships may provide biomarkers for better risk assessment and monitoring of treatment response. The most relevant agonists carried by either functional or dysfunctional HDLs as well as their cellular responders are interesting targets for drug development.

References

1. Rosenson RS. The high-density lipoprotein puzzle. Why classic epidemiology, genetic epifemiology, and clinical trials conflict? Arterioscler Vasc Thromb Biol. 2016; 36: 777-82.
2. Boden WE, Probsteld JL, Anderson T, Chaitman BR, Desvignes-Nickens P, Koprowicz K, *et al.* Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy. N Engl J Med. 2011; 365: 2255-67.
3. Landray MJ, Haynes R, Hopewell JC, Parish S, Aung T, Tomson J, *et al.* Effects of extended-release niacin with laropiprant in high-risk patients. N Engl J Med. 2014; 371: 203-12.
4. Anderson TJ, Boden WE, Desvigne-Nickens P, Fleg JL, Kashyap ML, McBride R, *et al.* Safety profile of extended-release niacin in the AIM-HIGH trial. N Engl J Med. 2014; 371: 288-90.
5. Schwartz GG, Olsson AG, Abt M, Ballantyne CM, Barter PJ, Brumm J, *et al.* Effects of dalcetrapib in patients with a recent acute coronary syndrome. N Engl J Med. 2012; 367: 2089-99.
6. Barter PJ, Cauleld M, Eriksson M, Grundy SM, Kastelein JJ, Komajda M, *et al.* Effects of torcetrapib in patients at high risk for coronary events. N Engl J Med. 2007; 357: 2109-22.
7. PRNewswire [Internet]. Lilly Provides Update on Evacetrapib Phase 3 Trial [updated 2015 Jul 27; cited 2018 Jan 12]. Available from: https://www.prnewswire.com/news-releases/lilly-provides-update-on-evacetrapib-phase-3-trial-300118811.html
8. Nicholls SJ, Ruotolo G, Brewer HB, Kane JP, Wang MD, Krueger KA, *et al.* Cholesterol efflux capacity and pre-beta-1 HDL concentrations are increased in dyslipidemic patients treated with evacetrapib. J Am Coll Cardiol. 2015; 66: 2201-10.
9. Rosenson RS, Brewer HB Jr. New challenges for HDL-modifying therapies as a strategy to lower cardiovascular disease events in statin-treated patients. Cardiovasc Drugs Ther. 2015; 29: 1-3.
10. Rosenson RS, Brewer HB Jr, Chapman MJ, Fazio S, Hussain MM, Kontush A, *et al.* HDL measures, particle heterogeneity, proposed nomenclature, and relation to atherosclerotic cardiovascular events. Clin Chem. 2011; 57: 392-410.
11. Rosenson RS, Brewer HB Jr, Davidson WS, Fayad ZA, Fuster V, Goldstein J, *et al.* Cholesterol efflux and atheroprotection: advancing the concept of reverse cholesterol transport. Circulation. 2012; 125: 1905-19.
12. Camont L, Lhomme M, Rached F, Le Goff W, Nègre-Salvayre A, Salvayre R, *et al.* Small, dense high-density lipoprotein-3 particles are enriched in negatively charged phospho-lipids: relevance to cellular cholesterol efflux, antioxidative, antithrombotic, anti-inflammatory, and antiapoptotic functionalities. Arterioscler Thromb Vasc Biol. 2013; 33: 2715-23.
13. Rohatgi A, Khera A, Berry JD, Givens EG, Ayers CR, Wedin KE, *et al.* HDL cholesterol efflux capacity and incident cardiovascular events. N Engl J Med. 2014; 371: 2383-93.
14. Saleheen D, Scott R, Javad S, Zhao W, Rodrigues A, Picataggi A, *et al.* Association of HDL cholesterol efflux capacity with incident coronary heart disease events: a prospective case-control study. Lancet Diabetes Endocrinol. 2015; 3: 507-13.
15. Rosenson RS, Brewer HB Jr, Ansell B, Barter P, Chapman MJ, Heinecke JW, *et al.* Translation of high-density lipoprotein function into clinical practice: current prospects and future challenges. Circulation. 2013; 128: 1256-67.
16. Rosenson RS, Brewer HB Jr, Ansell BJ, Barter P, Chapman MJ, Heinecke JW, *et al.* Dysfunctional HDL and atherosclerotic cardiovascular disease. Nat Rev Cardiol. 2016; 13: 48-60.

17. Huang Y, DiDonato JA, Levison BS, Schmitt D, Li L, Wu Y, *et al.* An abundant dysfunctional apolipoprotein A1 in human atheroma. Nat Med. 2014; 20: 193-203.
18. Shao B, Tang C, Sinha A, Mayer PS, Davenport GD, Brot N, *et al.* Humans with atherosclerosis have impaired ABCA1 cholesterol efflux and enhanced high-density lipoprotein oxidation by myeloperoxidase. Circ Res. 2014; 114: 1733-42.
19. Rader DJ, Alexander ET, Weibel GL, Billheimer J, Rothblat GH. The role of reverse cholesterol transport in animals and humans and relationship to atherosclerosis. J Lipid Res. 2009; 50(Suppl): S189-94.
20. Rader DJ, Tall AR. Is it time to revise the HDL cholesterol hypothesis? Nat Med 2012; 18: 1344-6.
21. Shah AS, Tan L, Long JL, Davidson WS. Proteomic diversity of high density lipoproteins: our emerging understanding of its importance in lipid transport and beyond. J Lipid Res. 2013; 54: 2575-85.
