http://eprints.gla.ac.uk/226114/

High-density lipoprotein's vascular protective functions in metabolic and cardiovascular disease - could extracellular vesicles be at play?

Jack D. Beazer[1] ✉ , Patamat Patanapirunhakit[1], Jason M. R. Gill[1], Delyth Graham[1], Helen Karlsson[2], Stefan Ljunggren[2], Monique T. Mulder[3] and Dilys J. Freeman[1]

1. Institute of Cardiovascular and Medical Science, College of Medical Veterinary and Life Science, University of Glasgow, Wolfson Link Building, University Avenue, Glasgow, G12 8QQ, United Kingdom.

2. Occupational and Environmental Medicine Centre, Department of Health, Medicine and Caring Sciences, Linköping University, Linköping, Sweden

3. Section of Pharmacology, Vascular and Metabolic Diseases, Department of Internal Medicine, Erasmus University Medical Centre, Rotterdam, The Netherlands

✉ j.beazer.1@research.gla.ac.uk

Abstract

High-density lipoprotein (HDL) is a circulating complex of lipids and proteins known primarily for its role in reverse cholesterol transport and consequent protection from atheroma. In spite of this, therapies aimed at increasing HDL concentration do not reduce the risk of cardiovascular disease (CVD), and as such focus has shifted towards other HDL functions protective of vascular health – including vaso-dilatory, anti-inflammatory, antioxidant and anti-thrombotic actions. It has been demonstrated that in disease states such as CVD and conditions of insulin resistance such as type 2 diabetes mellitus (T2DM), HDL function is impaired owing to changes in the abundance and function of HDL-associated lipids and proteins, resulting in reduced vascular protection. However, the gold standard density ultracentrifugation technique used in the isolation of HDL also co-isolates extracellular vesicles (EVs). EVs are ubiquitous cell-derived particles with lipid bilayers that carry a number of lipids, proteins and DNA/RNA/miRNAs involved in cell to cell communication. EVs transfer their bioactive load through interaction with cell surface receptors, membrane fusion and endocytic pathways and have been implicated in both cardiovascular and metabolic diseases – both as protective and pathogenic mediators. Given that studies using density ultracentrifugation to isolate HDL also co-isolate EVs, biological effects attributed to HDL may be confounded by EVs. We hypothesise that some of HDL's vascular protective functions in cardiovascular and metabolic disease, may be mediated by EVs. Elucidating the contribution of EVs to HDL functions will provide better understanding of vascular protection and function in conditions of insulin resistance and potentially provide novel therapeutic targets for such diseases.

Introduction

The identification of plasma high-density lipoprotein (HDL) concentration as an inverse predictor of cardiovascular risk harks back to the Framingham Heart Study of the 1970s [1, 2]. HDL is a circulating complex of lipids and proteins primarily known for its role in reverse cholesterol transport; the removal of excess cholesterol from peripheral tissues and subsequent transfer to the liver for excretion in bile [3]. However, intervention studies have consistently demonstrated that pharmacotherapy aimed at increasing HDL concentration, for example by cholesteryl ester transfer protein inhibition, does not reduce the risk of cardiovascular disease (CVD) [4, 5]. As such focus has shifted towards HDL functionality, independent of concentration, that protects endothelial function and vascular health, such as vaso-dilatory, anti-inflammatory, antioxidant, and anti-thrombotic actions (Figure 1.). A landmark proteomic study of HDL in 2005 initially identified 13 proteins associated with HDL [6], but given technological advances, such as in liquid-chromatography mass spectrometry, this total now exceeds over 100 proteins [7]. It has been demonstrated that in disease states like CVD, type 2 diabetes mellitus (T2DM) and the metabolic complication of pregnancy pre-eclampsia, HDL function is impaired owing to changes in the abundance and function of HDL associated lipids and proteins [8, 9].

Figure 1 – An overview of HDL and EV vascular effects.
HDL and EVs have overlapping vascular functional effects mediated through distinct mechanisms. This may lead to confounding in studies where HDL and EVs are not adequately separated.

However, the gold standard density ultracentrifugation (DUC) technique used to isolate HDL from plasma has been shown to co-isolate extracellular vesicles (EVs), owing to their overlapping densities [10]. EVs are ubiquitous cell-derived particles with lipid bilayers characterised by their size; from smallest to largest are exosomes, microparticles and apoptotic bodies. Exosomes are formed through the endocytosis of cell interior multivesicular bodies and can carry lipids, proteins and DNA/RNA/miRNA that can mediate cell to cell communication. Microparticles and apoptotic bodies are formed directly from the cell membrane, with the latter blebbing from cells undergoing apoptosis. All EV subtypes can be found in a variety of body fluids and can be derived from multiple origins including endothelial cells, platelets, immune cells and adipose tissue [11]. EVs transfer their bioactive load through interaction with cell surface receptors, membrane fusion and endocytic pathways and as such have been implicated in cardiovascular and metabolic diseases as both protective and pathogenic mediators [12]. As studies using DUC to isolate HDL also co-isolate EVs, biological effects attributed to HDL may be confounded by EVs (Figure 1). This may in part explain the lack of efficacy of therapeutics aimed at increasing HDL concentration to reduce CVD risk and posits EVs as an alternative therapeutic target in metabolic disease and consequent cardiovascular risk. In the present hypothesis article, current knowledge of HDL and EVs in CVD and metabolic disease is briefly discussed and the evidence for EVs mediating some HDL functions summarised.

HDL protects the vascular endothelium in health, but this is impaired in cardiovascular and metabolic disease.

