

Application of microdosimetry on biological physics for ionizing radiation

Dandan Chen(陈丹丹), Liang Sun(孙亮)
Citation: Chin. Phys. B . 2018, 27(2): 028701. **doi:** 10.1088/1674-1056/27/2/028701

Journal homepage: http://cpb.iphy.ac.cn; http://iopscience.iop.org/cpb

What follows is a list of articles you may be interested in

In vitro three-dimensional cancer metastasis modeling: Past, present, and future

Wei-jing Han(韩伟静), Wei Yuan(袁伟), Jiang-rui Zhu(朱江瑞), Qihui Fan(樊琪慧), Junle Qu(屈军乐), Li-yu Liu(刘雳宇), on behalf of the U.S.--China Physical Sciences-Oncology Alliance
Chin. Phys. B . 2016, 25(1): 018709. **doi:** 10.1088/1674-1056/25/1/018709

TOPICAL REVIEW — Soft matter and biological physics

Lipoprotein in cholesterol transport: Highlights and recent insights into its structural basis and functional mechanism*

Shu-Yu Chen(陈淑玉)†, Na Li(李娜)†, Tao-Li Jin(金桃丽), Lu Gou(缑璐), Dong-Xiao Hao(郝东晓), Zhi-Qi Tian(田芷淇), Sheng-Li Zhang(张胜利), and Lei Zhang(张磊)‡

Department of Applied Physics, Xi'an Jiaotong University, Xi'an 710049, *China*

(Received 31 August 2017; revised manuscript received 4 December 2017; published online 5 January 2018)

Lipoproteins are protein-lipid macromolecular assemblies which are used to transport lipids in circulation and are key targets in cardiovascular disease (CVD). The highly dynamic lipoprotein molecules are capable of adopting an array of conformations that is crucial to lipid transport along the cholesterol transport pathway, among which high-density lipoprotein (HDL) and low-density lipoprotein (LDL) are major players in plasma cholesterol metabolism. For a more detailed illustration of cholesterol transport process, as well as the development of therapies to prevent CVD, here we review the functional mechanism and structural basis of lipoproteins in cholesterol transport, as well as their structural dynamics in the plasma lipoprotein (HDL and LDL) elevations, in order to obtain better quantitative understandings on structure–function relationship of lipoproteins. Finally, we also provide an approach for further research on the lipoprotein in cholesterol transport.

Keywords: cholesterol transport, high-density lipoprotein (HDL), low-density lipoprotein (LDL), cholesteryl ester transfer protein (CETP)

PACS: 87.14.Cc, 87.14.E–, 87.15.H–, 87.64.Ee **DOI:** 10.1088/1674-1056/27/2/028702

1. Introduction

Current insight in epidemiology implicated that lipoproteins with high triglycerides are the strong and independent predictors of CVD, the leading cause of mortality globally.[1,2] Typically, a lipoprotein is the biochemical assembly consisting of proteins and triacylglycerols and/or cholesterol esters, which transports hydrophobic lipids around the body.[3] According to the density/size, lipoproteins could be classified into high-, low-, intermediate-, very low-density and ultra-low-density lipoproteins (HDL, LDL, IDL, VLDL, and chylomicrons).[4] Among them, HDL and LDL are two important nodes in lipid digestion & transport, and the plasma cholesterol metabolism. HDL carries LDL cholesterol away from the arteries and back to the liver, and might correlate with better cardiovascular health.[5,6] However, plasma levels of LDL cholesterol (LDL-C), IDL cholesterol (IDL-C), and VLDL cholesterol (VLDL-C) are causally associated with CVD and all-cause mortality. Therefore, a great deal has transpired with regard to lipoprotein-mediated cholesterol transport (or cholesterol metabolism) in the human body.

The widely acknowledged concept of lipoprotein is referred to lipoprotein particle metabolism, which can be divided into two pathways, exogenous and endogenous (Fig. 1).[7] Inside the enterocytes (LDL), synthesized cholesterols enter the VLDLs which contain a large amount of triglyceride, under the co-action of lipoprotein lipase (LPL) and hepatic lipase (HL).[8,9] With the increasing of cholesterol, VLDLs evolve into IDLs, and about half of the generated IDLs are rapidly enzymatically processed by liver cells. The remaining IDLs continue to hydrolyze triglycerides and eventually evolve into LDL, which are the primary plasma carriers of cholesterol for delivery to the tissues of the body.[10,11] However, the higher plasma LDL level could induce atherosclerosis and other cardiovascular diseases.[12] Therefore, the understanding for functional mechanism of LDL transport cholesterol and its pathogenesis become crucial for human health research.

Current observational experiments lead to the idea that the HDL protein particle (endogenous pathway) accumulates cholesteryl esters and returns to the liver where cholesterol is removed by reverse cholesterol transport (RCT) (Fig. 1).[6,13,14] In generally, the RCT consists of three major steps, firstly, biogenesis of HDL, apoA-I, is produced primarily in the liver and intestine, further obtaining lipids and cholesterol via ATP-binding cassette transporter A1 (ABCA1) expressed by hepatocytes and enterocytes, thereby generating nascent discoidal HDLs.[15] Secondly, nascent discoidal HDL acquires additional phospholipids and unesterified cholesterol from peripheral cells throughout the ABCA1 activities. After it is transferred to blood via the lymphatic system, free cholesterol of nascent discoidal HDL interacts with the enzyme lecithin-cholesteryl acyltransferase (LCAT) to generate

*Project supported by the National Natural Science Foundation of China (Grant Nos. 11504287 and 11774279).