22. Toth PP, Barter PJ, Rosenson RS, Boden WE, Chapman MJ, Cuchel M, *et al.* High-density lipoproteins: a consensus statement from the National Lipid Association. J Clin Lipidol. 2013; 7: 484-525.
23. Heinecke JW. Small HDL promotes cholesterol efflux by the ABCA1 pathway in macrophages: implications for therapies targeted to HDL. Circ Res. 2015; 116: 1101-3.
24. Otvos JD. Measurement of lipoprotein subclass profiles by nuclear magnetic resonance spectroscopy. Clin Lab. 2002; 48 : 171-80.
25. Caulfield MP, Li S, Lee G, Blanche PJ, Salameh WA, Benner WH, *et al.* Direct determination of lipoprotein particle sizes and concentrations by ion mobility analysis. Clin Chem. 2008; 54: 1307-16.
26. Hutchins PM, Ronsein GE, Monette JS, Pamir N, Wimberger J, He Y, *et al.* Quantification of HDL particle concentration by calibrated ion mobility analysis. Clin Chem. 2014; 60: 1393-401.
27. Ronsein GE, Heinecke JW. Time to ditch HDL-C as a measure of HDL function? Curr Opin Lipidol. 2017; 28: 414-8.
28. Vaisar T, Tang C, Babenko I, Hutchins P, Wimberger J, Suffredini AF, *et al.* Inflammatory remodeling of the HDL proteome impairs cholesterol efflux capacity. J Lipid Res. 2015; 56: 1519-30.
29. Ronsein GE, Reyes-Soffer G, He Y, Oda M, Ginsberg H, Heinecke JW. Targeted proteomics identifies paraoxonase/arylesterase 1 (PON1) and apolipoprotein Cs as potential risk factors for hypoalphalipoproteinemia in diabetic subjects treated with fenofibrate and rosiglitazone. Mol Cell Proteomics. 2016; 15: 1083-93.
30. Marsillach J, Becker JO, Vaisar T, Hahn BH, Brunzell JD, Furlong CE, *et al.* Paraoxonase-3 is depleted from the high-density lipoproteins of autoimmune disease patients with subclinical atherosclerosis. J Proteome Res. 2015; 14: 2046-54.
31. Gordon SM, Davidson WS, Urbina EM, Dolan LM, Heink A, Zang H, *et al.* The effects of type 2 diabetes on lipoprotein composition and arterial stiffness in male youth. Diabetes. 2013; 62: 2958-67.
32. Ronsein GE, Pamir N, von Haller PD, Kim DS, Oda MN, Jarvik GP, *et al.* Parallel reaction monitoring (PRM) and selected reaction monitoring (SRM) exhibit comparable linearity, dynamic range and precision for targeted quantitative HDL proteomics. J Proteomics. 2015; 113: 388-99.
33. Henderson CM, Vaisar T, Hoofnagle AN. Isolating and quantifying plasma HDL proteins by sequential density gradient ultracentrifugation and targeted proteomics. Methods Mol Biol. 2016; 1410: 105-20.
34. Khera AV, Cuchel M, de la Llera-Moya M, Rodrigues A, Burke MF, Jafri K, *et al.* Cholesterol efflux capacity, high-density lipoprotein function, and atherosclerosis. New Engl J Med. 2011; 364: 127-35.
35. Voight BF, Peloso GM, Orho-Melander M, Frikke-Schmidt R, Barbalic M, Jensen MK, *et al.* Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study. Lancet. 2012; 380: 572-80.
36. von Eckardstein A, Rohrer L. HDLs in crises. Curr Opin Lipidol. 2016; 27: 264-73.
37. Chang TI, Streja E, Moradi H. Could high-density lipoprotein cholesterol predict increased cardiovascular risk? Curr Opin Endocrinol Diabetes Obes. 2017; 24: 140-7.
38. Fazio S, Pamir N. HDL particle size and functional heterogeneity. Circ Res. 2016; 119: 704-7.
39. Camont L, Chapman MJ, Kontush A. Biological activities of HDL subpopulations and their relevance to cardiovascular disease. Trends Mol Med. 2011; 17: 594-603.
40. Kuller L, Arnold A, Tracy R, Otvos J, Burke G, Psaty B, *et al.* Nuclear magnetic resonance spectroscopy of lipoproteins and risk of coronary heart disease in the Cardiovascular Health Study. Arterioscler Thromb Vasc Biol. 2002; 22: 1175-80.
41. Rosenson RS, Otvos JD, Freedman DS. Relations of lipoprotein subclass levels and low-density lipoprotein size to progression of coronary artery disease in the Pravastatin Limitation of Atherosclerosis in the Coronary Arteries (PLAC-I) trial. Am J Cardiol. 2002; 90: 89-94.
42. Garvey WT, Kwon S, Zheng D, Shaughnessy S, Wallace P, Hutto A, *et al.* Effects of insulin resistance and type 2 diabetes on lipoprotein subclass particle size and concentration determined by nuclear magnetic resonance. Diabetes Metab Res Rev. 2003; 52: 453-62.
43. Festa A, Williams K, Hanley AJG, Otvos JD, Goff DC, Wagenknecht LE, *et al.* Nuclear magnetic resonance lipoprotein abnormalities in prediabetic subjects in the insulin resistance atherosclerosis study. Circulation. 2005; 111: 3465-72.
44. Goff DC Jr, D'Agostino RB Jr, Haffner SM, Otvos JD. Insulin resistance and adiposity influence lipoprotein size and subclass concentrations. Results from the insulin resistance atherosclerosis study. Metabolism. 2005; 54: 264-70.