The vascular protection mediated by HDL has been shown to employ a variety of pathways other than reverse cholesterol transport (Figure 2). Anti-inflammatory and vaso-dilatory effects of HDL are largely attributed to the stimulation of endothelial nitric oxide synthase (eNOS), increasing the availability of nitric oxide (NO) and inducing vasodilation. NO also plays a role in the prevention of inflammation, through the inhibition of expression of adhesion molecules (vascular cell adhesion molecule-1 [VCAM-1], and intercellular adhesion molecule-1 [ICAM-1]) in response to tumour necrosis factor-α (TNFα) signalling. NO stimulation by HDL has been attributed to apolipoprotein-AI (ApoAI) acting through scavenger-receptor B1 (SRB1) and the apolipoprotein M (ApoM)-anchored lysophospholipid sphingosine-1-phospate (S1P) through its receptor S1PR-1 [13, 14]. HDL antithrombotic actions are also linked with NO stimulation, through improved blood flow due to NO-mediated vasodilation, coupled with antagonised platelet activation via platelet activating factor and thromboxane A_2 downregulation [15]. There is evidence for antithrombotic functions of HDL beyond NO stimulation, such as HDL reducing the propensity for von-Willebrand factor to become hyper-adhesive and efficiently bind platelets [16]. HDL bound paraoxonase-1 (PON-1) acts to prevent the oxidation of low-density lipoprotein and other lipids, attenuating the effects of oxidative stress. HDL PON-1 has also been shown to reduce the activity of the oxidised low-density lipoprotein receptor LOX-1, reducing downstream reactive oxygen species production [17].

Cardiovascular and metabolic disease have both been shown to adversely affect vascular protection by HDL, though the cause/effect relationship is yet to be fully established [18]. HDL from coronary artery disease (CAD) patients inhibited eNOS stimulation due to reduced PON-1 and was unable to prevent adhesion molecule expression in response to TNFα (Figure 2) [17]. CAD-derived HDL had lower S1P content and a reduced ability to limit inflammation in vascular smooth muscle cells compared to healthy HDL, which could be recovered through S1P enrichment [19]. The cholesterol efflux capacity and anti-inflammatory ability of HDL is impaired in myocardial infarction (MI) patients, with the degree of dysfunction correlated with the likelihood of future cardiac events and the severity of MI [20, 21]. The antioxidant functions of HDL were diminished in chronic heart failure patients and were linked to clinical outcomes in terms of disease severity [22].

Figure 2 –HDL-mediated vascular protection.

HDL has multiple mechanisms of action protecting the vasculature, predominantly in an eNOS dependent manner. Apolipoprotein AI (ApoAI) binds scavenger receptor B1 (SRB1) and induces the Akt pathway, leading to downstream endothelial nitric oxide (eNOS) activation and nitric oxide (NO) production. The same pathway is activated when apolipoprotein M (ApoM) bound sphingosine-1-phosphate (S1P) acts on S1P receptor 1/3. HDL bound PON-1 prevents the oxidation of low-density lipoprotein (LDL) and reduces oxidised-LDL receptor (LOX-1) activation, preventing the subsequent generation of reactive oxygen species (ROS) that inhibit NO action. The generation of NO inhibits NFκB-mediated adhesion molecule synthesis (vascular cell and intercellular adhesion molecule 1, VCAM-1 / ICAM-1) in response to tumour necrosis factor alpha and other cytokines through their respective receptors.

Metabolic disease is associated with dyslipidaemia, obesity and insulin resistance resulting in increased CVD risk. In T2DM, vascular inflammation, oxidative stress and advanced glycation end products due to hyperglycaemia ultimately accrue and provoke vascular dysfunction and atheroma [23]. Glycation of HDL bound proteins and enzymes in T2DM reduces protective effects of HDL due to conformational changes in those glycated proteins [24]. Much like in coronary artery disease, T2DM HDL had reduced ability to stimulate eNOS and prevent TNFα induced NFκB inflammatory responses in endothelial cells [25]. These changes were observed alongside lower PON-1 antioxidant activity and were associated with hyperglycaemia [26]. Of note, South Asians have increased risk of T2DM and CVD with the onset of disease occurring at younger age and lower BMI compared to white Europeans [27] and there is evidence of impaired anti-oxidant and anti-inflammatory properties of HDL in this population [28]. The HDL lipidome is also altered in T2DM, particularly with regard to S1P content. HDL S1P was lower in HDL from diabetes patients impairing protective intracellular pathways which can be reversed through enrichment of HDL with S1P in a dose-dependent manner [29, 30], though there is some disagreement as to whether HDL S1P/ApoM or plasma S1P/ApoM is associated with CVD in T2DM in a study of African Americans with diabetes [31]. Other alterations to the HDL lipidome in T2DM subjects with dyslipidaemia include a substantial (+77%) increase in triglyceride content and smaller HDL particles with elevated ceramide, known to be linked with inflammation and insulin resistance [32].

These impairments in HDL vascular protection in those at risk of CVD can be ameliorated through lifestyle interventions. A randomised controlled trial where participants at risk of cardiovascular disease adhered to a Mediterranean diet rich with olive oil or nuts for a year showed improvements in HDL cholesterol efflux, PON-1 activity and improved vaso-dilatory function through nitric oxide stimulation [33]. A similar diet undertaken by men with metabolic syndrome for five weeks provoked some changes in the HDL proteome (namely inter-α-trypsin inhibitor heavy chain H4) linked to an improvement in the anti-inflammatory effects of HDL with no other changes in HDL function [34]. In a comparable cohort of men, a high-fibre, low-fat diet changed HDL from an inflammatory to anti-inflammatory state as well as increasing the activity of platelet-activating factor acetylhydrolase (PAF-AH) [35]. Evidence is conflicting on weight loss and improvements in HDL function; adolescents following gastric sleeve weight loss surgery were seen to have better HDL cholesterol efflux and anti-oxidant activity compared to their pre-surgery baseline, while a study of diet and low intensity exercise induced weight loss in obese women with or without diabetes had no impact on HDL functional metrics [36, 37].