†These authors contributed equally to this work.

‡Corresponding author. E-mail: zhangleio@xjtu.edu.cn

© 2018 Chinese Physical Society and IOP Publishing Ltd

cholesteryl esters (CE). The cholesteryl esters gather in the center of HDL and turn nascent discoidal HDL into spherical HDL (sHDL).[16] The final step is the uptake of HDL cholesterol by liver via binding to HDL receptors scavenger receptor B1 (SR-BI)[17] or other unidentified HDL receptor (HDLR).[18]

Up to now, it is widely accepted that intervention to raise HDL cholesterol (HDL-C) and promote the RCT process, might reduce or even eliminate the accumulation of cholesterol in arterial blood vessels to protect against CVD. However, cholesteryl esters can be transferred between the two pathways, exogenous and endogenous, with the help of cholesteryl ester transfer protein (CETP), which transits cholesteryl esters (CE) and triglycerides (TG) between VLDL/LDL and HDL (Fig. 1).[19,20] This motion might reduce the HDL-medicated cholesterol transport and the level of HDL protein particles. Currently, pharmacological inhibition of CETP is being regarded as a way of increasing HDL levels and preventing CVD.[19,20]

As mentioned above, HDL, LDL, CETP might be three vital nodes during the cholesterol transport (lipoprotein particle metabolism) that closely related to CVD. At present, the schematic illustrating of cholesterol transport process is becoming clear, but may be incomplete. In this review, we will focus on the structural basis and functional mechanism of lipoproteins in cholesterol transport, especially their conformation dynamics. Firstly, we will cover how HDL, LDL and CETP induce the metabolisms during cholesterol transport. Secondly, we will summarize a recent process in the spatial information of the three lipoproteins, especially the elevations of plasma HDL and LDL, and shine a light on the assembly processes of lipoprotein particles and the substrates dynamics exchanges. It will prove valuable for an in-depth understanding on structure–function relationship of lipoproteins, even the correlation between various lipoprotein classes and cardiovascular risk.

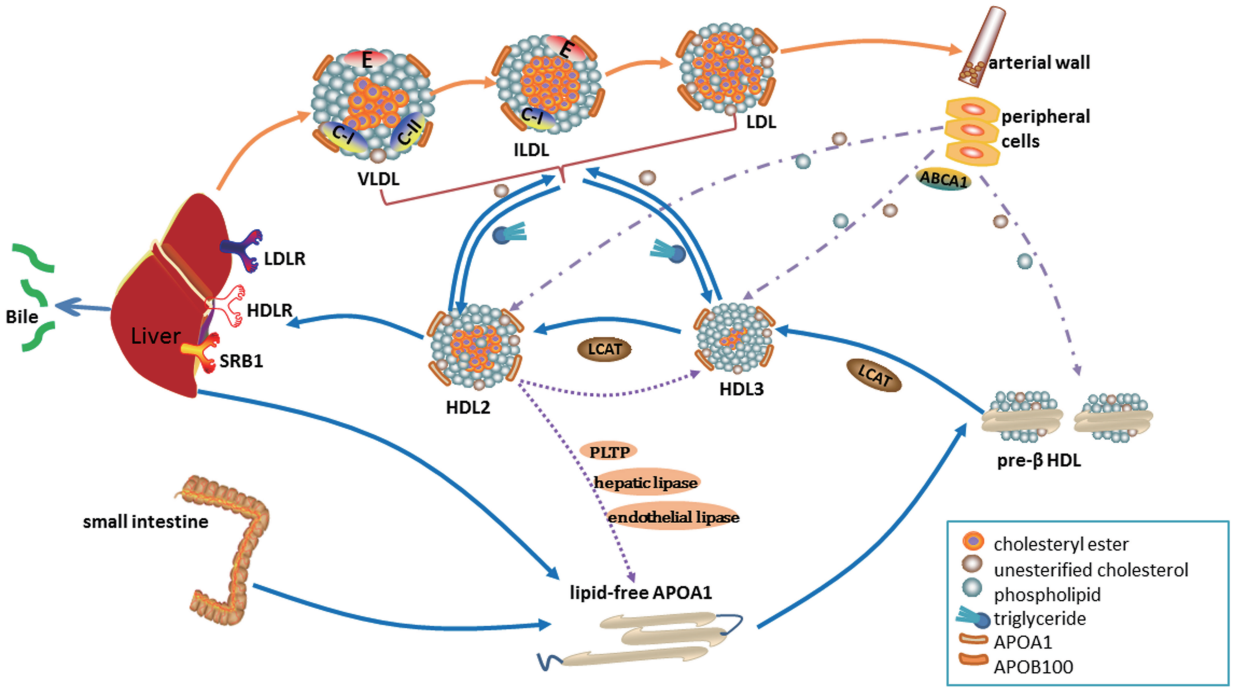


Fig. 1. (color online) Cholesterol metabolism pathways in the human body.

