Chapter

Structure of Lipoproteins and Their Capacity for Lipid Exchange: Relevance for Development of Atherosclerosis and Its Treatment by HDL Therapy

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Abstract

Atherosclerosis, the largest killer in the western world, arises from build-up of plaques at the artery walls and can result in cardiovascular disease. Low- and high-density lipoproteins are involved in the disease development by depositing and removing lipids to and from macrophages at the artery wall. These processes are complex and not fully understood. Thus, determining the specific roles of the different lipoprotein fractions involved is of fundamental importance for the treatment of the disease. In this chapter, we present the state of the art in lipoprotein structure with focus on the comparison between normolipidemic and hypertriglyceridemic individuals. Then we discuss lipid transfer between lipoproteins and receptor-free cellular membranes. Although these models lack any receptor, key clinical observations are mirrored by these, including increased ability of HDL to remove lipids, in contrast to the ability of LDL to deposit them. Also effects of saturated and unsaturated lipids in the presence and absence of cholesterol are revised. These models can then be used to understand the difference in functionality of lipoproteins from individuals showing different lipid profiles and have the potential to be used also for the development of new HDL therapies.

Keywords: lipoprotein structure, SAXS, lipid exchange, lipid transfer, neutron scattering

1. Introduction

This chapter starts by discussing the main compositional and structural properties of lipoproteins, which are water-soluble, heterogeneous nanoparticles responsible for carrying lipids, cholesterol and triglycerides in the body. The differences in their composition and structure are strictly related to how the different lipoproteins are produced and what their roles are in the body. We then move on to discuss the relationship between how lipoprotein type and lipoprotein subclass relate with the risk to develop atherosclerosis. We review recent evidence that suggests differences in low-density lipoprotein (LDL) overall size, shape and protein layer thicknesses within small dense LDL subfractions of normolipidemic and hyper triglyceridemic

individuals. This is of importance since structural differences across a certain lipoprotein class or subclass are not taken into account in clinical studies and this might explain controversies in the role of, for example, small dense LDL in the development of atherosclerosis. We then move on to discuss how lipoprotein capacity for lipid transfer and lipid exchange has been studied along the years and focus on recent results that quantify these abilities using simplistic model membranes lacking specific receptors. Despite the simplicity of these model systems, the results mirror those obtained for cholesterol efflux and in clinicals studies. Finally, we discuss advances in plaque remodelling therapies based on the engineering of nanoparticles mimicking nascent high-density lipoprotein (HDL) particles with focus on the challenges for the formulation of therapies that are effective in the clinics.

2. Lipoproteins are nanoparticles, made of lipids and apolipoproteins

2.1 Lipoprotein metabolism

Lipoproteins are the carriers of fat in the body. Plasma lipoproteins are secreted mostly by the liver and the intestine, whereas lipoproteins carrying lipids in the central nervous system are secreted mainly by the glial cells [1]. In **Figure 1**, the two ways by which plasma lipoproteins are formed are summarised. Chylomicrons, the largest and the least dense lipoprotein type, are produced in the intestine after a meal. Their size varies and depends on the amount of fat consumed during a meal. For instance, during fasting, chylomicrons are small and contain low amounts of triglycerides whereas a high-fat meal will result in the formation of larger particles,

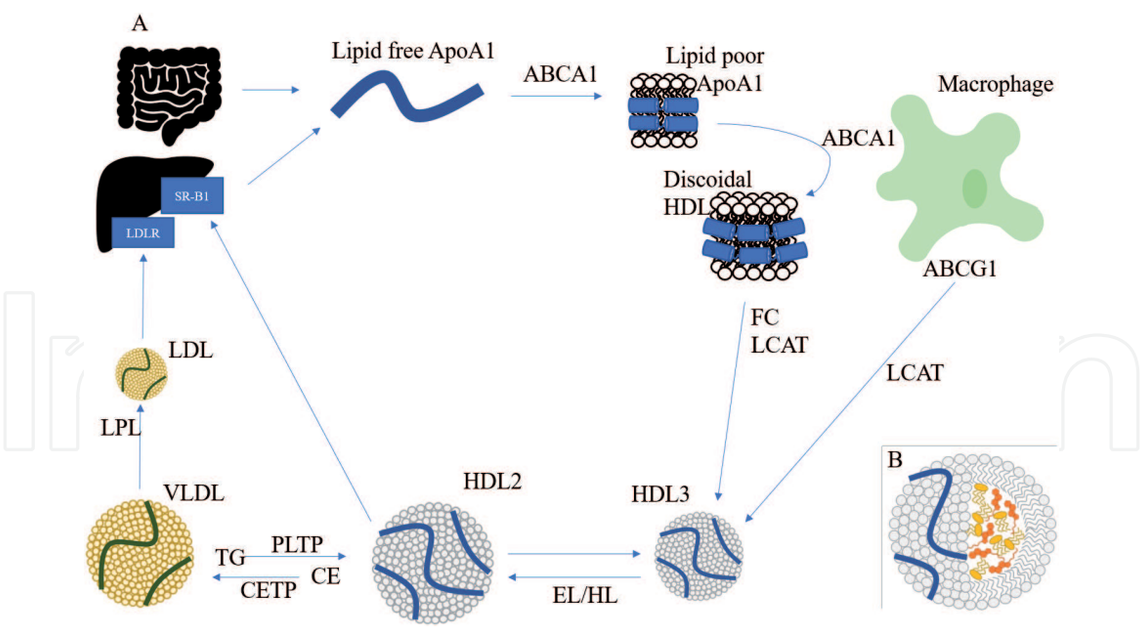

Figure 1.
(A) Lipoprotein metabolism: Lipid-free ApoA1 is produced by both the intestine and the liver. It gains phospholipids and cholesterol via *the ATP binding cassette A1 (ABCA1) to form lipid-poor ApoA1; it gains further lipids from peripheral tissues to form nascent discoidal HDL, which obtains free cholesterol (FC) from macrophages* via *interaction with ABCA1 and ABCG1 transporters. The FC is esterified by lecithin-cholesterol acyltransferase (LCAT) to form mature spherical HDL. Mature HDL can interact with the scavenger receptor class B type-1 (SR-B1) in the liver resulting in the exchange of unesterified cholesterol in both directions. Cholesteryl esters (CE) are transferred to VLDL* via *the cholesteryl ester transfer protein (CETP) for eventual uptake by the LDLR in the liver. The progression from VLDL to LDL occurs* via *hydrolysis by lipoprotein lipases (LPL). Phospholipids and triglycerides (TG) are transferred to HDL from VLDL* via *the phospholipid transfer protein (PLTP) resulting in HDL remodelling. Hepatic and endothelial lipases (HL/EL) also promote HDL remodelling. Insert: B. lipoprotein structure with a core of cholesterol esters and triglycerides.*

rich in triglycerides [2]. They undergo hydrolysis of triglycerides resulting in chylomicron remnants which are cleared *via* the liver [3]. Then, very low-density lipoproteins (VLDL) are produced in the liver. VLDL are the next least dense lipoprotein type, rich in triglycerides and their size depends on the production of triglycerides in the liver: the higher the production, the larger the secreted VLDL. These particles are hydrolysed in muscle and adipose tissues by lipoprotein lipases (LPL) resulting in the removal of the triglycerides and the formation of intermediate density lipoprotein (IDL), which are lipoprotein particles rich in cholesterol [2]. Further hydrolysis *via* LPL occurs and results in the formation of LDL, which is even richer in cholesterol and accounts for the principal carrier of cholesterol in circulation.