In terms of exercise, a three-month bicycle ergometer training programme in metabolic syndrome patients returned cholesteryl ester transfer protein activity to normal, non-metabolic syndrome levels while increasing PON-1 activity [38]. A 10 week walk/run training programme in those with the metabolic syndrome increased HDL PON-1 activity, while also attenuating adhesion molecule expression and monocyte adhesion in endothelial cells incubated with participant HDL. The same study also found HDL reduced the inhibition of NO production by eNOS in endothelial cells treated with TNF-α. These effects occurred independently of HDL concentration, in fact, HDL levels did not increase in the participants after 10 weeks of training [39]. Cardiac rehabilitation,

through diet education and a five-month exercise programme involving brisk walking 3-5 times per week, led to 9.4% increase in HDL cholesterol efflux in acute coronary syndrome patients [40]. After 15 weeks of aerobic exercise in chronic heart failure patients, HDL better stimulated eNOS-mediated NO production compared to pre-training baseline levels with no accompanying changes in proteomics [41].

Extracellular vesicles in cardiovascular and metabolic disease

Extracellular vesicles have both protective and pathogenic effects in the vasculature in cardiovascular and metabolic disease, which may be expected given their diverse cellular origins, cargoes and subtype. Interestingly, EVs have also been posited to regulate metabolism, which has been reviewed by Fatima et al [42]. In pathophysiology, circulating EV levels increase [12]. Much like HDL however, the cause/effect relationship between EVs and cardiometabolic disease is unclear. EVs can be anti-inflammatory; quiescent endothelium derived EVs (EEVs) reduce monocyte activation through miRNA 10a *in vitro* and in a mouse model of peritonitis [43]. EEVs can also deliver miRNAs 126 and 222 locally to the endothelium, where they promote endothelial repair [44] and a reduction in ICAM-1 expression [45] respectively, though these functions were impaired with diabetes mellitus-like hyperglycaemia. Shear-stressed human umbilical vein endothelial cell (HUVEC) EVs have atheroprotective effects, mediated through the transfer of miRNA 143/145 to smooth muscle cells and preventing their de-differentiation. In a mouse model of atherosclerosis miRNA 143/145 containing EVs reduced atherosclerotic lesion size by two-fold [46]. EEVs from TNFa stimulated HUVECs limit palmitate-induced oxidative stress through the transfer and stimulation of eNOS [47]. In contrast, EVs derived in a similar fashion can evoke inflammatory responses in HUVECs while inducing a mixture of pro- and anti-inflammatory responses in THP-1 monocyte like cells, dependent upon the chemokines and cytokines transferred including IL-6, IL-8, CCL-4 and CCL-5 [48].

In terms of pathogenic EV effects, endothelial and platelet-derived exosomes isolated from cerebrovascular disease patient plasma contain higher levels of select pro-atherogenic proteins compared to healthy controls [49]. Platelet EVs released during myocardial infarction contain miRNA-320b leading to increased ICAM-1 expression on endothelial cells [50], while platelet EV miRNA-142-3p causes aberrant endothelial proliferation in hypertension [51]. Exosomes from activated monocytes induce ICAM-1 and IL-6 expression in endothelial cells in an NFκB dependent manner, linking chronic inflammation and cardiovascular pathology [52]. Metabolic syndrome patient plasma EVs inhibit eNOS activity through the inhibitory threonine-145 phosphorylation site in endothelial cells and reduce endothelial dependent contraction in mouse aortic rings post EV injection *in vivo*, though these EVs did not alter the expression of cytokines as described in other studies [53]. HUVECs cultured in high glucose conditions produce a higher concentration of EVs that are pro-coagulant, induce endothelial ROS production and impair endothelial relaxation. These EVs have a distinct proteomic profile compared to control media cultured HUVEC EVs, containing proteins related to oxidation-reduction processes, haemostasis and protein complex formation not present in the controls [54]. Stimulating HUVECs with high glucose in tandem with angiotensin II

produces EVs that decrease eNOS expression in mouse aortic rings through the MEK/ERK pathway, impairing NO mediated relaxation [55]; taken together these studies offer EVs as a potential link between diabetic pathophysiology and vascular dysfunction.

There is evidence for EVs mediating the positive vascular/metabolic effects of exercise, particularly as a number of factors known to be released during exercise (so-called 'exerkines') can be found in EVs [56]. A six-month aerobic exercise regimen in sedentary African American men and women reduced the plasma concentration of endothelial EVs and IL-6 and improved flow mediated dilation of the brachial artery [57]. Exercised mouse serum EVs administered intramyocardially prior to ischaemia/reperfusion injury reduced the resulting infarct size to a greater extent compared to EVs from sedentary mice, and prevented cardiomyocyte apoptosis *in vitro* [58]. Moderate exercise in mice increases circulating EV number and miRNA 126 carried by endothelial progenitor cell EVs, and these EVs improved survival in endothelial cells cultured with high glucose in hypoxic conditions [59]. Longer term exercise is also associated with improved EV function; student rowers who regularly trained for more than one year had a 1.8-fold increase in endothelial exosomal miRNA-342-5p, found to prevent apoptosis in cultured cardiomyocytes in a caspase 9 and JNK2 dependent manner. Shear stress of the endothelium occurs during exercise, and this is associated with increases in miRNA-342-5p containing exosomes released from HUVECS [60]. In contrast, a study of pre-hypertensive individuals undertaking 6 months of aerobic exercise found that the long-term exercise associated sheer stress reduced the number of circulating EVs, owing to increased endothelial mitochondrial biogenesis [61].

Are extracellular vesicles responsible for some of HDL's vascular functions?