2. Function of lipoproteins in cholesterol transport

2.1. HDL function

HDL is a cardioprotective lipoprotein, at least in part, because of its ability to mediate RCT. The original hypothesis[21] suggests that HDL is protective lipoprotein against atherosclerosis, which is supported by a series of animal studies.[22–24] Thereafter, the classic HDL cholesterol hypothesis is questionable and is gradually being replaced by the HDL function hypothesis.[25] In this view, HDL function instead of HDL-C has a causal relation to atheroprotection, although this hypothesis is waiting to be verified.

In vivo and *in vitro* studies have revealed several potentially anti-atherogenic properties of HDL.[26,27] One proof is that HDL can remove cholesterol from macrophages in arterial walls in the RCT pathway.[28] Besides, apolipoprotein A1 (apoA-I, 28-kD, 243 residues), the major protein component of HDL particles, mediates many HDL functions, such as HDL-mediated process of RCT, cardioprotective anti-inflammatory, anti-oxidative and anti-thrombotic properties.[29] The highly dynamic apoA-I molecules are capa-

ble of adopting an array of conformations through remodeling HDL that is crucial to lipid transport during the RCT process. Further studies show that mutations in apoA-I induce varied types of dyslipidemias.[30] This is because defective apoA-I prevents the process of HDL remodeling and further affects normal RCT pathway, leading to increasing susceptibility to CVD.

2.2. LDL function

Cholesterol is an indispensable matter for non-hepatic cells to form membrane, which is carried from the liver to body cells. In this transport process, LDL works as a vehicle for cholesterol transportation between the liver and cells to maintain a constant cholesterol supply in the human body. There is about 70% plasma cholesterol contained in LDL in a normal person. A body cell, which needs the cholesterol, produces an LDL receptor (LDL-R) on its surface, which forms coated endocytic vesicles that carry LDL into the cell.[31] Hence, the endocytosis of cholesterol-rich LDL is mediated by LDL-R and thus the plasma level of LDL is maintained. These results further indicated that the major function of LDL-Rs is protecting against atherogenesis in normal expression.[32]

However, in some abnormal conditions, which are distinct from LDL-R-mediated endocytosis, LDL may induce over-accumulation of cholesterol to form foam cells, resulting in the development of atherosclerosis.[33] The apo-B48 (apoprotein B48) and apo-B100 (apoprotein B100) located in the surface of LDL particles tend to interact with extracellular material, which make LDL particles easy to bind with blood vessel intima.[34] LDL particles trapped in the intima are susceptible to be modified, such as acetylation, enzymatic digestion and oxidation. The ox-LDL can promote lipoprotein aggregation[35,36] and provoke inflammation by recruiting the circulating monocytes to the site followed invade the vessel wall and differentiate into macrophages.[35,37] Then scavenger function of macrophages is promoted to phagocytose ox-LDL particles hardly within degradation to form the macrophage-derived foam cells. Moreover, ox-LDL trapped in the intima as foreign material can induce smooth muscle cells (SMC) to eliminate it by migrating from the middle layer of the artery wall into the inner layer.[38] However, after phagocytosis, the liberated cholesterol of LDL cannot be hydrolyzed, with forming the SMC-derived foam cells. Finally, these foam cells are changed to produce atherosclerotic plaque.[39]

2.3. CETP function

Apart from the HDL-mediated and LDL-mediated pathways, CETP plays an essential role in cholesterol transport. Human CETP mediates the net transfer of cholesterol ester from HDL to apolipoprotein B (apoB)-containing lipoproteins. Meanwhile, HDLs are catalyzed by CETP, in connection with various lipases [lipoprotein lipase (LpL), hepatic lipase (HL), and endothelial lipase (EL)] and phospholipid transfer protein (PLTP), to form lipid-poor HDL particles and shed from HDL lipid-free apoA-I.

CETP acts as a medium between lipoproteins for elevating plasma LDL-C (or VLDL-C) level and lowering HDL-C level.[40] Up to now, there are three hypotheses of CETP-mediated cholesterol transport mechanism: (i) the shuttle model: the CETP shuttles between lipoproteins, transporting HDL cholesterol ester to LDL (or VLDL) in exchange for the glycerin phospholipids to return;[41] (ii) the tunnel model: a ternary complex in which CETP bridges two lipoproteins and neutral lipid is transported from one side to the other;[42] (iii) the dimer tunnel model: a modified tunnel model that the hydrophobic tunnel is replaced by CETP dimer.[43] Based on the above mentioned assumptions, a series of CETP inhibitors have been investigated clinically, such as torcetrapib, dalcetrapib, evacetrapib, and anacetrapib.[44–46] However, current inhibitors represent the turbulent beginning of CETP inhibition and an increased mortality rate related to off-target effects and lack of efficacy.[47–49] Accompanying adverse effects call for a deeper exploration of the mechanism for CETP-mediated lipid transfer.

3. Structural basis of lipoproteins in cholesterol transport

3.1. HDL structure

Although the heterogeneity of HDL prevents its structural analysis at high-resolution level, in the past 20 years, significant progress has been made in overcoming the dynamic characteristics of HDL, with a series of proposed models. In the following text, we summarize a recent process in the structure of the three main states of HDL including lipid-free apoA-I, discoidal and spherical HDL. All the structural information makes it better to understand why HDL has compositional heterogeneity and the identification of their structure–function relationship, as well as the role of HDL subfractions on CVD protection.