45. Kathiresan S, Otvos JD, Sullivan LM, Keyes MJ, Schaefer EJ, Wilson PW, et al. Increased small low-density lipoprotein particle number: a prominent feature of the metabolic syndrome in the Framingham Heart Study. Circulation. 2006; 113: 20-9.
46. Otvos JD, Collins D, Freedman DS, Shalaurova I, Schaefer EJ, Mcnamara JR, *et al.* Low-density lipoprotein and high-density lipoprotein particle subclasses predict coronary events and are favorably changed by gemfibrozil therapy in the Veterans Affairs High-Density Lipoprotein Intervention Trial. Circulation. 2006; 113: 1556-63.
47. Mora S, Szklo M, Otvos JD, Greenland P, Psaty BM, Goff DC, *et al.* LDL particle subclasses. LDL particle size, and carotid atherosclerosis in the Multi-Ethnic Study of Atherosclerosis (MESA). Atherosclerosis. 2007; 192: 211-7.
48. Mora S, Otvos JD, Rifai N, Rosenson RS, Buring JE, Ridker PM. Lipoprotein particle profiles by nuclear magnetic resonance compared with standard lipids and apolipoproteins in predicting incident cardiovascular disease in women. Circulation. 2009; 119: 931-9.
49. van der Steeg WA, Holme I, Boekholdt SM, Larsen ML, Lindahl C, Stroes ES, *et al.* High-density lipoprotein cholesterol, high-density lipoprotein particle size, and apolipoprotein A-I: significance for cardiovascular risk: the IDEAL and EPIC-Norfolk studies. J Am Coll Cardiol. 2008; 51: 634-42.
50. El Harchaoui K, Arsenault BJ, Franssen R, Despres JP, Hovingh GK, Stroes ES, *et al.* High-density lipoprotein particle size and concentration and coronary risk. Ann Intern Med. 2009; 150: 84-93.

51. Kontush A. HDL particle number and size as predictors of cardiovascular disease. Front Pharmacol. 2015; 6: 218. doi: 10.3389/fphar.2015.00218.
52. Kontush A, Lhomme M, Chapman MJ. Unraveling the complexities of the HDL lipidome. J Lipid Res. 2013; 54: 2950-63.
53. Pirillo A, Norata GD, Catapano AL. High-density lipoprotein subfractions – What the clinicians need to know. Cardiology. 2013; 123: 116-25.
54. Norata GD, Pirillo A, Ammirati E, Catapano AL. Emerging role of high density lipoproteins as a player in the immune system. Atherosclerosis. 2012; 220: 11–21.
55. Drew BG, Rye KA, Duffy SJ, Barter P, King-well BA. The emerging role of HDL in glucose metabolism. Nat Rev Endocrinol. 2012; 8: 237-45.
56. Singh SA, Andraski AB, Pieper B, Goh W, Mendivil CO, Sacks FM, *et al.* Multiple apolipoprotein kinetics measured in human HDL by high-resolution/accurate mass parallel reaction monitoring. J Lipid Res. 2016; 57: 714-28.
57. Singh SA, Miyosawa K, Aikawa M. Mass spectrometry meets the challenge of understanding the complexity of the lipoproteome: recent findings regarding proteins involved in dyslipidemia and cardiovascular disease. Exp Rev Proteomics. 2015; 12: 519-32.
58. Singh SA, Aikawa M. Unbiased and targeted mass spectrometry for the HD: proteome. Curr Opin Lipidol. 2017; 28: 68. doi: 10.1097/mol.0000000000000374.
59. Rye KA, Barter PJ. Regulation of high-density lipoprotein metabolism. Circ Res. 2014; 114: 143-56.
60. Stoffel W, Krüger E, Deutzmann R. Cell-free translation of human liver apolipoprotein AI and AII mRNA. Processing of primary translation products. Hoppe Seylers Z Physiol Chem. 1983; 364: 227-37.
61. Chau P, Fielding PE, Fielding CJ. Bone morphogenetic protein-1 (BMP-1) cleaves human proapolipoprotein A1 and regulates its activation for lipid binding. Biochemistry. 2007; 46: 8445-50.
62. Zhu J, Gardner J, Pullinger CR, Kane JP, Thompson JF, Francone OL. Regulation of apoAI processing by procollagen C-proteinase enhancer-2 and bone morphogenetic protein-1. J Lipid Res. 2009; 50: 1330-9.
63. Gillard BK, Lin HY, Massey JB, Pownall HJ. Apolipoproteins A-I, A-II and E are independently distributed among intracellular and newly secreted HDL of human hepatoma cells. Biochim Biophys Acta. 2009; 1791: 1125-32.
64. Kiss RS, McManus DC, Franklin V, Tan WL, McKenzie A, Chimini G, Marcel YL. The lipidation by hepatocytes of human apolipoprotein A-I occurs by both ABCA1-dependent and -independent pathways. J Biol Chem. 2003; 278: 10119-27.
65. Ji A, Wroblewski JM, Cai L, de Beer MC, Webb NR, van der Westhuyzen DR. Nascent HDL formation in hepatocytes and role of ABCA1, ABCG1, and SR-BI. J Lipid Res. 2012; 53: 446-55.
66. Maric J, Kiss RS, Franklin V, Marcel YL. Intracellular lipidation of newly synthesized apolipoprotein A-I in primary murine hepatocytes. J Biol Chem. 2005; 280: 39942-9.