A key issue in the study of both HDL and EVs is their co-isolation when using density ultracentrifugation (DUC), and the consequent risk of mis-interpreting experimental outcomes. HDL and EVs co-isolate due to overlapping densities in the range of 1.063-1.21g/ml and although EVs only account for ~1% of the particles in a given HDL fraction isolated using DUC, they are much larger in diameter and therefore account for around 10 times the volume. Without further processing of HDL fractions after DUC, EV contamination will remain [10]. As such, studies on HDL that have not undertaken additional purification steps subsequent to DUC do not take into consideration potentially confounding EVs, thereby potentially inappropriately attributing beneficial or detrimental EV effects to HDL. It should be noted that it is particularly difficult to completely separate HDL and EVs, even when using multistep purification processes, and the post isolation processes can alter the biological activity and integrity of both HDL and EVs. It has been demonstrated that another lipoprotein, LDL, can bind to EVs leading to their co-isolation [62] though it is unclear whether HDL directly interacts with EVs in a similar manner. While density ultracentrifugation is the standard technique employed for lipoprotein isolation, EV isolation techniques are much more varied and include antibody-based techniques (including immuno-magnetic isolation), size-exclusion chromatography (SEC) and kit-based procedures that may affect the types and quality of EVs isolated, as well as the degree of HDL contamination. Of the EV isolation techniques, SEC can effectively separate purified fractions of larger EVs from HDL and plasma proteins but with the

caveat of lower yield than DUC. Smaller EVs isolated using SEC may still be contaminated by HDL, given the pore size of 75nm in the columns used in these studies being much larger than HDL and small EVs [63, 64]. A study comparing methods of EV isolation found that kit-based procedures and centrifugation alone were not adequate to deplete lipoproteins. A combination of isolation techniques is required to deplete lipoproteins in serum samples though this leads to a limited EV yield [65]. As such, studies on extracellular vesicles from plasma/serum samples which have employed DUC or kit-based procedures alone may be confounded by HDL.

Very few studies have directly compared HDL and EVs. HDL, like EVs, has been proposed to carry miRNAs. Isolated and purified HDL carries an miRNA profile distinct from both EVs and LDL. HDL appears to transport miRNA from a variety of cellular origins while EVs contain miRNA only from their parent cells [66]. A comprehensive lipidomic comparison of lipoproteins and EVs established distinct lipidomic profiles of HDL and EVs, where EVs contain eight-times less lipid compared to lipoproteins but are enriched in lysoglycerophospholipids and have higher relative sphingolipid content (particularly sphingomyelin) compared to HDL. This study did however find markedly different EV lipid compositions to previous EV only lipidomic studies, particularly the enrichment in lysoglycerophospholipids [67]. This may be due to the EV isolation technique; previous EV only lipidomic studies employed ultracentrifugation which as discussed can lead to contamination by other plasma particles like HDL. Although steps to understand the separate roles of HDL and EVs in health and disease have been undertaken, these studies are somewhat confounded by the issues associated with HDL and EVs co-isolating, and the potential problems encountered when further purifying HDL or EV fractions (Table 1).

There is considerable overlap in some of the protective and detrimental functions of HDL and EVs in cardiovascular and metabolic disease, particularly in terms of anti-oxidative, anti-inflammatory and atheroprotective functions (Figure 1). There is also overlap in the mechanisms by which these functions take place, including miRNA transfer and eNOS stimulatory pathways, and that impaired HDL and EV functionality in cardiometabolic disease can be recovered with exercise. Given that a large number of the studies cited used ultracentrifugation which leads to co-isolation of HDL and EVs without stringent measures, it follows that there may be some confounding taking place, and that the biological effects described could be misattributed to either HDL or EVs. It may also be the case that HDL and EVs do indeed share these effects, but one particle may have dominant effects over the other in changing disease states and progression. Even in studies where HDL and EVs have been separated with further processing, there are some potential issues. The effect of fed and fasting states on EV function and circulating concentration is unclear, though it has been shown that adipose derived EVs have altered cargo in response to nutrition status [68]. It is difficult to pinpoint the cellular sources of circulating EVs given that there is little consensus regarding tissue specific markers and considerable overlap in the markers currently used [69].

Particle to be isolated	Isolation Method	Advantages	Limitations
HDL	Density Ultracentrifugation	'Gold-standard' method for HDL isolation [70] Enables isolation of HDL subspecies separately	Co-isolation of EVs and plasma proteins [10] Shear forces may damage/remove HDL surface-bound proteins [71] Buffers used may interfere with downstream analysis, due to high salt concentration or oxidation [71] Lengthy process [70] Low throughput process limited by centrifuge capacity [71]
	Size Exclusion Chromatography	Fast; only requires a single step Economical	SEC eluate is enriched for HDL but still contains contaminants [70] Co-isolation of similar molecular weight plasma proteins [70]
	Immunoaffinity	More specific than DUC and SEC Scalable for high-throughput applications [71]	Typically uses antibodies to ApoAI which can be found on non-HDL particles [70] Newer method that requires further characterisation
Extracellular Vesicles	Density Ultracentrifugation	Well established and easy to perform [72]	Co-isolation of HDL and other plasma proteins[64] Lengthy process Risk of damage to EV integrity Low throughput [72]
	Size Exclusion Chromatography	Fast; only requires a single step [64] Removal of the majority of plasma contaminants [64]	Dilute EVs across fractions require pooling, reducing purity [65] Co-isolation of similar sized plasma proteins Smaller EVs co-isolated with HDL [63]
	Immunoaffinity	Results in highly pure EV samples [69]	Only captures a subset of EVs carrying sufficient target protein Best suited to small sample volumes Not suited to downstream functional assays due to tight antibody binding [69]
	Commercial kit-based procedures	Convenient Fast High EV yield [65]	High degree of lipoprotein and plasma protein contamination [65]

Table 1 - Summary of the advantages and limitations of HDL and EV isolation methods.
A combination of isolation methods is usually required to isolate purified fractions of HDL and EVs, in order to account for the limitations of each individual method.

Hypothesis

Given that HDL and EVs co-isolate in DUC as well as in commonly used kit-based EV isolation protocols, there is the possibility that EV effects are being attributed to HDL. This may also occur in the other direction, where HDL effects are being attributed to EVs. There has been little direct comparison between HDL and EVs despite this co-isolation. Overlapping functions of HDL and EV on the endothelium in both health and disease have been described in separate studies while exercise has been shown to recover perturbed HDL and EV function. As such, we hypothesise that some of HDL's vascular protective functions are in fact mediated by EVs, and that some EV functions may be mediated by HDL.