3.1.1. Lipid-free apoA-I

Lipid-free apoA-I plays a major role in cholesterol metabolism because its structural plasticity is the origin of HDL model diversity. The structure of full-length lipid-free apoA-I in native states still remains unknown because of its flexibility. The first x-ray crystal structure of the N-terminal truncated lipid-free protein, Δ(1-43)apoA-I, was published in 1997 by Borhani *et al.*[50] This structure suggests a "horseshoe-shape" organization in which two highly α-helical apoA-I molecules form an antiparallel dimer, being regarded as the significant initial model ("double-belt" model) for comprehending the structure of apoA-I on HDL subclasses

(Fig. (2(b)).[51] Subsequent crystal organization of lipid-free Δ(1-43)apoA-I revealed that apoA-I could accommodate a four-helix bundle.[52–54] This four-helix structural information was further verified by the crystal structure of full-length lipid-free apoA-I,[55,56] as well as chemical cross-linking combined with mass spectrometry (CCL/MS).[57] However, the structural model of full-length apoA-I was out of step with many physical biochemical measurements, such as α-helical content, hinting the structure of apoA-I may be altered during crystallization processes.[58,59]

Besides, antiparallel helical dimers were observed in the crystal structures of the N- and the C-terminally truncated proteins, with many similarities (such as 5/5 repeat register, an inherent property of apoA-I dimer, see Figs. 2(b) and 2(c)). Overall, two legitimate crystal structures of apoA-I; both are truncated forms and represent a lipid-bound and intermediate state.[50,60] The amphipathic α-helix is the structural motif that enables apoA-I to stabilize all HDL subclasses through the conformation change. In the lipid-free state, the N-terminal two thirds of the molecule constitute a dynamic, four-helix bundle, and the helical segments unfold and refold in seconds, making it possible for apoA-I remodeling in different HDL subclasses. The C-terminal third of the protein forms an intrinsically disordered domain that mediates initial binding to phospholipid surfaces, which occurs with coupled α-helix formation.

Despite numerous efforts having been devoted to the apoA-I structure, there remains some confusions, including the solution conformation of free apoA-I, and the atom-detail conformations of apoA-I N-terminal on various HDL. X-ray experiment revealed that the helices bundle at the N-terminal of apoA-I are far more stable than could be achieved in isolation, indicating mutually stabilizing interactions of helix bundle.[61,62] Meanwhile, the dynamic helical structure is unfolding and refolding in seconds, resulting in the remodeling of apoA-I, during the formation of various HDL particles.

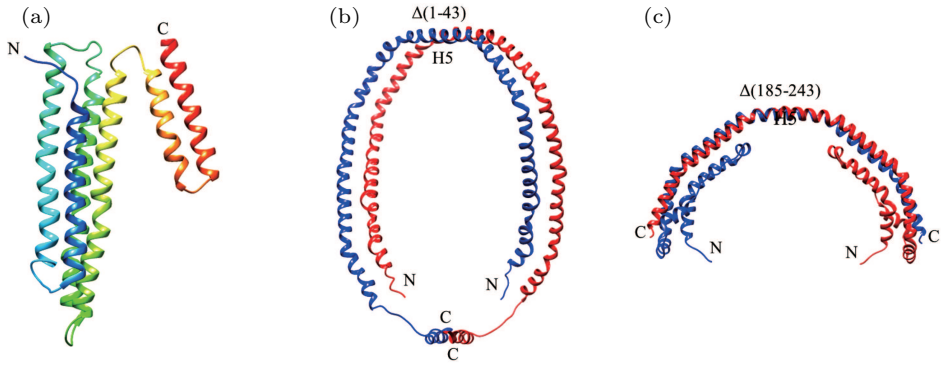


Fig. 2. (color online) Comparison of the three crystal structures of lipid-free apoA-I, (a) the x-ray crystal structure of full-length lipid-free apoA-I (PDB: 2A01) by Ajees *et al.*,[55] (b) the x-ray crystal structure of the N-terminal truncated $\Delta(1-43)$ apoA-I dimer (PDB: 1AV1),[50] (c) the x-ray crystal structure of the C-terminal truncated $\Delta(185-243)$ apoA-I dimer (PDB: 3R2P).[60]

3.1.2. Discoidal HDL

Due to the heterogeneity of human plasma HDL, reconstituted HDL particle (rHDL) is an ideal *in vivo* model system, which possesses most of the properties of native lipoprotein complexes such as LCAT activation, lipid transfer, and receptor binding.[63–65]

In 1999, Segrest *et al.* came up with the original double-belt model for Δ(1–43)apoA-I. In this model, the two antiparallel monomers of apoA-I in which each helix 5 segments directly oppose each other[66,67] and the hydrophobic face of the amphipathic α-helix is in close contact with the fatty acid acyl chains.[68] The resulting orientation of the registry that left to interface in which helix 5 of both molecules is identical to that of Borhani's crystal structure.[50] Furthermore, Bhat *et al.* discovered that the N- and C-terminal 40–50 residues doubled back on the molecule and thus refined as the "belt and buckle".[69] In 2006, Martin and colleagues[70] observed that self-rotation coupling feature of residues 134–145 was coincident with a looping region that brought in a partial opening between the parallel belts. This "looped belt" model was also suggested to be a potential site by which LCAT could access the cholesterol and phospholipid acyl chains.