HDL formation and metabolism, on the other hand, starts with the production of apolipoprotein A1 (ApoA1) in the intestine and liver [4]. ApoA1 initial lipidation starts with the addition of both phospholipids and cholesterol from peripheral tissues *via* its interaction with the ATP-binding cassette A1 (ABCA1) transporter. Further lipid uptake mediated by ABCA1 and ABCG1 transporters leads to the formation of the "nascent", discoidal HDL. Free cholesterol is then esterified by lecithin-cholesterol acyltransferase (LCAT), resulting in the formation of mature, spherical HDL with a core full of cholesterol esters [4]. Both nascent and mature HDL can interact with the scavenger receptor class B type-1 (SR-B1) in the liver and undergo transfer of cholesterol esters towards SR-B1 and exchange of unesterified cholesterol in both directions [5]. The transfer of cholesterol esters also occurs *via* the cholesteryl ester transfer protein (CETP) to VLDL and LDL for eventual uptake by the LDL receptor (LDLR) in the liver [6]. The transfer of phospholipids and triglycerides from VLDL to HDL is facilitated by phospholipid transfer protein (PLTP), resulting in HDL remodelling. The hydrolysis of HDL phospholipids and triglycerides *via* hepatic and endothelial lipases (HL and EL) also results in HDL remodelling [7].

2.2 Lipoprotein composition

Lipoproteins are very heterogeneous in composition and differ in terms of their proportions of constituent proteins, cholesterol/cholesteryl esters, triglycerides and phospholipids (**Table 1**). The proteins responsible for the stability of the lipoprotein structure and their function in lipid transport and metabolism are known as apolipoproteins. Apolipoproteins vary in their size and overall structure, but they share a structure rich in amphipathic alpha helices, which are prone to interact with lipids and fats in aqueous environments such as plasma. In general, the larger the particle the higher the content ratio between lipids to proteins, which makes them less dense. Lipoproteins differ also in the main apolipoprotein they present. Apolipoprotein B100 (ApoB100) [9] is present only in VLDL, IDL

	VLDL	LDL	HDL
Diameter/ nm	30–80	18–25	5–12
Cholesterol/% (w/w)	~6	~7	~4
Phospholipids/% (w/w)	~17	~21	~29
Triglycerides/% (w/w)	~55	~6	~4
Protein/% (w/w)	~8	~20	~50

Table 1.
Biochemical composition and size of major lipoprotein types [8].

and LDL, since they are all formed from chylomicrons. ApoB100 is one of the largest proteins known (~4500 residues) and therefore is irreversibly bound to these particles. The rest of the apolipoproteins are reversible and exchange between the different lipoprotein types. For example, Apolipoprotein C (ApoC) and Apolipoprotein E (ApoE) are commonly found in VLDL and LDL [10]. The main protein present in HDL is ApoA1, contributing to about 70% of total protein content in all HDL [10]. The second most abundant is Apolipoprotein A2 (ApoA2) [10] followed by a combination of various other proteins including ApoC, E and Apolipoprotein J. While almost all HDL contain ApoA1 [11], the remaining apolipoproteins vary across different HDL types and subclasses [10].

2.3 Lipoprotein classification

As discussed previously, lipoprotein types vary drastically in their biochemical composition, which determines their size and density (**Table 1**). To further increase the degree of complexity, there are more subdivisions to these main categories. For example, using ultra-centrifugation, LDL can be subdivided in six subfractions which range from the large buoyant LDL (lbLDL) subfractions to the small dense LDL (sdLDL) subfractions [12, 13]. As their names suggest, these subfractions differ in size and density. For HDL, there are generally five distinct subpopulations with slight variations in composition, but most notably differences in size and density. HDL was first described by differences in density according to ultra-centrifugation techniques categorising HDL into two distinct groups [14]: i) HDL2, which is lower in density due to a higher lipid content (1.063–1.125 g mL^{-1}), and ii) HDL3, which is slightly higher in density owing to its higher protein content (1.125–1.21 g mL^{-1}). These groups can be further categorised by their size, using polyacrylamide gradient gel electrophoresis (GGE) resulting in five additional subclasses ranging in size from 7.2–12.0 nm in diameter [15]. By means of GGE, HDL particles can also be classified based on their surface charge into particles that migrate to either pre- β - or α-positions. Pre- β-position corresponds to lipid-free ApoA1, lipid-poor ApoA1, and most discoidal HDLs, whereas spherical, mature HDL migrate to α -position [16].

2.4 Lipoprotein structure

The structure of lipoproteins was studied already in the late 1970s by small angle X-ray scattering (SAXS) [13, 17–19] and in the early 1980s by small angle neutron scattering (SANS) [20]. It was then proposed their core-shell structure today widely accepted: lipoproteins are nanoparticles that consist of a core of cholesterol esters and triglycerides, with an outer monolayer of lipids and cholesterol, all encased by apolipoproteins. For the cholesterol richer LDL, a gel-liquid phase transition occurs below body temperature, T_m. In the early SAXS-based structure, the core was thought to organise in cholesteryl rich concentric layers maintaining a spherical structure [13] below T_m. More recently, cryogenic transmission electron microscopy (cryo-EM) was used to demonstrate that LDL particles are ellipsoidal having a stack of parallel cholesteryl ester layers below T_m [21–23]. The SAXS model for LDL was then revised in 2017 to account for LDL super-ellipsoid shape and planar lamellar ordered cholesteryl ester layer packing in the core below T_m (**Figure 2A**), and spherical LDL with the melted core above T_m [25] (**Figure 2B**). In contrast to cryo-EM, SAXS allows the probing of a large number of LDL particles in a relatively short time and in a physiological-like environment. Therefore, larger and systematic studies can be done providing a range of structural detail that is unprecedented: not only the size but also the shape of the overall particle can be determined, as well as the inner structure of the core and the outer protein shell.