Future experimental work

Despite the development of novel techniques, such as the use of agarose gel electrophoresis [73], the separation of HDL from EVs remains a challenge. Purified HDL and EVs should undergo proteomic analysis to ensure that EV proteins are not being assigned to HDL (and vice versa) and to understand how both proteomes change in cardiometabolic disease. To enhance these proteomic studies, functional readouts of both HDL and EVs can also aid the clarification of biological effects undertaken by each, such as *in vitro* inflammatory cell assays and *ex vivo* vessel wire myography. Further understanding of the degree to which HDL and EVs are involved in cardiometabolic diseases can be achieved through regression analyses of vascular readouts with HDL and EVs, such as soluble VCAM-1 as a marker of vascular inflammation [74]. A transcriptomics approach may reveal changes in miRNAs associated with HDL and EVs in disease.

Clinical Implications

Clarifying HDL and EV roles in vascular protection may highlight one or the other as a more likely therapeutic target in cardiometabolic disease. It may explain to some degree why HDL cholesterol raising therapies are not as effective as expected, should it be found that EVs are actually performing some HDL-associated protective functions. Recombinant HDL and HDL mimetics are increasingly being investigated as potential therapies for cardiovascular risk with varying success. Understanding the molecular and functional changes in HDL in cardiometabolic disease may reveal new candidates for recombinant HDL components and improve their efficacy [75, 76].

Acknowledgements and Funding

References

1. Kannel WB, Castelli WP, Gordon T, McNamara PM (1971) Serum cholesterol, lipoproteins, and the risk of coronary heart disease. The Framingham study. Ann Intern Med 74:1–12

2. Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR (1977) High density lipoprotein as a protective factor against coronary heart disease: The Framingham study. Am J Med 62:707–714

3. Roheim PS (1986) Atherosclerosis and lipoprotein metabolism: Role of reverse cholesterol transport. Am J Cardiol 57:3C-10C

4. Lincoff AM, Nicholls SJ, Riesmeyer JS, et al (2017) Evacetrapib and Cardiovascular Outcomes in High-Risk Vascular Disease. N Engl J Med 376:1933–1942

5. Khera A V, Patel PJ, Reilly MP, Rader DJ (2013) The Addition of Niacin to Statin Therapy Improves High-Density Lipoprotein Cholesterol Levels But Not Metrics of Functionality. J Am Coll Cardiol 62:1909–1910

6. Karlsson H, Leanderson P, Tagesson C, Lindahl M (2005) Lipoproteomics II: Mapping of proteins in high-density lipoprotein using two-dimensional gel electrophoresis and mass spectrometry. Proteomics 5:1431–1445

7. Ljunggren SA, Helmfrid I, Norinder U, Fredriksson M, Wingren G, Karlsson H, Lindahl M (2017) Alterations in high-density lipoprotein proteome and function associated with persistent organic pollutants. Environ Int 98:204–211

8. Kajani S, Curley S, McGillicuddy FC (2018) Unravelling hdl—looking beyond the cholesterol surface to the quality within. Int J Mol Sci 19:1–23

9. Einbinder Y, Biron-Shental T, Agassi-Zaitler M, Tzadikevitch-Geffen K, Vaya J, Khatib S, Ohana M, Benchetrit S, Zitman-Gal T (2018) High-density lipoproteins (HDL) composition and function in preeclampsia. Arch Gynecol Obstet 298:405–413

10. Yuana Y, Levels J, Grootemaat A, Sturk A, Nieuwland R (2014) Co-isolation of extracellular vesicles and high-density lipoproteins using density gradient ultracentrifugation. J Extracell Vesicles 3:23262

11. Pardo F, Villalobos-Labra R, Sobrevia B, Toledo F, Sobrevia L (2018) Extracellular vesicles in obesity and diabetes mellitus. Mol Aspects Med 60:81–91

12. Jansen F, Li Q, Pfeifer A, Werner N (2017) Endothelial- and Immune Cell-Derived Extracellular Vesicles in the Regulation of Cardiovascular Health and Disease. JACC Basic to Transl Sci 2:790–807

13. Kimura T, Tomura H, Mogi C, et al (2006) Role of scavenger receptor class B type I and sphingosine 1-phosphate receptors in high density lipoprotein-induced inhibition of adhesion molecule expression in endothelial cells. J Biol Chem 281:37457–37467

14. Galvani S, Sanson M, Blaho VA, Swendeman SL, Conger H, Dahlbäck B, Kono M, Proia RL, Smith JD, Hla T (2015) HDL-bound sphingosine 1-phosphate acts as a biased agonist for the endothelial cell receptor S1P1 to limit vascular inflammation. Sci Signal 8:ra79--ra79

15. Mineo C, Deguchi H, Griffin JH, Shaul PW (2006) Endothelial and antithrombotic actions of HDL. Circ Res 98:1352–1364

16. Chung DW, Chen J, Ling M, et al (2016) High-density lipoprotein modulates thrombosis by preventing von Willebrand factor self-Association and subsequent platelet adhesion. Blood 127:637–645

17. Besler C, Heinrich K, Rohrer L, et al (2011) Mechanisms underlying adverse effects of HDL on eNOS-activating pathways in patients with coronary artery disease. J Clin Invest 121:2693–2708

18. Lemmers RFH, van Hoek M, Lieverse AG, Verhoeven AJM, Sijbrands EJG, Mulder MT (2017) The anti-inflammatory function of high-density lipoprotein in type II diabetes: A systematic review. J Clin Lipidol 11:712-724.e5

19. Keul P, Polzin A, Kaiser K, Gräler M, Dannenberg L, Daum G, Heusch G, Levkau B (2019) Potent anti-inflammatory properties of HDL in vascular smooth muscle cells mediated by HDL-S1P and their impairment in coronary artery disease due to lower HDL-S1P: A new aspect of HDL dysfunction and its therapy. FASEB J 33:1482–1495

20. Annema W, Willemsen HM, de Boer JF, et al (2016) HDL function is impaired in acute myocardial infarction independent of plasma HDL cholesterol levels. J Clin Lipidol 10:1318–1328

21. Dullaart RPF, Annema W, Tio RA, Tietge UJF (2014) The HDL anti-inflammatory function is impaired in myocardial infarction and may predict new cardiac events independent of HDL cholesterol. Clin Chim Acta 433:34–38