In 2008, Wu and colleagues used mass spectrometry (MS) to determine the structure of lipid-free and lipid-bound apoA-I on 104-Å rHDL and proposed a "solar flares" model.[71] This model shows that the C-terminal of both apoA-I molecules interact with each other, and the distal C-terminal residues of helix 10 are exposed to the solvent. These loops, amino acids 159–178, are suggested as LCAT binding sites because this area shows reduced deuterium exchange when LCAT appears. However, this conclusion is different from the recent HDX study on similar particles which failed to support the existence of the looped solar flares.[72]

Different from the models discussed above that rHDLs are discoidal in shape, Wu *et al.*[73] proposed a new model called double superhelix (DSH) apoA-I which is significantly different from other normal models. This model suggests an open helical shape in which particles resemble an ellipsoid rather than a disc. Although the model shape is different, the interface interaction between two apoA-I molecules is the

same as the 5/5 double-belt model but the DSH model did not show interaction in C-terminal.

The feasibility of DSH model is still under debate because molecular dynamics research showed that this model rapidly collapsed to a disc-shaped structure.[74] With the ability of directly imaging a particle's structure, transmission electron microscopy (EM) is an alternative powerful approach for investigating structures of lipoproteins. In 2011, by direct visualization of individual particles, Zhang *et al.*[75] used two different EM methods of negative stain and cryo-EM tomography to reveal that apoA-I/HDL particles (both plasma HDL and 7.8 nm, 8.4 nm, 9.6 nm of rHDLs) are discoidal in shape. Furthermore, this conclusion was also confirmed by Stephen C Murray[76] using cryo-EM reconstruction method. Recently, Bibow S *et al.*[77] presented three-dimensional structure of shortened construct of human apoA-I in reconstituted discoidal HDL particles by a combination of nuclear magnetic resonance (NMR), electron paramagnetic resonance (EPR) and transmission electron microscopy (TEM) data. These results showed that the rHDL particles form a double belt in an antiparallel fashion and a gross "right-to-right" rotation of the helices after lipidation was proposed necessarily to adjust a hydrophobic interior. All the above-mentioned evidence supported that the reconstituted apoA-I/HDL particles are possibly discoidal in shape. Therefore, the DSH model is strongly unfavorable.

More recently, Phillips *et al.*[62,72] described the position and stability of α-helical segments in full-length human apoA-I on a model of large (9.6 nm) and small (7.8 nm) discoidal HDL particles. They suggested that the nonhelical regions in lipid-free apoA-I (residues 45–53, 66–69, 116–146, and 179–236) change conformation from random coil to α-helix so that nearly the entire apoA-I molecule adopts a helical structure.

According to the above descriptions, we can summarize that when apoA-I bound to the lipid, it involved conformation change from random coil to α-helix in the C-terminal domain. Discoidal HDL are stabilized by two apoA-I molecules wrapped around the edge of the disc in an antiparallel, double-belt arrangement so that the hydrophobic PL acyl chains are protected from exposure to water. These apoA-I molecules are in a highly dynamic state and adapt to discs of different sizes by certain segments forming loops that detach reversibly from the particle surface.

Based on the structure model of lipid-free and lipid-bound apoA-I, Mei and Atkinson also proposed HDL formation mechanism as shown in Fig. 3. Firstly, the monomeric apoA-I forms a helix bundle in which the C-terminal domain exposed hydrophobic surface. Then, the C-terminal domain of monomeric apoA-I binds the lipid to form a helical structure. This binding process promotes the formation of dimers. Finally, lipids will be full of double loops and the remainder of C-terminal domain will be transferred into helical conformation, thus leading to the formation of a discoidal, nascent HDL. In the same year, Segrest *et al.*[78] also discovered that the basic driving force for the formation of nascent discoidal HDL is a PL pump-induced surface density increase.

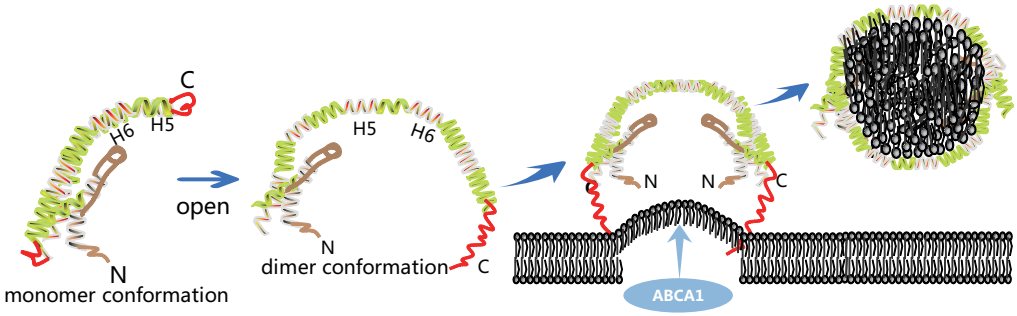


Fig. 3. (color online) The formation mechanism of HDL through dimerization regulated by the H5 region. The left shows the monomer open conformation transfer to dimer conformation of apoA-I in solution regulated by the H5 region. The right shows intermediate state that full-length apoA-I dimer model binds lipid to form the final HDL state.

3.1.3. Spherical HDL

Despite the fact that spherical HDL accounts for the majority of HDL particles in human plasma, its structure is hardly known compared to lipid-free apoA-I and discoidal HDL. Recent developments greatly advanced our understanding of the model of both natural and reconstituted spherical HDL.