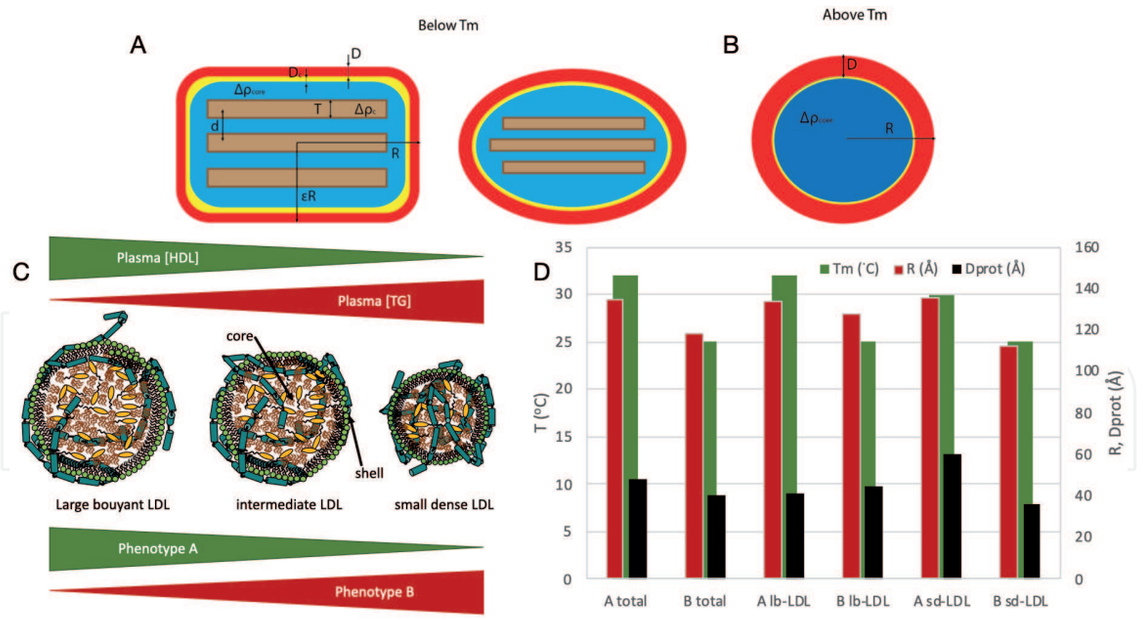

Figure 2.
The updated LDL model based on SAXS data below (A) and above T_m *(B) describes a superellipsoid of revolution or a sphere with core-shell structure, respectively. LDL phenotype A described a LDL sample enriched with lbLDL while phenotype B describes a LDL fraction enriched with sdLDL. Phenotype B is linked to a higher risk to develop atherosclerosis (C) Some structural parameters for total LDL fractions as well as LDL subfractions lbLDL and sdLDl for subject A presenting normal lipid serum values and subject B presenting high triglyceride serum values (D) Data in D is taken from Jakubauskas* et al *2020 [24].*

3. Lipoproteins and atherosclerosis

Atherosclerosis is a chronic inflammatory disease considered to be the largest killer in western countries [26]. It is characterised by the plaque build-up in artery walls and can lead to cardiovascular diseases (CVD), giving rise to ischemic heart disease and strokes [27]. It is accepted that structural and compositional differences between HDL and LDL play a large role in their contributions to atherosclerosis [28]. The plaque build-up originates from LDL deposition into the artery walls [29], which are then oxidised and cause the upregulation of adhesion molecules on the surface of the endothelium of the arteries and the recruitment of monocytes to the forming lesion [30]. These then differentiate in macrophages which express scavenger receptors on their surface that mediate the uptake of even more LDL. This leads to the transformation of macrophages into foam cells, and the formation in the arteries of plaques that consist of apoptotic and necrotic macrophages, cholesterol crystals and other extracellular components [31]. Upon the rupture of these plaques, thrombus material enters the blood stream and can lead to heart attacks or other CVD related phenomena. As opposite to the role of LDL in atherosclerosis development, HDL have been instead shown to play a preventative role by a process known as reverse cholesterol transport (RCT) [32]. By this process, cholesterol is removed from the lipid-filled foam cells at the artery wall and deposited in the liver where it is cleared from the body [33–35]. The cholesterol removal occurs *via* efflux by various means, including passive aqueous diffusion or active, receptor/transporter mediated transfer [36]. Additionally, the presence of HDL has been shown to prevent the oxidation of the LDL and therefore helps prevent the development to atherosclerosis [37]. While both lipoprotein types are characterised by a lipid binding activity, their abilities to exchange lipids and to transport cholesterol differ drastically. Indeed, LDL mainly deposit lipids and cholesterol to artery walls, whereas the main role of HDL in the blood is to catalyse cholesterol efflux thereby removing excess cholesterol from macrophages

trapped at artery walls [28]. Therefore, HDL and LDL are commonly known as 'good' and 'bad' cholesterol respectively.

There are several controversies regarding the role of HDL and LDL in the development of atherosclerosis. On one hand, increased levels of HDL are correlated to reduced atherosclerotic risk due to increased RCT efficiency [35] and, in some other cases, shown to have a neutral [38] or even negative correlation [39] to the prevention of atherosclerotic development. On the other hand, high LDL levels are related to high risk for development of atherosclerosis even though the total cholesterol LDL concentration often fails as a CVD biomarker [40]. This could be due to the pro-atherogenic effect of LDL [41] being counteracted by the anti-atherogenic effect of HDL [42]. Indeed, the ratio between LDL and HDL has been suggested as a better biomarker than their individual serum levels [40].

3.1 Different atherogenic capacity of lipoprotein subclasses

The LDL subclass distribution varies significantly within the population with two main LDL phenotypes [43]: the healthy one (phenotype A), enriched with lbLDL subfractions, and the unhealthy one (phenotype B), enriched with sdLDL subfractions [44] (**Figure 2C**). LDL size is an alleged marker for CVD risk since there is a positive correlation between LDL size and HDL concentration, and a negative correlation between LDL size and triglyceride concentration [43]. However, it is unclear whether it is the LDL concentration or their particle size which is the key parameter to predict CVD risk. Interestingly, there is some genetic, age and sex related predisposition for sdLDL. For example, a low-fat diet can contribute to a phenotype B in individuals with a genetic predisposition for sdLDL [45].

Recently, sdLDL fractions of normal and hyper triglyceridemic subjects were shown to have similar biochemical composition but different sizes and protein rich shell layer thicknesses [24] (**Figure 2D**). This suggests that LDL size and structure are not directly a consequence of composition and diet, mirroring the data found for individuals with a predisposition for sdLDL [45]. In particular, the protein rich shell layer thickness seems a very important parameter to consider since the protein conformation within this layer is determinant for further interaction with receptors and other biomolecules in the body. These structural analyses raise the following questions: are sdLDL subclasses from healthy and hyper triglyceridemic subjects equally pro-atherogenic? If not, is this due to the differences in the presence of specific apolipoproteins or post translational modifications in the protein-rich shell?

Finally, measuring total plasma cholesterol concentrations in clinics is *per se* insufficient to reliably determine the LDL atherogenic potential in a particular individual, as the LDL size-distribution component is not accounted for. The detailed understanding of LDL ultrastructure seems necessary to evaluate the atherogenicity of the individual LDL subfractions with the ultimate aim to develop effective diagnostic and therapeutic tools against CVD. Indeed, the SAXS analysis of the total LDL fraction and its subfractions shows that SAXS has the potential to discern between LDL phenotypes directly from the total LDL measurement [24] (**Figure 2D**), which could be used to extract information on the sdLDL structural parameters between subjects with a range of clinical conditions.

The role of the different HDL subclasses in the development of atherosclerosis is less studied and the results are controversial. A 2015 study found no evidence for any changes in HDL subclasses profiling for individuals with nonalcoholic fatty liver disease (NAFLD) [46], a disease associated with increased cardiometabolic risk. Another study found that HDL2b subclass was enriched in Hispanic women with LDL phenotype B [47]. Preliminary results from our group suggest that HDL main fractions and subfractions size differs between normolipidemic and

hypertriglyceridemic individuals. These contrasting results indicate that more research is needed to understand the differences in HDL subclasses across various lipid metabolism disorders. Differences in HDL and LDL subclass structure rather than their relative proportion might be relevant for their function and their role in the development of atherosclerosis. Structural studies in combination with detailed compositional analysis by means of lipidomics and proteomics, as well as post-translational modifications, are needed to advance the field.