22. Schrutka L, Distelmaier K, Hohensinner P, Sulzgruber P, Lang IM, Maurer G, Wojta J, Hülsmann M, Niessner A, Koller L (2016) Impaired High-Density Lipoprotein Anti-Oxidative Function Is Associated With Outcome in Patients With Chronic Heart Failure. J Am Heart Assoc. https://doi.org/10.1161/JAHA.116.004169

23. Srivastava RAK (2018) Dysfunctional HDL in diabetes mellitus and its role in the pathogenesis of cardiovascular disease. Mol Cell Biochem 440:167–187

24. Sun J, Shen Y, Lu A, Lu L, Shen W (2013) Glycation of high-density lipoprotein in type 2 diabetes mellitus. Chin. Med. J. (Engl). 126:

25. Vaisar T, Couzens E, Hwang A, Russell M, Barlow CE, DeFina LF, Hoofnagle AN, Kim F (2018) Type 2 diabetes is associated with loss of HDL endothelium protective functions. PLoS One 13:1–16

26. Ebtehaj S, Gruppen EG, Parvizi M, Tietge UJF, Dullaart RPF (2017) The anti-inflammatory function of HDL is impaired in type 2 diabetes: Role of hyperglycemia, paraoxonase-1 and low grade inflammation. Cardiovasc Diabetol 16:1–9

27. Sattar N, Gill JMR (2015) Type 2 diabetes in migrant south Asians: mechanisms, mitigation, and management. Lancet Diabetes Endocrinol 3:1004–1016

28. Bakker LEH, Boon MR, Annema W, et al (2016) HDL functionality in South Asians as compared to white Caucasians. Nutr Metab Cardiovasc Dis 26:697–705

29. Brinck J., Thomas A, Lauer E, et al (2016) Diabetes Mellitus Is Associated With Reduced High-Density Lipoprotein Sphingosine-1-Phosphate Content and Impaired High-Density Lipoprotein Cardiac Cell Protection. Arterioscler Thromb Vasc Biol 36:817–824

30. Tong X, Lv P, Mathew A V, et al (2014) The compensatory enrichment of sphingosine -1- phosphate harbored on glycated high-density lipoprotein restores endothelial protective function in type 2 diabetes mellitus. Cardiovasc Diabetol 13:82

31. Liu M, Frej C, Langefeld CD, et al (2019) Plasma apoM and S1P levels are inversely associated with mortality in African Americans with type 2 diabetes mellitus. J Lipid Res 60:1425–1431

32. Ståhlman M, Fagerberg B, Adiels M, Ekroos K, Chapman JM, Kontush A, Borén J (2013) Dyslipidemia, but not hyperglycemia and insulin resistance, is associated with marked alterations in the HDL lipidome in type 2 diabetic subjects in the DIWA cohort: Impact on small HDL particles. Biochim Biophys Acta - Mol Cell Biol Lipids 1831:1609–1617

33. Hernáez Á, Castañer O, Elosua R, et al (2017) Mediterranean Diet Improves High-Density Lipoprotein Function in High-Cardiovascular-Risk Individuals. Circulation 135:633–643

34. Richard C, Couture P, Desroches S, Nehmé B, Bourassa S, Droit A, Lamarche B (2014) Effect of an Isoenergetic Traditional Mediterranean Diet on the High-Density Lipoprotein Proteome in Men with the Metabolic Syndrome. Lifestyle Genomics 7:48–60

35. Roberts CK, Ng C, Hama S, Eliseo AJ, Barnard RJ (2006) Effect of a short-term diet and exercise intervention on inflammatory/anti-inflammatory properties of HDL in overweight/obese men with cardiovascular risk factors. J Appl Physiol 101:1727–1732

36. Davidson WS, Inge TH, Sexmith H, Heink A, Elder D, Hui DY, Melchior JT, Kelesidis T, Shah AS (2017) Weight loss surgery in adolescents corrects high-density lipoprotein subspecies and their function. Int J Obes 41:83–89

37. Aicher BO, Haser EK, Freeman LA, Carnie A V, Stonik JA, Wang X, Remaley AT, Kato GJ, Cannon III RO (2012) Diet-Induced Weight Loss in Overweight or Obese Women and Changes in High-Density Lipoprotein Levels and Function. Obesity 20:2057–2062

38. Casella-Filho A, Chagas ACP, Maranhão RC, Trombetta IC, Cesena FHY, Silva VM, Tanus-Santos JE, Negrão CE, da Luz PL (2011) Effect of Exercise Training on Plasma Levels and Functional Properties of High-Density Lipoprotein Cholesterol in the Metabolic Syndrome. Am J Cardiol 107:1168–1172

39. Sang H, Yao S, Zhang L, et al (2015) Walk-Run Training Improves the Anti-Inflammation Properties of High-Density Lipoprotein in Patients With Metabolic Syndrome. J Clin Endocrinol Metab 100:870–879

40. Furuyama F, Koba S, Yokota Y, Tsunoda F, Shoji M, Kobayashi Y (2018) Effects of Cardiac Rehabilitation on High-Density Lipoprotein-mediated Cholesterol Efflux Capacity and Paraoxonase-1 Activity in Patients with Acute Coronary Syndrome. J Atheroscler Thromb 25:153–169

41. Adams V, Besler C, Fischer T, et al (2013) Exercise training in patients with chronic heart failure promotes restoration of high-density lipoprotein functional properties. Circ Res 113:1345–1355

42. Fatima F, Nawaz M (2017) Long distance metabolic regulation through adipose-derived circulating exosomal miRNAs: A trail for RNA-based therapies? Front Physiol 8:1–13

43. Njock M-S, Cheng HS, Dang LT, Nazari-Jahantigh M, Lau AC, Boudreau E, Roufaiel M, Cybulsky MI, Schober A, Fish JE (2015) Endothelial cells suppress monocyte activation through secretion of extracellular vesicles containing antiinfl ammatory microRNAs. Blood 125:3202–3212