Based on the double-belt model in discoidal HDL, Davidson and colleagues[79] first proposed a trefoil model of apoA-I in spherical HDL particles by the elegant chemical cross-linking and mass spectrometry. In this model, half of each apoA-I molecule in the double-belt arrangement is bent 60° out of the plane of the particle and the spherical geometry is achieved by addition of a third apoA-I molecule bent in the same way. Importantly, the trefoil model still showed the same interactions as in discs with two apoA-I molecules. This model also suggests that the hinging of the Δ(1–43)apoA-I molecule occurs near residues 133 and 233[79] which is different from the hinging of the full-length protein conformation, meanwhile, the trefoil model is assumed to occur near residues 65 and 185.[80] However, more work would be required to conclusively demonstrate that this motion occurs at these sites. However, the solution structure of identical recon-

stituted sHDL was also determined by means of small angle neutron scattering.[81] The low-resolution molecular envelope of the protein component is most consistent with the helical dimer with a hairpin (HdHp) model according to the analyses of cross-linking and mass spectrometry. Different from the intermolecular contacts in the trefoil model, the interactions within the hairpinned apoA-I are intramolecular, though the same sequences interact in all molecules.

It should be noted that the first detailed molecular model which is related to LpA-I HDL that only contains apoA-I fractions isolated from human plasma has been proposed by Huang.[82] They studied 5 density subfractions of normal human plasma HDL and further isolated particles only containing apoA-I. These particles range in diameter from 8.8 nm to 11.2 nm and contain 3–5 apoA-I molecules. They found that apoA-I adopts intermolecular interactions in plasma HDL which is very similar to those of the double-belt and trefoil models derived from reconstituted systems. Thus, they concluded that apoA-I adopts a common structural organization, characterized by distinct intermolecular contacts, regardless of size and shape or natural versus synthetic method of production. Furthermore, in the case of having the same number of apoA-I molecules, Segrest *et al.*[83] found that circulating sHDL and reconstituted sHDL contain approximately the same amount of core lipid, while circulating sHDL has obviously less surface lipid monolayers, which indicated that the apoA-I package on natural spheres is much closer than the typical recombinant particles. More recently, Chetty *et al.*[72] have applied hydrogen exchange (HX) and MS methods to compare apoA-I secondary structure in discoidal and spherical HDL particles. They demonstrated that the size of apoA-I in both 10-nm spherical LpA-I particle and discoidal HDL are very similar. In addition, they pointed out the length of this partially disordered segment is similar to that of rHDL discs of 8 nm in diameter, suggesting that apoA-I on these spheres is more tightly packed than in the 96-Å discs. This is consistent with higher number of apoA-I molecules on the surface.

Overall, these studies reinforced the idea that global apoA-I conformation does not change significantly between particles of different shapes or origins. The protein maintains similar protein–protein contacts when a HDL disc changes to a sphere as LCAT converts free cholesterol to cholesteryl ester.

3.2. LDL structure

Because of the relevance with various diseases, the structure of LDL is a focus of interest. The most effective and conclusive structural analyses of LDL have been generated by cryo-EM combined with single particle technology and small angle scattering model reconstruction technology.[84] Meanwhile, a variety of other physico-chemical techniques were used in the structural studies, including differential scanning calorimetry, various spectroscopy and magnetic resonance imaging.

3.2.1. Overall LDL structure

The primary character of low-density lipoproteins (LDLs) in nature is heterogeneousness, which includes difference in density ($\sim$ 1.019–1.063), shape, size (diameter $\sim$ 18 nm–25 nm, mean 22 nm), surface charge and chemical composition.[85] A general consensus is that LDL particles all have two compartments, an amphipathic surface phospholipid monolayer that is surrounded by one single copy of apoB-100, and a hydrophobic lipid-cholesteryl esters core.[86] The structure and physical function of LDLs predominantly depend on the core–lipid composition and the conformation of the apoB-100. Meanwhile, both particle size and shape are significant determinants of its function. For example, small, dense LDL has been shown to be more susceptible to non-specific binding to cell surface rather than binding to LDLR, thus increasing the risk for initial atherosclerosis,[87] and subtle changes of particle diameter could alter conformation of apoB-100, further changing surface charge of the particle.[88]

3.2.2. Lipid core of LDL

A polar lipid core of LDL mainly consists of cholesteryl esters, some triglycerides, and some free-cholesterol. Specifically, LDL is the only biological macromolecules known today whose structural changes are strikingly related to physiological temperature.[89] By using x-ray and neutron small angle scattering technology, it has been demonstrated that lipids located in the core show order arranged to a liquid–crystalline phase below the critical temperature. Contrarily, the lipids organized in an oil-like fluid show a disordered state above the critical temperature.[90,91] The general range of transition temperature is 15 °C$\sim$ 35 °C, and this value correlates well with individual LDL particle and its ratio of cholesteryl esters to triglycerides: the greater the ratio, the higher the critical temperature (Fig. 4).[92,93] Some studies on LDL had shown that the overall structure of LDL is a classical spherical particle when core structure is composed of radial cholesteryl esters arranged into a concentric spherical shell.[94,95] However, the subsequent studies proposed the core-located lipids present in the liquid–crystalline state within an ellipsoidal shape particle by cryo-EM. The internal frozen-lipid core is revealed not centered radially but an organized three-layer structure. The distance between the layers corresponds well to the length of cholesteryl esters.[95,96]