67. Nagata KO, Nakada C, Kasai RS, Kusumi A, Ueda K. ABCA1 dimermonomer interconversion during HDL generation revealed by single- molecule imaging. Proc Natl Acad Sci USA. 2013; 110: 5034-9.
68. Phillips MV. Molecular mechanisms of cellular cholesterol efflux. J Biol Chem. 2014; 289: 24020-9.
69. Kimura T, Sato K, Malchinkhuu E, Tomura H, Tamama K, Kuwabara A, *et al.* High-density lipoprotein stimulates endothelial cell migration and survival through sphingosine 1-phosphate and its receptors. Arterioscler Thromb Vasc Biol. 2003; 23: 1283-8.
70. Nofer JR, van der Giet M, Tölle M, Wolinska I, von Wnuck Lipinski K, Baba HA, *et al.* HDL induces NO-dependent vasorelaxation via the lysophospholipid receptor S1P3. J Clin Invest. 2004; 113: 569-81.
71. Christoffersen C, Obinata H, Kumaraswamy SB, Galvani S, Ahnström J, Sevvana M, *et al.* Endothelium-protective sphingosine-1-phosphate provided by HDL- associated apolipoprotein M. Proc Natl Acad Sci USA. 2011; 108: 9613-8.
72. Galvani S, Sanson M, Blaho VA, Swendeman SL, Obinata H, Conger H, *et al.* HDL-bound sphingosine 1-phosphate acts as a biased agonist for the endothelial cell receptor S1P1 to limit vascular inflammation. Sci Signal. 2015; 8: ra79. doi: 10.1126/scisignal.aaa2581.
73. Wilkerson BA, Grass GD, Wing SB, Argraves WS, Argraves KM. Sphingosine 1-phosphate (S1P) carrier-dependent regulation of endothelial barrier: high density lipoprotein (HDL)-S1P prolongs endothelial barrier enhancement as compared with albumin-S1P via effects on levels, traf cking, and signaling of S1P1. J Biol Chem. 2012; 287: 44645-53.
74. Blaho VA, Galvani S, Engelbrecht E, Liu C, Swendeman SL, Kono M, *et al.* HDL-bound sphingosine-1-phosphate restrains lymphopoiesis and neuroinflammation. Nature. 2015; 523: 342-6.
75. Levkau B. HDL-S1P: cardiovascular functions, disease-associated alterations, and therapeutic applications. Front Pharmacol. 2015; 6: 243. doi: 10.3389/fphar.2015.00243.
76. Galvani S, Hla T. Quality versus quantity. Arterioscler Thromb Vasc Biol. 2017; 37: 1018-9.
77. Esteller A. Physiology of bile secretion. World J Gastroenterol. 2008; 14: 5641-9.
78. Paulusma CC, Folmer DE, Ho-Mok KS, de Waart DR, Hilarius PM, Verhoeven AJ, Oude Elferink RP. ATP8B1 requires an accessory protein for endoplasmic reticulum exit and plasma membrane lipid flippase activity. Hepatology. 2008; 47: 268-78.
79. Paulusma CC, Groen A, Kunne C, Ho-Mok KS, Spijkerboer AL, Rudi de Waart D, *et al.* Atp8b1 deficiency in mice reduces resistance of the canalicular membrane to hydrophobic bile salts and impairs bile salt transport. Hepatology. 2006; 44: 195-204.
80. Rottiers V, Näär AM. MicroRNAs in metabolism and metabolic disorders. Nat Rev Mol Cell Biol. 2012; 13: 239-50.
81. Michell DL, Vickers KC. Lipoprotein carriers of micrornas. Biochim Biophys Acta. 2016; 1861(12 Pt B): 2069-74.
82. Canfran-Duque A, Lin CS, Goedeke L, Suarez Y, Fernandez-Hernando C. Micro-RNAs and high-density lipoprotein metabolism. Arterioscler Thromb Vasc Biol. 2016; 36: 1076-84.
83. Martinez LO, Jacquet S, Esteve JP, Rolland C, Cabezón E, Champagne E, *et al.* Ectopic beta-chain of ATP synthase is an apolipoprotein A-I receptor in hepatic HDL endocytosis. Nature. 2003; 421: 75-9.
84. Jacquet S, Malaval C, Martinez LO, Sak K, Rolland C, Perez C, *et al.* The nucleotide receptor P2Y13 is a key regulator of hepatic high-density lipoprotein (HDL) endocytosis. Cell Mol Life Sci. 2005; 62: 2508-15.
85. Goffinet M, Tardy C, Boubekeur N, Cholez G, Bluteau A, Oniciu DC, *et al.* P2Y13 receptor regulates HDL metabolism and atherosclerosis in vivo. PLoS One. 2014; 9: e95807. doi: 10.1371/journal.pone.0095807.
86. Fabre AC, Malaval C, Ben Addi A, Verdier C, Pons V, Serhan N, *et al.* P2Y13 receptor is critical for reverse cholesterol transport. Hepatology. 2010; 52: 1477-83.
87. Serhan N, Cabou C, Verdier C, Lichtenstein L, Malet N, Perret B, *et al.* Chronic pharmacological activation of P2Y13 receptor in mice decreases HDL-cholesterol level by increasing hepatic HDL uptake and bile acid secretion. Biochim Biophys Acta. 2013; 1831: 719-25.

88. Lichtenstein L, Serhan N, Annema W, Combes G, Robaye B, Boeynaems JM, *et al.* Lack of P2Y13 in mice fed a high cholesterol diet results in decreased hepatic cholesterol content, biliary lipid secretion and reverse cholesterol transport. Nutr Metab. 2013; 10: 67. doi: 10.1186/1743-7075-10-67.