4. Determining lipid exchange capacity by lipoproteins

As already discussed, LDL function is counteracted by HDL function, and lipid exchange is a key process for their functions. Lipid exchange is known to be independent of protein exchange [48–51]. Lipid exchange (where no net transfer takes place) and lipid transfer between lipoproteins of different types (*i.e.* between HDL and LDL) are known to occur, both *in vitro* and *in vivo*, for phospholipids, cholesterol, and sphingomyelin [52–54]. It also occurs between lipoproteins and lipid microemulsions [20, 55], lipid vesicles [56, 57], and cells [58]. The older studies used chemical analysis and focused on net changes in lipoprotein particle composition upon a given equilibration time. Therefore, these studies did not monitor the exchange processes directly. Only the work by Maric *et al.* [57] measured the kinetics of lipid exchange directly between lipoproteins and lipid vesicles making use of deuterated lipids and SANS.

It is well accepted that there are several pathways for lipid transfer and cholesterol uptake. The main pathway is through receptor-mediated endocytosis, in which lipoprotein binding to LDL receptors takes place for ApoB100 and ApoE containing lipoproteins [59, 60]. Another mechanism involves scavenger receptor class B family (SR-B) receptors [61], that mediate the selective core lipid transfer from lipoprotein particles to cells and tissues. Lipid exchange and lipid transfer can potentially occur *via* lipoprotein endocytosis and subsequent transcytosis in endothelial cells [62, 63]. Finally, direct lipid exchange or lipid transfer from lipoprotein particles to the cell membrane also occurs, as discussed above. Recently, spontaneous lipid transfer to receptor free model membranes was demonstrated by cryo-EM, fluorescence cross correlation spectroscopy and spectral imaging, regardless of lipoprotein type [64]. In particular, lipid transfer affected the model membrane properties, with HDL having the most dramatic effect in lipid packing and collective lipid diffusion.

4.1 Quantification of lipid exchange by lipoproteins

Quantification of lipid exchange and transfer is challenging, and examples were scarce in literature until recently. Browning *et al*. [65] presented a new protocol to follow interactions of human lipoproteins with model membranes using neutron reflection. HDL and LDL from healthy male adults were incubated with model phospholipid membranes in physiological-like conditions. The study showed that HDL was able to remove more lipids from the model membranes than LDL, whilst LDL deposited more lipids than HDL. The lipid exchange studies on simplified model systems mirrored the function of lipoproteins in the body!

Following reports using the same protocol showed that the inclusion of charged lipids in the model membranes led to unaffected quantity of lipids deposited but increased lipid removal by HDL [66]. The lipid exchange did not seem to affect the lipoprotein structure, as determined by SANS [57]. Then, the ability of the level of lipid unsaturation and of the presence of cholesterol to affect the lipid exchange capacity of lipoproteins was investigated [67]. The presence of acyl chain

unsaturation dramatically decreased the quantities of lipids deposited and removed by the lipoproteins, regardless of the lipoprotein type (**Figure 3**). In other words, HDL and LDL have a greater affinity for saturated rather than unsaturated lipids, though LDL to a lesser extent. The difference in ease of lipid removal between the lipoprotein types could be explained by variance in the specific protein-lipid interactions. Different conformations of ApoA1 (the main protein in HDL) were shown to have varying binding affinities to unsaturated lipid vesicles [68]. The increased ease for saturated lipid removal is likely due to the increased mobility of said lipids within the fluid model membrane: saturated phospholipids were shown to have greater mobility than their unsaturated counterparts in both gel and fluid phases [69].

The presence of cholesterol also had a large impact on the amounts of lipids exchanged and removed [67] (**Figure 3**). In the bilayers comprising saturated lipids, the presence of cholesterol decreased the amounts of lipids exchanged and removed through HDL whereas, in the case of the unsaturated lipids, the presence of cholesterol made little difference when interacting with HDL. LDL, on the other hand, did not follow the same pattern. Indeed, including 10 mol% cholesterol in the saturated bilayer increased the amount of lipids exchanged. Upon further increasing to 20 mol%, a similar value was found to that of the saturated bilayer alone. For the unsaturated model membranes, the presence of 20 mol% cholesterol also gave similar values to that of the bilayer alone. However, in terms of removal, for both the saturated and unsaturated bilayers, the presence of cholesterol increasingly reduced the quantity of lipids removed. It is possible that the presence of cholesterol may inhibit the mobility of phospholipids [70] and cause it to localise preferentially towards saturated lipids [71]. However, there were no clear systematic changes

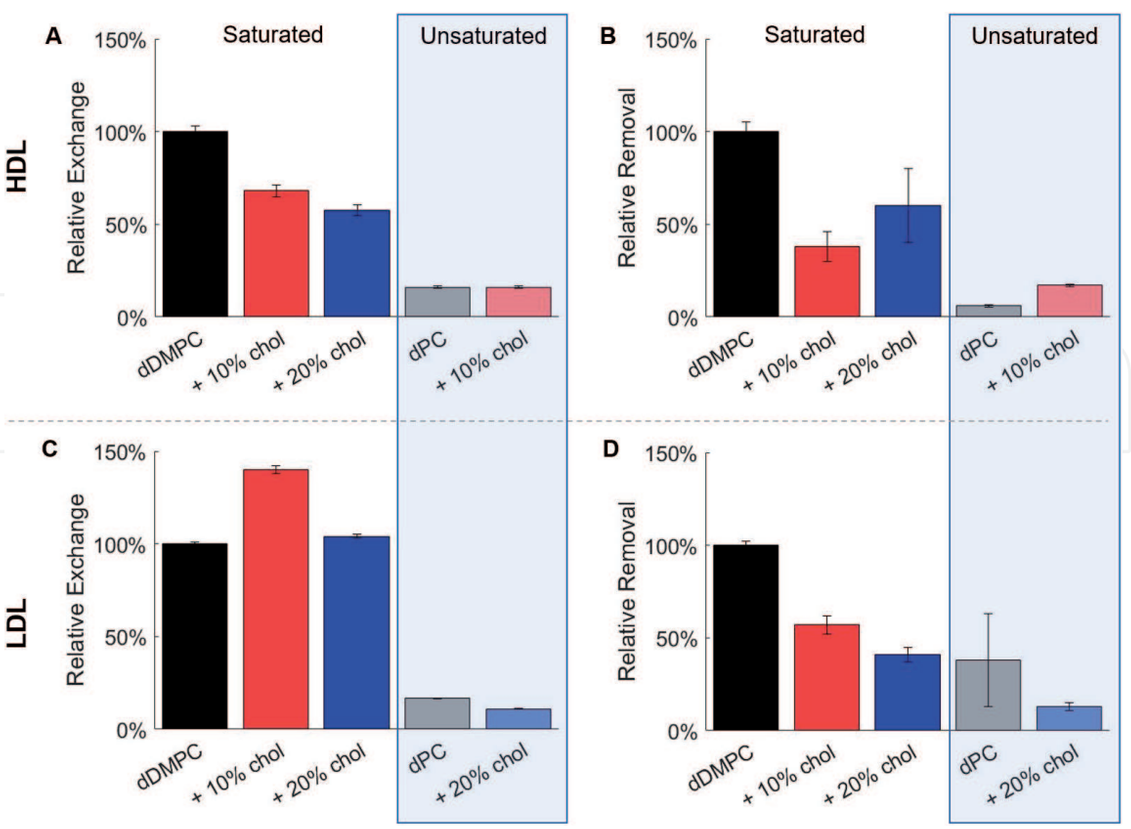

Figure 3.
Relative lipid exchange and removal for HDL or LDL particles interacting with membranes formed with saturated (dDMPC) and unsaturated (dPC) phospholipids with and without cholesterol. Results are shown for HDL (A, B) and LDL (C, D) regarding exchange (A, C) and removal (B, D). Replotted with permission from Waldie et al *2020 [67].*

when cholesterol was incorporated into the bilayer, thus it is difficult to pinpoint the exact cause of the changes seen. LDL has also been shown to preferentially bind to rafts of saturated lipid with cholesterol [72]. Since no reduction or increase in exchange was seen due to cholesterol, it is likely that the specific lipoprotein-lipid interactions determine the final quantities of lipids exchanged and removed.