Based on the above mentioned studies, it seems reasonable to speculate that the change of temperature might indirectly change the shape of LDL particles from roughly spherical to ellipsoid.[84] In view of this, many efforts have been made to explore the structure of LDL at different temperatures, such as 4 °C, 6 °C,[96–98] and 37 °C.[99]

3.2.3. ApoB-100 in LDL

The apoB-100, consisting of 4536 amino acid residues and accounting for ~20% of the weight of overall LDL, is the only protein component of LDL. The apoB-100 is wrapped around the phospholipid monolayer on the surface of LDL particle and forms an irregular ring shape. In this shape, the N-terminal and the C-terminal of the apoB-100 touch each other, in which the N-terminal forms a protruding globular structure.[100]

A more generally accepted structural model of apoB-100 generated by recent computational technology is called "pentapartite" structure, in which the apoB-100 is defined as five consecutive domains, NH2-$\beta\alpha$1-β1-α2-β2-α3-COOH, corresponding to different functions respectively.[98] More recently, Ren *et al.* published a new LDL reconstruction in which the lipid core is revealed as an organized three-layer structure by single particle technology. In their model, the apoB-100 includes a pair of "paddles" configurations with several long "fingers" extensions which have similar length and interval.[101]

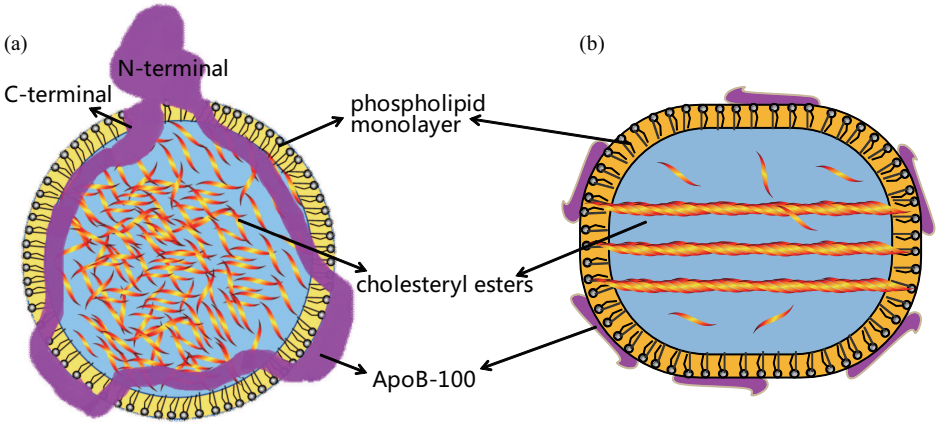


Fig. 4. (color online) The overall structure and core structure of LDL (a) above the critical temperature. (b) below the critical temperature.

3.3. CETP structure

The crystal structure of CETP, reported by Qiu *et al.* in 2007, has greatly promoted our understanding of the structure and function of CETP.[20] CETP is a hydrophobic transfer protein composed of 476 amino acids and reveals a so-called banana-shape (the size is 135 Å×30 Å×35 Å). The structure includes two different β-barrel structures in N- and C-terminals respectively, and a central β-sheet with a ~60 Å-long hydrophobic central cavity, which can hold two phospholipids and two cholesterol molecules. Moreover, the two phospholipid molecules that located in two pores near the central domain expose the hydrophilic terminal to the aqueous environment and hydrophobic terminal to the hydrophobic cavity, [Figs. 5(a) and 5(b)].

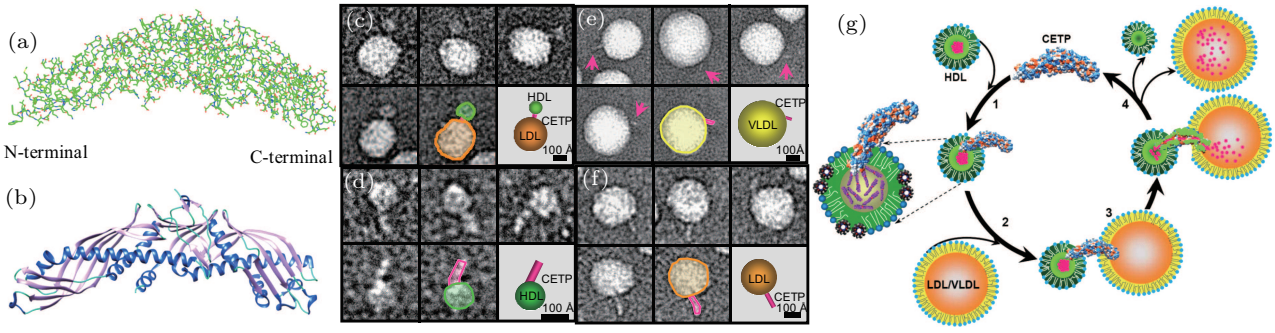


Fig. 5. (color online) The crystal structure of CETP (PDB: 2OBD) and three-dimensional density maps of CETP binging lipoproteins, (a) atom figure of CETP, (b) secondary structure of CETP, (c) ternary complexes of HDL-CETP-LDL in cryo-EM micrographs, (d)–(f) the CETP insert into HDL, VLDL, LDL respectively in cryo-EM micrographs, (g) the tunnel model of CETP-mediated lipid transfer, (Ref. [105]).