44. Jansen F, Yang X, Hoelscher M, et al (2013) Endothelial Microparticle – Mediated Transfer of MicroRNA-126 Promotes Vascular Endothelial Cell Repair via SPRED1 and Is Abrogated in Glucose-Damaged Endothelial Microparticles. 2026–2038

45. Jansen F, Yang X, Baumann K, Przybilla D, Schmitz T, Flender A, Paul K, Alhusseiny A, Nickenig G, Werner N (2015) Endothelial microparticles reduce ICAM-1 expression in a microRNA-222-dependent mechanism Cell culture and EMP generation. 19:2202–2214

46. Hergenreider E, Heydt S, Tréguer K, et al (2012) Atheroprotective communication between endothelial cells and smooth muscle cells through miRNAs. Nat Publ Gr 14:249–256

47. Mahmoud AM, Wilkinson FL, McCarthy EM, Moreno-Martinez D, Langford-Smith A, Romero M, Duarte J, Alexander MY (2017) Endothelial microparticles prevent lipid-induced endothelial damage via Akt/eNOS signaling and reduced oxidative stress. FASEB J 31:4636–4648

48. Hosseinkhani B, Kuypers S, van den Akker NMS, Molin DGM, Michiels L (2018) Extracellular vesicles work as a functional inflammatory mediator between vascular endothelial cells and immune cells. Front Immunol. https://doi.org/10.3389/fimmu.2018.01789

49. Goetzl EJ, Schwartz JB, Mustapic M, Lobach I V., Daneman R, Abner EL, Jicha GA (2017) Altered cargo proteins of human plasma endothelial cell–derived exosomes in atherosclerotic cerebrovascular disease. FASEB J 31:3689–3694

50. Gidlöf O, Van Der Brug M, Öhman J, Gilje P, Olde B, Wahlestedt C, Erlinge D (2013) Platelets activated during myocardial infarction release functional miRNA, which can be taken up by endothelial cells and regulate ICAM1 expression. Blood 121:3908–3917

51. Bao H, Chen YX, Huang K, Zhuang F, Bao M, Han Y, Chen XH, Shi Q, Yao QP, Qi YX (2018) Platelet-derived microparticles promote endothelial cell proliferation in hypertension via miR-142-3p. FASEB J 32:3912–3923

52. Tang N, Sun B, Gupta A, Rempel H, Pulliam L (2016) Monocyte exosomes induce adhesion molecules and cytokines via activation of NF-κB in endothelial cells. FASEB J 30:3097–3106

53.   Agouni A, He A, Ducluzeau PH, Mostefai HA, Draunet-busson C, Leftheriotis G, Heymes C, Martinez MC (2008) Endothelial Dysfunction Caused by Circulating Microparticles from Patients with Metabolic Syndrome. Am J Pathol 173:1210–1219

54.   Burger D, Turner M, Xiao F, Munkonda MN, Akbari S, Burns KD (2017) High glucose increases the formation and pro-oxidative activity of endothelial microparticles. Diabetologia 60:1791–1800

55.   Taguchi K, Hida M, Narimatsu H, Matsumoto T, Kobayashi T (2017) Glucose and angiotensin II-derived endothelial extracellular vesicles regulate endothelial dysfunction via ERK1/2 activation. Pflugers Arch Eur J Physiol 469:293–302

56.   Safdar A, Tarnopolsky MA (2018) Exosomes as Mediators of the Systemic Adaptations to Endurance Exercise. Cold Spring Harb Perspect Med 8:1–24

57.   Babbitt DM, Diaz KM, Feairheller DL, et al (2013) Endothelial activation microparticles and inflammation status improve with exercise training in African Americans. Int J Hypertens. https://doi.org/10.1155/2013/538017

58.   Bei Y, Xu T, Lv D, et al (2017) Exercise-induced circulating extracellular vesicles protect against cardiac ischemia–reperfusion injury. Basic Res Cardiol 112:1–15

59.   Ma C, Wang J, Liu H, Chen Y, Ma X, Chen S, Chen Y, Bihl J, Yang Y (2018) Moderate Exercise Enhances Endothelial Progenitor Cell Exosomes Release and Function. Med Sci Sports Exerc 50:2024–2032

60.   Hou Z, Qin X, Hu Y, et al (2019) Longterm Exercise-Derived Exosomal miR-342-5p: A Novel Exerkine for Cardioprotection. Circ Res 124:1386–1400

61.   Kim JS, Kim B, Lee H, Thakkar S, Babbitt DM, Eguchi S, Brown MD, Park JY (2015) Shear stress-induced mitochondrial biogenesis decreases the release of microparticles from endothelial cells. Am J Physiol - Hear Circ Physiol 309:H425–H433

62.   Sódar BW, Kittel Á, Pálóczi K, et al (2016) Low-density lipoprotein mimics blood plasma-derived exosomes and microvesicles during isolation and detection. Sci Rep 6:1–12

63.   Baranyai T, Herczeg K, Onódi Z, et al (2015) Isolation of exosomes from blood plasma: Qualitative and quantitative comparison of ultracentrifugation and size exclusion chromatography methods. PLoS One 10:1–13

64.   Böing AN, van der Pol E, Grootemaat AE, Coumans FAW, Sturk A, Nieuwland R (2014) Single-step isolation of extracellular vesicles by size-exclusion chromatography. J Extracell Vesicles. https://doi.org/10.3402/jev.v3.23430

65.   Brennan K, Martin K, FitzGerald SP, O'Sullivan J, Wu Y, Blanco A, Richardson C, Mc Gee MM (2020) A comparison of methods for the isolation and separation of extracellular vesicles from protein and lipid particles in human serum. Sci Rep 10:1–13

66.   Vickers KC, Palmisano BT, Shoucri BM, Shamburek RD, Remaley AT (2011) MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. Nat Cell Biol 13:423–435

67.   Sun Y, Saito K, Saito Y (2019) Lipid profile characterization and lipoprotein comparison of extracellular vesicles from human plasma and serum. Metabolites 9:10–14