To further look into this, LDL with differing compositions were used against the model membranes. It was found that with increasing density of LDL, *i.e.* increasing percentage of protein, greater levels of exchange were observed [67]. These findings can be explained by the difference in ApoB100 (the main protein in LDL) found on smaller, more dense LDL particles. Denser LDL particles contain regions with altered epitopes [73] that may house part of the lipid-binding region of LDL [74] and thus affect their binding and interactive activities.

Access to the neutron beam is limited and highly specialised personnel are needed for these experiments. Methodology based on attenuated total reflection Fourier transfer infrared spectroscopy (ATR-FTIR) seems an accessible complementary alternative method to determine lipid exchange [75], which is far more accessible and exists in many laboratories as a benchtop technique.

5. Plaque remodelling by HDL therapy

As already discussed in Section 2, and as shown by Framingham Heart Study [76, 77] and later on by the Atherosclerosis Risk in Communities study [78], there is strong inverse association between HDL-cholesterol levels and CVD risk. Changes in lifestyle, such as weight loss, increase of physical activity, reduction in saturated fat intake, and low alcohol intake can alone increase HDL-cholesterol levels by up to 20%. However, lifestyle changes alone are not sufficient to prevent and reduce mortality in atherosclerosis. Thus, over the years there have been many pharmacological interventions aimed at raising circulatory HDL-cholesterol levels while decreasing LDL-cholesterol levels [79–81]. Nevertheless, these interventions have not resulted in the expected reduction in CVD events, suggesting that HDL functionality, rather than changes in HDL levels *per se* might have a role in the disease progression. For this reason, the focus on anti-atherosclerotic solutions shifted to therapies that use the ability of HDL and its main apolipoprotein (ApoA1) to remove cholesterol from macrophages and that have thus the potential to reduce the plaques size and the steric burden at the arterial wall. One of these approaches led to the use of ApoA1 Milano, a natural variant of ApoA1 endowed with higher ability to stimulate cholesterol efflux and better anti-inflammatory and plaque-stabilising properties with respect to the wild-type protein [82]. Infusions of the Milano variant in complex with phospholipids (ETC-216) were used in patients with high CVD risk and resulted in the reduction of the atheroma volume in one study [83] but a new formulation (MDCO-216) did not result in plaque regression in patients with acute coronary syndromes and on statin treatment. Treatments based on wild-type ApoA1 have also been explored. CER-001 is a formulation of human recombinant ApoA1, sphingomyelin and phospholipid. It regressed atherosclerosis and increased RCT in mice [84] but failed to regress plaques in patients on statins with acute coronary syndrome and high plaque burden [85]. Short-term infusions of another formulation of HDL with wild-type ApoA1 and phospholipids (CSL111) showed improvement in the plaque characterisation index and quantitative coronary score, but the positive effects were accompanied by hepatotoxicity, thus the clinical development halted [86]. Later on, a new formulation (CSL-112) was developed, and its administration has been found to increase the RCT with no adverse effects in humans so far [87]. A phase III clinical study is currently ongoing and is expected

to conclude in 2022 (NCT03473223), assessing the potential benefits of CSL112 in reducing adverse cardiovascular events in subjects with acute coronary syndrome.

ApoA1 mimetic peptides have also been explored in the treatment of the adverse effects of atherosclerosis. These have the advantage to be structurally simpler than the native full-length protein, although retaining the same biological functions, and have the potential to be administered orally instead of *via* injection. One of these ApoA1 mimetic peptides is Rev-D4F, which reduced atherosclerotic lesion area and macrophage content at the lesion site in $Apoe^{-/-}$ mice, while also decreasing LDL oxidation [88]. Another ApoA1 mimetic, RG54, showed increased glucose tolerance, was able to stimulate cholesterol efflux from macrophages, and prevented the formation of atherosclerotic plaques in $Apoe^{-/-}$ mice [89]. Finally, 2F*, a photoactivatable ApoA1 mimetic peptide, was able to increase cholesterol efflux in stably transfected baby hamster kidney cells [90]. Although very promising, these peptides have been explored only from a pre-clinical point of view and their efficacy in humans and lack of side effects have still to be proved.

Another apolipoprotein that has been suggested as a good candidate for HDL therapy is ApoE. ApoE is well known for its atheroprotective properties including the ability to induce RCT from peripheral cells to the liver [91] and to stimulate cholesterol efflux from macrophages thus, in turn, preventing the formation of foam cells in the development to atherosclerosis [92, 93]. Various studies have shown ApoE to have an increased ability to protect against atherosclerosis compared to ApoA1 [94]. The Ac-hE18A-NH2 ApoE mimetic has been shown to have a superior ability than the 4F ApoA1 mimetic to reduce atherosclerotic lesions in $Apoe^{-/-}$ mice [95]. The Ac-hE18A-NH2 mimetic has also demonstrated more effective anti-inflammatory properties than the 4F mimetic [96]. Most studies that have been carried out with the use of ApoE peptide mimetics are only in the pre-clinical trial stage and have not yet been tested in clinical studies for therapeutic use [94].

The variability of the outcomes of the pre-clinical and clinical studies described above points towards the need for a deeper understanding of the functionality (RCT and fat exchange, for example) and the structure of the HDL particles before considering them as a potential therapeutic for CVD. For example, a very recent study shows that the type of lipids used in the rHDL formulation has a significant effect on their ability to mediate cholesterol efflux [97], with saturated lipids having the greatest potential for cholesterol efflux. This mimics the results for fat exchange on simplistic models presented in **Figure 3** and discussed in Section 4.1 in which saturated fats are more easily taken up by lipoproteins than unsaturated ones or those in the presence of cholesterol. Thus, not only the type of apolipoprotein but also the type of lipids used in the formulation have a fundamental role in the functionality of the rHDL and need to be further explored.

6. Conclusions

In this chapter we discussed the composition, function and structure of the two most studied lipoprotein types: LDL and HDL. In particular, we presented an updated model for determining the ultrastructure of LDL based on SAXS data that potentially enables determination of LDL phenotype from the total fraction measurement while highlighting structural differences in the small dense LDL subfraction between individuals with normal and high plasma serum triglyceride levels. Such differences, unknown until recently, may explain the different atherogenic potential of small dense LDL subfractions between different individuals and help unravel the controversies related to their atherogenic potential. Moreover, the capacity of lipoprotein fractions and subfractions to transfer lipids and cholesterol

might be linked to these structural differences. Therefore, techniques that enable quantifying lipid exchange and lipid transfer in a reproducible and systematic manner are needed. We present recent data by fluorescence spectroscopy and neutron scattering showing how model cellular membranes lacking receptors can be used in the quantification of lipid exchange and lipid transfer by lipoproteins. In particular, the methodology might be especially useful when designing HDL therapy nanoparticles which require efficient removal of both saturated lipids and cholesterol from atherosclerotic plaque. Even though HDL therapy is quite promising in pre-clinics, it still has to show its potential in clinical studies.