89. Arakaki N, Kita T, Shibata H, Higuti T. Cell-surface Hþ-ATP synthase as a potential molecular target for anti-obesity drugs. FEBS Lett. 2007; 581: 3405-9.
90. Howard AD, Verghese PB, Arrese EL, Soulages JL. The beta-subunit of ATP synthase is involved in cellular uptake and resecretion of apoA-I but does not control apoA-I-induced lipid efflux in adipocytes. Mol Cell Biochem. 2011; 348: 155-64.
91. Lee H, Jun DJ, Suh BC, Choi BH, Lee JH, Do MS, *et al.* Dual roles of P2 purinergic receptors in insulin-stimulated leptin production and lipolysis in differentiated rat white adipocytes. J Biol Chem. 2005; 280: 28556-63.
92. Radojkovic C, Genoux A, Pons V, Combes G, de Jonge H, Champagne E, *et al.* Stimulation of cell surface F1-ATPase activity by apolipoprotein A-I inhibits endothelial cell apoptosis and promotes proliferation. Arterioscler Thromb Vasc Biol. 2009; 29: 1125-30.
93. Cavelier C, Ohnsorg PM, Rohrer L, von Eckardstein A. The beta-chain of cell surface F0F1 ATPase modulates ApoA-I and HDL transcytosis through aortic endothelial cells. Arterioscler Thromb Vasc Biol. 2012; 32: 131-9.
94. Tran-Dinh A, Diallo D, Delbosc S, Varela-Perez LM, Dang Q, Lapergue B, *et al.* HDL and endothelial protection. Br J Pharmacol. 2013; 169: 493-511.
95. Mineo C, Deguchi H, Griffin JH, Shaul PW. Endothelial and antithrombotic actions of HDL. Circ Res. 2006; 98: 1352-64.
96. Erlinge D, Burnstock G. P2 receptors in cardiovascular regulation and disease, Purinergic Signal. 2008; 4: 85-7.
97. Martinez LO, Najib S, Perret B, Cabou C, Lichtensein L. Ecto-F1-ATPase/P2Y pathways in metabolic and vascular functions of high density lipoproteins. Atherosclerosis. 2015; 238: 89-100.
98. Edmondson AC, Braund PS, Stylianou IM, Khera AV, Nelson CP, Wolfe ML, *et al.* Dense genotyping of candidate gene loci identifies variants associated with high-density lipoprotein cholesterol. Circ Cardiovasc Genet. 2011; 4: 145-55.
99. Van Capelleveen JC, Bochem AE, Motazacker MM, Hovingh GK, Kastelein JJP. Genetics of HDL-C: a causal link to atherosclerosis? Curr Atheroscler Rep. 2013; 15: 326. doi: 10.1007/s11883-013-0326-8.
100. Tuteja S, Rader DJ. High-density lipoproteins in the prevention of cardiovascular disease: changing the paradigm. Nature. 2014; 96: 48-56.
101. Goodman AL, Gordon JI. Our unindicted coconspirators: human metabolism from a microbial perspective. Cell Metab. 2010; 12:111-6.
102. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, DuGar B, *et al.* Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature. 2011; 472: 57-63.
103. Tang WH, Wang Z, Levison BS, Koeth RA, Britt EB, Fu X, *et al.* Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. N Engl J Med. 2013; 368: 1575-84.
104. Koeth RA, Wang Z, Levison BS, Buffa JA, Org E, Sheehy BT, *et al.* Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. Nat Med. 2013; 19: 576-85.
105. Nakaya K, Takiguchi S, Ikewaki K. A new frontier for reverse cholesterol transport. The impact of intestinal microbiota on reverse cholesterol transport. Arterioscler Thromb Vasc Biol. 2017; 37: 385-6.
106. Mineo C, Shaul PW. Regulation of signal transduction by HDL. J Lipid Res. 2013; 54: 2315-24.
107. Nofer JR. Signal transduction by HDL: agonists, receptors, and signaling cascades. Exp Pharmacol. 2015; 224: 229-56.
108. Duffy D, Rader DJ. Emerging therapies targeting high-density lipoprotein metabolism and reverse cholesterol transport. Circulation. 2006; 113: 1140-50.
109. Barter PJ, Nicholls S, Rye KA, Anantharamaiah GM, Navab M, Fogelman AM. Antiinflammatory properties of HDL. Circ Res. 2004; 95: 764-72.
110. Cockerill GW, Rye K-A, Gamble JR, Vadas MA, Barter PJ. High-density lipoproteins inhibit cytokine-induced expression of endothelial cell adhesion molecules. Arterioscler Thromb Vasc Biol. 1995; 15: 1987-94.
111. Bisoendial RJ, Hovingh GK, Levels JH, Lerch PG, Andresen I, Hayden MR, *et al.* Restoration of endothelial function by increasing high-density lipoprotein in subjects with isolated low high-density lipoprotein. Circulation. 2003; 107: 2944-8.
112. Seetharam D, Mineo C, Gormley AK, Gibson LL, Vongpatanasin W, Chambliss KL, *et al.* High-density lipoprotein promotes endothelial cell migration and reendothelialization via scavenger receptor-B type I. Circ Res. 2006; 98: 63-72.
113. Tso C, Martinic G, Fan WH, Rogers C, Rye KA, Barter PJ. High-density lipoproteins enhance progenitor-mediated endothelium repair in mice. Arterioscler Thromb Vasc Biol. 2006; 26: 1144-9.