68.   Crewe C, Joffin N, Rutkowski JM, Kim M, Zhang F, Towler DA, Gordillo R, Scherer PE (2018) An Endothelial-to-Adipocyte Extracellular Vesicle Axis Governed by Metabolic State. Cell 175:695-708.e13

69.   Ramirez MI, Amorim MG, Gadelha C, et al (2018) Technical challenges of working with extracellular vesicles. Nanoscale 10:881–906

70.   Ronsein GE, Vaisar T (2019) Deepening our understanding of HDL proteome. Expert Rev Proteomics 16:749–760

71.   Collier TS, Jin Z, Topbas C, Bystrom C (2018) Rapid Affinity Enrichment of Human Apolipoprotein A-I Associated Lipoproteins for Proteome Analysis. J Proteome Res 17:1183–1193

72. Coumans FAW, Brisson AR, Buzas EI, et al (2017) Methodological guidelines to study extracellular vesicles. Circ Res 120:1632–1648

73. Zhang Y, Deng Z, Lou D, Wang Y, Wang R, Hu R, Zhang X, Zhu Q, Chen Y, Liu F (2020) High-efficiency separation of Extracellular Vesicles from Lipoproteins in Plasma by Agarose Gel Electrophoresis. Anal Chem. https://doi.org/10.1021/acs.analchem.9b05675

74. Müller MM, Griesmacher A (2000) Markers of endothelial dysfunction. Clin Chem Lab Med 38:77–85

75. Darabi M, Guillas-Baudouin I, Le Goff W, Chapman MJ, Kontush A (2016) Therapeutic applications of reconstituted HDL: When structure meets function. Pharmacol Ther 157:28–42

76. Karalis I, Jukema JW (2018) HDL Mimetics Infusion and Regression of Atherosclerosis: Is It Still Considered a Valid Therapeutic Option? Curr Cardiol Rep 20:1–8

Figure 1

HDL

EXTRACELLULAR VESICLES
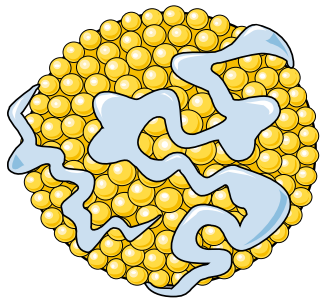
ANTI-INFLAMMATORY
Inhibition of adhesion molecule expression
Stimulation of nitric oxide synthesis

VASO-DILATORY
Stimulation of nitric oxide synthesis
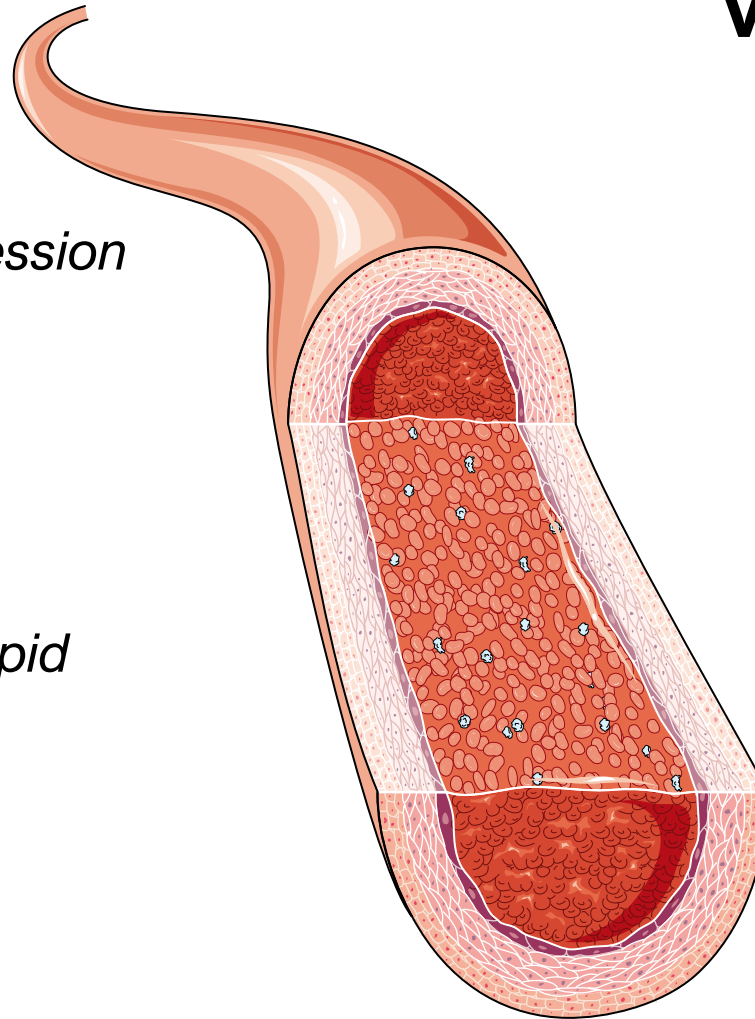
ANTI-OXIDANT
Inhibition of low-density lipoprotein, lipid and protein oxidation

ANTI-THROMBOTIC
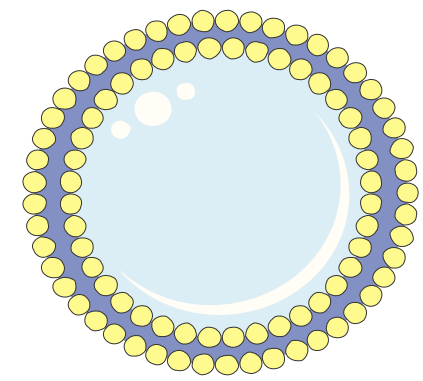*Platelet antagonism*
Vaso-dilation

ATHEROPROTECTIVE
Reverse cholesterol transport
All of the above.

BLOOD VESSEL

ANTI-INFLAMMATORY
Reduced monocyte activation
Cytokine/chemokine delivery
miRNA delivery

VASO-DILATORY
eNOS transfer
miRNA delivery

ANTI-OXIDANT
eNOS transfer

ATHEROPROTECTIVE
Prevents smooth muscle cell de-differentiation
miRNA delivery

Figure 2