Acknowledgements

The authors thank the Swedish Research Council for funding.

Conflict of interest

The authors declare no conflict of interest.

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References

[1] Ladu MJO, Reardon C, Eldik LVAN, Getz GS. Lipoproteins in the Central Nervous System. Ann N Y Acad Sci. 2009;903:167-75.

[2] Feingold KR, Grunfeld C. Introduction to Lipids and Lipoproteins [Internet]. Endotext. 2000. 1-19 p. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26247089

[3] Almer G, Mangge H, Zimmer A, Prassl R. Lipoprotein-Related and Apolipoprotein-Mediated Delivery Systems for Drug Targeting and Imaging. Curr Med Chem. 2015;22(31):3631-51.

[4] Zannis VI, Chroni A, Kypreos KE, Kan HY, Cesar TB, Zanni EE, et al. Probing the pathways of chylomicron and HDL metabolism using adenovirus-mediated gene transfer. Curr Opin Lipidol. 2004;15(2):151-66.

[5] Zannis VI, Chroni A, Krieger M. Role of apoA-I, ABCA1, LCAT, and SR-BI in the biogenesis of HDL. J Mol Med. 2006;84(4):276-94.

[6] Hafiane A, Genest J. HDL, Atherosclerosis, and Emerging Therapies. Cholesterol. 2013;2013: 891403.

[7] Kardassis D, Mosialou I, Kanaki M, Tiniakou I, Thymiakou E. Metabolism of HDL and its Regulation. Curr Med Chem. 2014;21(25):2864-80.

[8] Rensen PCN, Vrueh RLA De, Kuiper J, Bijsterbosch MK, Biessen EAL, Berkel TJC Van. Recombinant lipoproteins: lipoprotein-like lipid particles for drug targeting. Adv Drug Deliv Rev. 2001;47:251-76.

[9] Jonas A. Lipoprotein structure. In: Biochemistry of Lipids, Lipoproteins and Membranes. 2002. p. 483-504.

[10] von Eckardstein A, Kardassis D. High Density Lipoproteins. Vol. 224, Handbook of Experimental Pharmacology. 2015.

[11] Sacks FM, Jensen MK. From high-density lipoprotein cholesterol to measurements of function: Prospects for the development of tests for high-density lipoprotein functionality in cardiovascular disease. Arterioscler Thromb Vasc Biol. 2018;38(3):487-99.

[12] Austin MA, Breslow JL, Hennekens CH. Low-Density Lipoprotein Subclass Patterns and Risk of Myocardial Infarction. JAMA. 1988;260(13):1917-21.

[13] Baumstark MW, Kreutz W, Berg A, Frey I, Keul J. Structure of human low-density lipoprotein subfractions determined by X-ray small-angle scattering. BBA - Protein Struct Mol Enzymol. 1990;1037(1):48-57.

[14] de Lalla OF, Gofman JW. Ultracentrifugal Analysis of Serum Lipoproteins. In: Methods of Biochemical Analysis. 1954. p. 459-78.

[15] Nichols A V, Krauss RM, Musliner TA. Nondenaturing Polyacrylamide Gradient Gel Electrophoresis. Methods Enzymol. 1986;128:417-31.

[16] Castro GR, Fielding CJ. Early Incorporation of Cell-Derived Cholesterol into Pre-B-Migrating High-Density Lipoprotein. Biochemistry. 1988;27:25-9.

[17] Laggner P, Degovics G, Müller KW, Glatter O, Kratky O, Kostner G, et al. Molecular Packing and Fluidity of Lipids in Human Serum Low Density Lipoproteins. Hoppe Seylers Z Physiol Chem. 1977;358:771-8.

[18] Atkinson D, Deckelbaum RJ, Small DM, Shipley GG. Structure of

human plasma low-density lipoproteins: Molecular organization of the central core. PNAS. 1977;74(3):1042-6.

[19] Tardieu A, Mateu L, Sardet C, Weiss B, Luzzati V. Structure of Human Serum Lipoproteins in Solution II. Small-angle X-ray Scattering Study of HDL3 and LDL. J Mol Biol. 1976;101:129-53.

[20] Laggner P, Kostner GM, Rakusch U, Worcester D. Neutron Small Angle Scattering on Selectively Deuterated Human Plasma Low Density Lipoproteins. J Biol Chem. 1981;256(22):11832-9.

[21] Liu Y, Luo D, Atkinson D. Human LDL core cholesterol ester packing : three-dimensional image reconstruction and SAXS simulation studies. J Lipid Res. 2011;52:256-62.

[22] Orlova E V., Sherman MB, Chiu W, Mowri H, Smith LC, Gotto Jr AM. Three-dimensional structure of low density lipoproteins by electron cryomicroscopy. PNAS. 1999;96(July):8420-5.

[23] Kumar V, Butcher SJ, Öörni K, Engelhardt P, Heikkonen J, Kaski K, et al. Three-Dimensional cryoEM Reconstruction of Native LDL Particles to 16Å Resolution at Physiological Body Temperature. PLoS One. 2011;6(5):e18841.

[24] Jakubauskas D, Jansen M, Lyngsø J, Cheng Y, Skov Pederson J, Cárdenas M. Toward reliable low-density lipoprotein ultrastructure prediction in clinical conditions : A small-angle X-ray scattering study on individuals with normal and high triglyceride serum levels. Nanomedicine Nanotechnology, Biol Med. 2021;31:102318.

[25] Maric S, Lind TK, Lyngsø J, Cárdenas M, Pedersen JS. Modeling Small-Angle X-ray Scattering Data for Low-Density Lipoproteins: Insights into the Fatty Core Packing and Phase Transition. ACS Nano. 2017;11(1):1080-90.

[26] World Health Organization. Cardiovascular Disease [Internet]. 2017. Available from: https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds)

[27] Rader DJ, Daugherty A. Translating molecular discoveries into new therapies for atherosclerosis. Nature. 2008;451(7181):904-13.

[28] Badimon L, Vilahur G. LDL-cholesterol versus HDL-cholesterol in the atherosclerotic plaque: Inflammatory resolution versus thrombotic chaos. Ann N Y Acad Sci. 2012;1254(1):18-32.

[29] Ference BA, Ginsberg HN, Graham I, Ray KK, Packard CJ, Bruckert E, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement fromthe European Atherosclerosis Society Consensus Panel. Eur Heart J. 2017;38(32):2459-72.

[30] Nakashima Y, Raines EW, Plump AS, Breslow JL, Ross R. Atherosclerosis-Prone Sites on the Endothelium in the. Arterioscler Thromb Vasc Biol. 1998;18:842-51.

[31] Park YM, Febbraio M, Silverstein RL. CD36 modulates migration of mouse and human macrophages in response to oxidized LDL and may contribute to macrophage trapping in the arterial intima. J Clin Invest. 2009;119(1):136-45.

[32] Ouimet M, Barrett TJ, Fisher EA. HDL and Reverse Cholesterol Transport. Circ Res. 2019;124(10):1505-18.