114. Sumi M, Sata M, Miura SI., Rye KA, Toya N, Kanaoka Y, *et al.* Reconstituted high-density lipoprotein stimulates differentiation of endothelial progenitor cells and enhances ischemia-induced angiogenesis. Arterioscler Thromb Vasc Biol. 2007; 27: 813-18.
115. Yvan-Charvet L, Pagler T, Gautier EL, Avagyan S, Siry RL, Han S, *et al.* ATP-binding cassette transporters and HDL suppress hematopoietic stem cell proliferation. Science. 2010; 328: 1689-93.
116. Drew BG, Duffy SJ, Formosa MF, Natoli AK, Henstridge DC, Penfold SA, *et al.* High-density lipoprotein modulates glucose metabolism in patients with type 2 diabetes mellitus. Circulation. 2009; 119: 2103-11.
117. Soran H, Schofield JD, Liu Y, Durrington PN. How HDL protects LDL against atherogenic modification: paraoxonase 1 and other dramatis personae. Curr Opin Lipidol 2015; 26: 247-56.
118. Kruger AL, Peterson S, Turkseven S, Kaminski PM, Zhang FF, Quan S, *et al.* D-4F induces heme oxygenase-1 and extracellular superoxide dismutase, decreases endothelial cell sloughing, and improves vascular reactivity in rat model of diabetes. Circulation. 2005; 111: 3126-34.
119. Bussolati B, Ahmed A, Pemberton H, Landis RC, Di Carlo F, Haskard DO, *et al.* Bifunctional role for VEGF-induced heme oxygenase-1 in vivo: induction of angiogenesis and inhibition of leukocytic infiltration. Blood. 2004; 103: 761-6.
120. Tan JTM, Ng MKC, Bursill CA. The role of high-density lipoproteins in the regulation of angiogenesis. Cardiovasc Res. 2015; 106: 184-93.
121. Van der Stoep M, Korporaal JA, van Eck M. High-density lipoprotein as a modulator of platelet and coagulation responses. Cardiovasc Res. 2014; 103: 362-71.
122. Siebel Al, Heywood SE, Kingwell BA. HDL and glucose metabolism: current evidence and therapeutic potential. Front Pharmacol. 2015; 6: 258. doi: 10.3389/fphar.2015.00258.
123. Rye KA, Barter PJ, Cochran BJ. Apolipoprotein A-1 interactions with insulin secretion and production. Curr Opin Lipidol. 2016; 27: 8-13.
124. Lim S, Park YM, Sakuma I, Koh KK. How to control residual cardiovascular risk despite statin treatment: focusing on HDL-cholesterol. Int J Cardiol 2013; 166: 8-14.
125. Stone NJ, Robinson JG, Lichtenstein AH, Bairey Merz CN, Blum CB, Eckel RH, *et al.* 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in

adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation. 2014; 129: S1-45.

126. Hafiane A, Genest J. High density lipoproteins: measurement techniques and potential biomarkers of cardiovascular risk. BBA Clin. 2015; 3: 175-88.
127. Karathanasis SK, Freeman LA, Gordon SM, Remaley AT. The changing face of HDL and the best way to measure it. Clin Chem. 2017; 63: 196-210.
128. Asztalos BF, Tani M, Schaefer EJ. Metabolic and functional relevance of HDL subspecies. Curr Opin Lipidol. 2011; 22: 176-85.
129. Tall AR. Cholesterol efflux pathways and other poten- tial mechanisms involved in the athero-protective effect of high density lipoproteins. J Intern Med. 2008; 263: 256 –73.
130. de la Llera-Moya M, Drazul-Schrader D, Asztalos BF, Cuchel M, Rader DJ, Rothblat GH. The ability to promote efflux via ABCA1 determines the capacity of serum specimens with similar high-density lipoprotein choles- terol to remove cholesterol from macrophages. Arterioscler Thromb Vasc Biol. 2010; 30: 796-801.
131. Monette JS, Hutchins PM, Ronsein GE, Wimberger J, Irwin AD, Tang C, et al. Patients with coronary endothelial dysfunction have impaired cholesterol efflux capacity and reduced HDL particle concentration. Circ Res. 2016; 119: 83-90.
132. Kontush A, Lindahl M, Lhomme M, Calabresi L, Chapman MJ, Davidson WS. Structure of HDL: particle subclasses and molecular components. In: von Eckardstein A, Kardassis D, editors. Handbook of Experimental Pharmacology. Now York: Springer; 2015. p.3-51.
133. Rosales C, Davidson WS, Gillard BK, Gotto AM Jr, Pownall HJ. Speciated high-density lipoprotein biogenesis and functionality. Curr Atheroscler Rep. 2016; 18: 25. doi: 10.1007/s11883-016-0572-7.
134. Ballantyne CM, Miller M, Niesor EJ, Burgess T, Kallend D, Stein EA. Effect of dalcetrapib plus pravastatin on lipoprotein metabolism and high-density lipoprotein composition and function in dyslipidemic patients: results of a phase IIb dose-ranging study. Am Heart J. 2012; 163: 515-21.e3.
135. Krauss RM, Wojnooski K, Orr J, Geaney JC, Pinto CA, Liu Y, *et al.* Changes in lipoprotein subfraction concentration and composition in healthy individuals treated with the CETP inhibitor anacetrapib. J Lipid Res. 2012; 53: 540-7.