[33] Feig JE, Hewing B, Smith JD, Hazen SL, Fisher EA. HDL and Atherosclerosis Regression: Evidence

from Pre-clinical and Clinical Studies. Circ Res. 2014;114(1):205-13.

[34] Marques LR, Diniz TA, Antunes BM, Rossi FE, Caperuto EC, Lira FS, et al. Reverse cholesterol transport: Molecular mechanisms and the non-medical approach to enhance HDL cholesterol. Front Physiol. 2018;9(MAY):1-11.

[35] Rosenson RS, Brewer HB, Davidson WS, Fayad ZA, Fuster V, Goldstein J, et al. Cholesterol efflux and atheroprotection: Advancing the concept of reverse cholesterol transport. Circulation. 2012;125(15):1905-19.

[36] Yancey PG, Bortnick AE, Kellner-Weibel G, De la Llera-Moya M, Phillips MC, Rothblat GH. Importance of different pathways of cellular cholesterol efflux. Arterioscler Thromb Vasc Biol. 2003;23(5):712-9.

[37] Brites F, Martin M, Guillas I, Kontush A. Antioxidative activity of high-density lipoprotein (HDL): Mechanistic insights into potential clinical benefit. BBA Clin. 2017;8:66-77.

[38] 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(9841):572-80.

[39] Madsen CM, Varbo A, Nordestgaard BG. Extreme high high-density lipoprotein cholesterol is paradoxically associated with high mortality inmen and women: Two prospective cohort studies. Eur Heart J. 2017;38(32):2478-86.

[40] Fernandez ML, Webb D. The LDL to HDL Cholesterol Ratio as a Valuable Tool to Evaluate Coronary Heart Disease Risk. J Am Coll Nutr. 2008;27(1):1-5.

[41] Carmena R, Duriez P, Fruchart J. Atherogenic Lipoprotein Particles in Atherosclerosis. Circulation. 2004;109(Suppl III):2-7.

[42] Barter P. The role of HDL-cholesterol in preventing atherosclerotic disease. Eur Hear Journal, Suppl. 2005;7(F):4-8.

[43] Rizzo M, Berneis K. Low-density lipoprotein size and cardiovascular risk assessment. Q J Med. 2006;99:1-14.

[44] Kwiterovich PO. Clinical Relevance of the Biochemical , Metabolic , and Genetic Factors that Influence Low-Density Lipoprotein Heterogeneity. Am J Cardiol. 2002;90(8A):30-47.

[45] Dreon DM, Fernstrom HA, Williams PT, Krauss RM. LDL Subclass Patterns and Lipoprotein Response to a Low-Fat, High-Carbohydrate Diet in Women. Arterioscler Thromb Vasc Biol. 1997;17(4):707-14.

[46] Sonmez A, Nikolic D, Dogru T, Ercin CN, Genc H, Cesur M, et al. Low- and high-density lipoprotein subclasses in subjects with nonalcoholic fatty liver disease. J Clin Lipidol. 2015;9:576-82.

[47] Legro RS, Blanche P, Krauss RM, Lobo RA. Alterations in low-density lipoprotein and high-density lipoprotein subclasses among Hispanic women with polycystic ovary syndrome : influence of insulin and genetic factors. Fertil Steril. 1999;72(6):990-5.

[48] Bilheimer DW, Eisenberg S, Levy RI. The Metabolism of Very Low Density Lipoproteins I. Preliminary in vitro and in vivo Observations. BBA. 1972;260:212-21.

[49] Eisenberg S, Bilheimer DW, Levy RI. The Metabolism of Very Low Density Lipoproteins II. Studies on the Transfer of Apoproteins Between Plasma Lipoproteins. BBA. 1972;280:94-104.

[50] Eisenberg S, Rachmilewitz D. Metabolism of Rat Plasma Very Low Density Lipoprotein I. Fate in Cirulation of the Whole Lipoprotein. BBA. 1973;326:378-90.

[51] Eisenberg S, Rachmilewitz D. Metabolism of Rat Plasma Very Low Density Lipoprotein II. Fate in Circulation of Apoprotein Subunits. BBA. 1973;326:391-405.

[52] Illingworth DR, Portman OW. Independance of Phospholipid and Protein Exchange between Plasma Lipoproteins in vivo and in vitro. BBA. 1972;280:281-9.

[53] Kunkel HG, Bearn AG. Phospholipid Studies of Different Serum Lipoproteins Employing P32. Proc Soc Exp Biol Med. 1954;86(4):887-91.

[54] Illingworth DR, Portman OW. Exchange of phospholipids between low and high density lipoproteins of squirrel monkeys. J Lipid Res. 1972;13:220-7.

[55] Laggner P, Kostnert GM, Degovics G, Worcester DL. Structure of the cholesteryl ester core of human plasma low density lipoproteins : Selective deuteration and neutron small-angle scattering. Proc Natl Acad Sci U S A. 1984;81(July):4389-93.

[56] Jackson RL, Cardin AD, Barnhart RL, Johnson JD. Incorporation of Dipalmitoyl Phosphatidylcholine into Human Plasma Low Density Lipoproteins Effects on Composition and Structure. BBA. 1980;619:408-13.

[57] Maric S, Lind TK, Raida MR, Bengtsson E, Fredrikson GN, Rogers S, et al. Time-resolved small-angle neutron scattering as a probe for the dynamics of lipid exchange between human lipoproteins and naturally derived membranes. Sci Rep. 2019;9(7591):7591.

[58] Illingworth DR, Portman OW, Robertson AL, Magyar WA. The Exchange of Phospholipids Between Plasma Lipoproteins and Rapidly Dividing Human Cells Grown in Tissue Culture. BBA. 1973;306:422-36.

[59] Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. Science (80-). 1986;232(4746):34-47.

[60] Mahley RW, Ji Z. Remnant lipoprotein metabolism: key pathways involving cell-surface heparan sulfate proteoglycans and apolipoprotein E. J Lipid Res. 1999;40:1-16.

[61] Meyer JM, Graf GA, van der Westhuyzen DR. New Developments in Selective Cholesteryl Ester Uptake. Curr Opin Lipidol. 2014;24(5): 386-92.

[62] Zhang X, Sessa WC, Fernández-Hernando C. Endothelial Transcytosis of Lipoproteins in Atherosclerosis. Front Cardiovasc Med. 2018;5(September):130.

[63] Fung KYY, Lee W, Fairn G. Inhibition of Low density Lipoprotein Internalization and Transcytosis by HDL; an alternative role for "good" cholesterol. FASEB J. 2020;34:S1.

[64] Plochberger B, Sych T, Weber F, Novacek J, Axmann M, Stangl H. Lipoprotein Particles Interact with Membranes and Transfer Their Cargo without Receptors. Biochemistry. 2020;59:4421-8.

[65] Browning KL, Lind TK, Maric S, Malekkhaiat-Häffner S, Fredrikson GN, Bengtsson E, et al. Human Lipoproteins at Model Cell Membranes: Effect of Lipoprotein Class on Lipid Exchange. Sci Rep. 2017;7(7478).

[66] Browning KL, Lind TK, Maric S, Barker RD, Cárdenas M, Malmsten M. Effect of bilayer charge on lipoprotein lipid exchange. Colloids Surfaces B Biointerfaces. 2018;168:117-25.

[67] Waldie S, Sebastiani F, Browning K, Maric S, Lind TK, Yepuri N, et al. Lipoprotein ability to exchange and remove lipids from model membranes as a function of fatty acid saturation and presence of cholesterol. Biochim Biophys Acta - Mol Cell Biol Lipids. 2020;1865(10):158769.