136. Kuller LH, Grandits G, Cohen JD, Neaton JD, Prineas R. Lipoprotein particles, insulin, adiponectin, C-reactive protein and risk of coronary heart disease among men with metabolic syndrome. Atherosclerosis. 2007;195: 122–128.
137. Mackey RH, Greenland P, Goff DC Jr, Lloyd-Jones D, Sibley CT, Mora S. High-density lipoprotein cholesterol and particle concentrations, carotid atherosclerosis, and coronary events: MESA (Multi-Ethnic Study of Atherosclerosis). J Am Coll Cardiol. 2012; 60: 508-16.
138. Parish S, Offer A, Clarke R, Hopewell JC, Hill MR, Otvos JD, *et al.* Lipids and lipoproteins and risk of different vascular events in the MRC/BHF Heart Protection Study. Circulation. 2012; 125: 2469-78.
139. Mora S, Glynn RJ, Ridker PM. HDL cholesterol, size, particle number, and residual vascular risk after potent statin therapy. Circulation. 2013; 128: 1189-97.
140. Navab M, Hama SY, Anantharamaiah GM, Hassan K, Hough GP, Watson AD, *et al.* Normal high density lipoprotein inhibits three steps in the formation of mildly oxidized low density lipoprotein: steps 2 and 3. J Lipid Res. 2000; 41: 1495-508.
141. van Leuven SI, Hezemans R, Levels JH, Snoek S, Stokkers PC, Hovingh GK, *et al.* Enhanced atherogenesis and altered high density lipoprotein in patients with Crohn's disease. J Lipid Res. 2007; 48: 2640-6.
142. Bloedon LT, Dunbar R, Duffy D, Pinell-Salles P, Norris R, DeGroot BJ, *et al.* Safety, pharmacokinetics, and pharmacodynamics of oral apoA-I mimetic peptide D-4F in high-risk cardiovascular patients. J Lipid Res. 2008; 49: 1344-352.
143. Gebhard C, Rhainds D, Tardif JC. HDL and cardiovascular risk: is cholesterol in particle subclasses relevant? Eur Heart J 2015; 36: 10-2.
144. Sattler KJE, Elbasan Ş, Keul P, Elter-Schulz M, Bode C, Gräler MH, *et al.* Sphingosine 1-phosphate levels in plasma and HDL are altered in coronary artery disease. Basic Res Cardiol. 2010; 105: 821-32.
145. Argraves KM, Sethi AA, Gazzolo PJ, Wilkerson BA, Remaley AT, Tybjaerg-Hansen A, *et al.* S1P, dihydro-S1P and C24:1-ceramide levels in the HDL-containing fraction of serum inversely correlate with occurrence of ischemic heart disease. Lipids Health Dis. 2011; 10: 70. doi: 10.1186/1476-511x-10-70.
146. Xiong X, Liu H, Hua L, Zhao H, Wang D, Li Y. The association of HDL-apoCIII with coronary heart disease and the effect of statin treatment on it. Lipids Health Dis. 2015; 14: 127. doi: 10.1186/s12944-015-0129-8.
147. Chang PY, Lee CM, Hsu HC, Lin HJ, Chien KL, Chen MF, *et al.* Identification of the HDL-ApoCIII to VLDL-ApoCIII ratio as a predictor of coronary artery disease in the general population: the Chin-Shan Community Cardiovascular Cohort (CCCC) study in Taiwan. Lipids Health Dis. 2012; 11: 162. doi: 10.1186/1476-511x-11-162.
148. Besler C, Heinrich K, Rohrer L, Doerries C, Riwanto M, Shih DM, *et al.* Mechanisms underlying adverse effects of HDL on eNOS-activating pathways in patients with coronary artery disease. J Clin Invest. 2011; 121: 2693-708.
149. Kasumov T, Willard B, Li L, Li M, Conger H, Buffa JA, *et al.* 2H2O-based high-density lipoprotein turnover method for the assessment of dynamic high-density lipoprotein function in mice. Arterioscler Thromb Vasc Biol. 2013; 33: 1994-2003.
150. Martinez LO, Genoux A, Ferrieres J, Duparc T, Perret B. Serum inhibitory factor 1, high-density lipoprotein and cardiovascular diseases. Curr Opin Lipidol 2017; 28: 337-46.
151. Genoux A, Lichtenstein L, Ferrières J, Duparc T, Bongard V, Vervueren PL, *et al.* Serum levels of mitochondrial inhibitory factor 1 are independently associated with long-term prognosis in coronary artery disease: the GENES Study. BMC Med. 2016; 14: 125. doi: 10.1186/s12916-016-0672-9.
152. Sharrett AR, Ballantyne CM, Coady SA, Heiss G, Sorlie PD, Catellier D, *et al.* Coronary heart disease prediction from lipoprotein cholesterol levels, triglycerides, lipoprotein(a), apolipoproteins A-I and B, and HDL density subfractions: the atherosclerosis risk in communities (aric) study. Circulation. 2001; 104: 1108-13.
153. Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR. High density lipoprotein as a protective factor against coronary heart disease. The Framingham Study. Am J Med. 1977; 62: 707-14.
154. Gordon DJ, Probst eld JL, Garrison RJ, Neaton JD, Castelli WP, Knoke JD, *et al.* High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. Circulation. 1989; 79: 8-15.
155. Di Angelantonio E, Sarwar N, Perry P, Kaptoge S, Ray KK, Thompson A, *et al.* Major lipids, apolipoproteins, and risk of vascular disease. JAMA. 2009; 302: 1993-2000.