[68] Tricerri MA, Sanchez SA, Arnulphi C, Durbin DM, Gratton E, Jonas A. Interaction of apolipoprotein A-I in three different conformations with palmitoyl oleoyl phosphatidylcholine vesicles. J Lipid Res. 2002;43(2):187-97.

[69] Nanda H, García Sakai V, Khodadadi S, Tyagi MS, Schwalbach EJ, Curtis JE. Relaxation dynamics of saturated and unsaturated oriented lipid bilayers. Soft Matter. 2018;14(29):6119-27.

[70] Orädd G, Lindblom G, Westerman PW. Lateral diffusion of cholesterol and dimyristoylphosphatidylcholine in a lipid bilayer measured by pulsed field gradient NMR spectroscopy. Biophys J. 2002;83(5):2702-4.

[71] Pitman M, Suits F, MacKerell A, Feller S. Molecular-Level Organization of Saturated and Polyunsaturated Fatty Acids in a Phosphatidylcholine Bilayer Containing Cholesterol. Biochemistry. 2004;43(49):15318-28.

[72] Pike LJ. Rafts defined: A report on the Keystone symposium on lipid rafts and cell function. J Lipid Res. 2006;47(7):1597-8.

[73] Wang X, Pease R, Bertinato J, Milne RW. Well-defined regions of apolipoprotein B-100 undergo conformational change during its intravascular metabolism. Arterioscler Thromb Vasc Biol. 2000;20(5):1301-8.

[74] Segrest JP, Jones MK, De Loof H, Dashti N. Structure of apolipoprotein B-100 in low density lipoproteins. J Lipid Res. 2001;42(9):1346-67.

[75] Hall SCL, Clifton LA, Tognoloni C, Morrison KA, Knowles TJ, Kinane CJ, et al. Adsorption of a styrene maleic acid (SMA) copolymer-stabilized phospholipid nanodisc on a solid-supported planar lipid bilayer. J Colloid Interface Sci. 2020;574:272-84.

[76] 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(May):707-14.

[77] Castelli WP, Garrison RJ, Wilson PW, Abbott RD, Kalousdian S, Kannel WB. Incidence of Coronary Heart Disease and Lipoprotein Cholesterol Levels The Framingham Study. JAMA. 1986;256(20):2835-8.

[78] 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.

[79] 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(22):2089-99.

[80] Investigators A-H. Niacin in Patients with Low HDL Cholesterol Levels Receiving Intensive Statin Therapy. N Engl J Med. 2011;365(24):2255-67.

[81] Barter PJ, Caulfield M, Eriksson M, Grundy SM, Kastelein JJP, Komajda M, et al. Effects of Torcetrapib in Patients at High Risk for Coronary Events. N Engl J Med. 2007;357(21):2109-22.

[82] Ibanez B, Giannarelli C, Cimmino G, Santos-Gallego CG, Alique M, Pinero A, et al. Recombinant HDL Milano exerts greater anti-inflammatory and plaque stabilizing properties than HDL wild-type. Atherosclerosis. 2012;220:72-7.

[83] Nissen SE, Tsunoda T, Tuzco EM, Schoenhagen P, Cooper CJ, Yasin M, et al. Effect of Recombinant ApoA-1 Milano on Coronary Atherosclerosis in Patients with Acute Coronary Syndromes. JAMA. 2003;290(17):2292-300.

[84] Tardy C, Goffinet M, Boubekeur N, Ackermann R, Sy G, Bluteau A, et al. CER-001, a HDL-mimetic, stimulates the reverse lipid transport and atherosclerosis regression in high cholesterol diet-fed LDL-receptor deficient mice. Atherosclerosis. 2014;232:110-8.

[85] Nicholls SJ, Andrews J, Kastelein JJP, Merkely B, Nissen SE, Ray KK, et al. Effect of Serial Infusions of CER-001, a Pre-B High-Density Lipoprotein Mimetic, on Coronary Atherosclerosis in Patients Following Acute Coronary Syndromes in the CER-001 Atherosclerosis Regression Acute Coronary Syndrome Trial. JAMA. 2018;3(9):815-22.

[86] Tardif J-C, Grégoire J, L'Allier PL, Ibrahim R, Lespérance J, Heinonen TM, et al. Effects of Reconstituted High-Density Lipoprotein Infusions on Coronary Atherosclerosis. JAMA. 2007;297(15):1675-82.

[87] Gibson CM, Korjian S, Tricoci P, Daaboul Y, Yee M, Jain P, et al. Safety and Tolerability of CSL112, a Reconstituted, Infusible, Plasma-Derived Apolipoprotein A-I, After Acute Myocardial Infarction. Circulation. 2016;134:1918-30.

[88] Qin S, Kamanna VS, Lai JH, Liu T, Ganji SH, Zhang L, et al. Reverse D4F , an Apolipoprotein-AI Mimetic Peptide, Inhibits Atherosclerosis in ApoE-null Mice. J Cardiovasc Pharmacol Ther. 2012;17(3):334-43.

[89] Edmunds SJ, Liébana-García R, Nilsson O, Domingo-Espín J, Grönberg C, Stenkula KG, et al. ApoAI-derived peptide increases glucose tolerance and prevents formation of atherosclerosis in mice. Diabetologica. 2019;62:1257-67.

[90] Kawahara H, Miyashita N, Tachibana K, Tsuda Y, Morimoto K, Tsuji K, et al. A Photo-Activatable Peptide Mimicking Functions of Apolipoprotein A-I. Biol Pharm Bull. 2019;42(6):1019-24.

[91] Greenow K, Pearce NJ, Ramji DP. The key role of apolipoprotein E in atherosclerosis. J Mol Med. 2005;83:329-42.

[92] Zhu Y, Bellosta S, Langer C, Bernini F, Pitas RE, Mayley RW, et al. Low-dose expression of a human apolipoprotein E transgene in macrophages restores cholesterol efflux capacity of apolipoprotein E-deficient mouse plasma. PNAS. 1998;95:7585-90.

[93] Hayek T, Oiknine J, Brook JG, Aviram M. Role of HDL Apolipoprotein E in Cellular Cholesterol Efflux: Studies in Apo E Knockout Transgenic Mice. Biochem Biophys Res Commun. 1994;205(2):1072-8.

[94] Valanti EK, Dalakoura-Karagkouni K, Sanoudou D. Current and Emerging Reconstituted HDL-apoA-I and HDL-apoE Approaches to Treat Atherosclerosis. J Pers Med. 2018;8(4):1-12.

[95] Plump AS, Smith JD, Hayek T, Aalto-Setmla K, Walsh A, Verstuyft JG, et al. Severe hyercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. Cell. 1992;71(2):343-53.

[96] Li H, Reddick RL, Maeda N. Lack of ApoA-I Is Not Associated With Increased Susceptibility to Atherosclerosis in Mice. Arterioscler Thromb Vasc Biol. 1993;13:1814-21.

[97] Jebari-Benslaiman S, Uribe KB, Benito-Vicente A, Galicia-Garcia U, Larrea-Sebal A, Alloza I, et al. Cholesterol Efflux Efficiency of Reconstituted HDL Is Affected by Nanoparticle Lipid Composition. Biomedicines. 2020;8:373.