156. Barter P, Gotto AM, LaRosa JC, Maroni J, Szarek M, Grundy SM, *et al.* HDL cholesterol, very low levels of LDL cholesterol, and cardiovascular events. N Engl J Med. 2007; 357: 1301-10.

157. Ginsberg HN, Elam MB, Lovato LC, Crouse JR 3rd, Leiter LA, Linz P, *et al.* Effects of combination lipid therapy in type 2 diabetes mellitus. N Engl J Med. 2010; 362: 1563-74.
158. von Eckardstein A. Implications of torcetrapib failure for the future of HDL therapy: is HDL-cholesterol the right target? Expert Rev Cardiovasc Ther. 2010; 8: 345-58.
159. Besler C, Lüscher TF, Landmesser U. Molecular mechanisms of vascular effects of High-density lipoprotein: alterations in cardiovascular disease. EMBO Mol Med. 2012; 4: 251-68.
160. Luscher TF, Landmesser U, von Eckardstein A, Fogelman AM. High-density lipoprotein. Vascular protctive effcts, dysfunction, and potential as therapeutic target. Circ Res. 2014; 114: 171- 82.
161. Verges B, Florentin E, Baillot-Rudoni S, Petit JM, Brindisi MC, Pais de Barros JP, *et al.* Rosuvastatin 20 mg restores normal HDL-apoA-I kinetics in type 2 diabetes. J Lipid Res. 2009; 50: 1209-15.
162. Verdier C, Martinez LO, Ferrieres J, Elbaz M, Genoux A, Perret B. Targeting high-density lipoproteins: update on a promising therapy. Arch of Cardiol Dis. 2013; 106: 601-11.
163. Mineo C, Shaul PW. PON-dering differences in HDL function in coronary artery disease. J Clin Invest. 2011; 121: 2545-8.
164. Calkin AC, Drew BG, Ono A, Duffy SJ, Gordon MV, Schoenwaelder SM, *et al.* Reconstituted high-density lipoprotein attenuates platelet function in individuals with type 2 diabetes mellitus by promoting cholesterol efflux. Circulation. 2009; 120: 2095-104.
165. Murphy AJ, Bijl N, Yvan-Charvet L, Welch CB, Bhagwat N, Reheman A, *et al.* Cholesterol efflux in megakaryocyte progenitors suppresses platelet production and thrombocytosis. Nature Med. 2013; 19: 586-594.
166. Kontush A, Therond P, Zerrad A, Couturier M, Negre-Salvayre A, de Souza JA, *et al.* Preferential sphingosine-1-phosphate enrichment and sphingomyelin depletion are key features of small dense HDL3 particles: relevance to antiapoptotic and antioxidative activities. Arterioscler Thromb Vasc Biol. 2007; 27: 1843-9.
167. Patel S, Drew BG, Nakhla S, Duffy SJ, Murphy AJ, Barter PJ, *et al.* Reconstituted high-density lipoprotein increases plasma high-density lipoprotein anti-inflammatory properties and cholesterol efflux capacity in patients with type 2 diabetes. J Am Coll Cardiol. 2009; 53: 962-71.
168. De Souza JA, Vindis C, Nègre-Salvayre A, Rye K-A, Couturier M, Therond P, *et al.* Small, dense HDL 3 particles attenuate apoptosis in endothelial cells: pivotal role of apolipoprotein A-I. J Cell Mol Med. 2010; 14: 608-20.
169. Nieuwdorp M, Vergeer M, Bisoendial RJ, op 't Roodt J, Levels H, Birjmohun RS, *et al.* Reconstituted HDL infusion restores endothelial function in patients with type 2 diabetes mellitus. Diabetologia. 2008; 51: 1081-4.
170. Spieker LE, Sudano I, Hürlimann D, Lerch PG, Lang MG, Binggeli C, *et al.* High-density lipoprotein restores endothelial function in hypercholesterolemic men. Circulation. 2002; 105: 1399-402.
171. Besler C, Heinrich K, Rohrer L, Doerries C, Riwanto M, Shih DM, *et al.* Mechanisms underlying adverse effects of HDL on eNOS-activating pathways in patients with coronary artery disease. J Clin Invest. 2011; 121: 2693-708.
172. Barter PJ, Rye KA, Tardif JC, Waters DD, Boekholdt SM, Breazna A, *et al.* Effect of torcetrapib on glucose, insulin, and hemoglobin A1c in subjects in the Investigation of Lipid Level Management to Understand its Impact in Atherosclerotic Events (ILLUMINATE) trial. Circulation. 2011; 124: 555-62.
173. Drew BG, Duffy SJ, Formosa MF, Natoli AK, Henstridge DC, Penfold SA, *et al.* High-density lipoprotein modulates glucose metabolism in patients with type 2 diabetes mellitus. Circulation. 2009; 119: 2103-11.
174. Fryirs MA, Barter PJ, Appavoo M, Tuch BE, Tabet F, Heather AK, *et al.* Effects of high-density lipoproteins on pancreatic β-cell insulin secretion. Arterioscler Thromb Vasc Biol. 2010; 30: 1642-8.
175. Kingwell BA, Chapman MJ, Kontush A, Miller NE. HDL-targeted therapies progress, failure and future. Nat Rev Drug Discov. 2014; 13: 445-64.
176. Hernaez A, Farraz M, Fito M. Olive oil phenolic compounds and high-density lipoprotein function. Curr Opin Lipidol. 2016; 27: 47-53.
177. Annema W, von Eckardstein A. high-density lipoprotein-multifunctional but vulnerable protection from atherosclerosis. Circ J. 2013; 77: 2432-48.