Because of its special function to transfer cholesterol esters between HDL and LDL (or VLDL), the way CETP interacts with lipoproteins is essential. Qiu *et al.* investigated the mechanism of CETP-mediated lipid transfer. In their studies, the CETP shows a high binding affinity for nascent HDL and other lipoproteins to cover the lipoproteins surfaces owing to its proper curvature radius. They proposed a lipid transport mechanism, shuttle model. In this mechanism, the CETP in turn covers the surface of LDL (or VLDL) and HDL to swap LDL-cholesterol esters (or VLDL-cholesterol esters) with HDL-triglycerides. These steps are constantly recycled until the completion of the transport process, in which cholesterol esters move from LDL (or VLDL) to HDL.[20] This model based on the hydrophobic cavity of CETP and its feasi-

bility of binding to lipoproteins, explains the mechanism of CETP-mediated lipid transfer reasonably, but there are not complexes of CETP binding to lipoproteins in the cryo-EM micrographs intuitively to verify the authenticity of the model.

In 2012, a new mechanism was proposed by Zhang *et al.*[102] They studied human recombinant CETP with cryo-EM by using an optimized negative-staining (OpNS) EM protocol.[103,104] Applying the single-particle techniques, they obtained the 3D structure of CETP and the complexes of CETP binding to lipoproteins. In the 3D-map of complexes, they discovered the HDL-CETP binding structure, which appears to be formed by N-terminal of CETP inserted into HDL and the CETP-LDL (or CETP-VLDL) is formed by C-terminal of CETP inserted into LDL (or VLDL) [Figs. 5(c)–5(f)]. This conclusion was later confirmed by Geraldine *et al.* by using large-scale atomistic molecular dynamics.[105] The measurement of the protrusion from the lipoproteins surface shows that ~ 48 Å of the tapered N-terminal end of CETP penetrates the HDL surface and ~ 25 Å of the C-terminal end of CETP penetrates the LDL surface (~ 20 Å of the C-terminal end of CETP penetrates the VLDL surface) reaching the lipid–rich, lipoproteins core. Furthermore, Zhang *et al.* proposed the tunnel model of lipid transfer mediated by CETP.[102,106,107] In this model, both CETP terminals finish penetrating surface sites on lipoproteins, N-terminal to HDL and C-terminal to LDL (or VLDL). Then neutral lipids, including cholesterol esters and triglycerides, transfer through the hydrophobic tunnel at the core of the CETP (Fig. 5).

However, there are some discrepancies with the tunnel model mentioned above. Matthias *et al.* used the experiments which involve three monoclonal antibodies to demonstrate that the antibodies binding on both ends of CETP do not inhibit CETP's function of trans-shipment cholesterol esters, but the antibodies on the middle do.[108] In their research they supposed that the formation of the ternary tunnel complexes is not a mechanistic prerequisite by CETP to perform its functions. Hence, the real mechanism of CETP-mediated lipid transfer still remains to be studied and verified.

4. Conclusion and perspectives

In this focused review, we briefly summarized the functional mechanism and structural basis of lipoproteins (e.g., HDL, LDL, and CETP) in cholesterol transport, as well as their structural dynamics during the transport process. Furthermore, we reviewed the latest developments in the plasma lipoprotein (HDL and LDL) elevations, especially the conformational changes of lipoprotein particles involved in the maturation and remodeling of HDL/LDL. These digested data will allow investigators to obtain better quantitative understandings on structure–function relationship of lipoprotein, and be of benefit for a more detailed illustrating of cholesterol transport process, as well as the development of therapies to prevent CVD.

Though the schematic illustrating of cholesterol transport is well-documented, in these assays, the conformational dynamics of lipoproteins (e.g., HDL, LDL, and CETP) are not fully monitored. Due to this incapability of the current assays and highly heterogeneous nature of lipoprotein particles, the function of lipoprotein in cholesterol transport remains elusive with regard to many important questions, such as how the lipoprotein particle assembles and how the assembly modulates the neutral lipids dynamic exchanges at the molecular level. Cryo-EM, a rapidly developed technique in the past two decades, provides an advantaged platform for resolving these problems. In particular, Cryo-EM coupled with MD simulations have revealed several important mechanisms of CETP-mediated lipid exchange and metabolism with all-atom detail.[109,110] In addition, it is expected that further research could pay more attention to simultaneously monitor the dynamic structural change of lipoproteins and the dynamic mechanism of lipid transfer, especially the internal motivation of physical mechanism during the process of lipid transport.

Acknowledgment

We thank Dr. Zhiwei Yang for his help with instructive suggestions.

References

[1] Castelli W P 1996 *Atherosclerosis* **124** Suppl S1
[2] Gordon T, Castelli W P, Hjortland M C, Kannel W B and Dawber T R 1977 *Am. J. Med.* **62** 707
[3] Lusis A J and Pajukanta P 2008 *Nat. Genet.* **40** 129
[4] Fredrickson D S, Levy R I and Lees R S 1967 *Nutr. Rev.* **276** 34
[5] Kannel W B, Dawber T R, Friedman G D, Glennon W E and Mcnamara P M 1964 *Ann. Int. Med.* **61** 888
[6] Glomset J A, Janssen E T, Kennedy R and Dobbins J 1966 *J. Lipid Res.* **7** 638
[7] Kingwell B A, Chapman M J, Kontush A and Miller N E 2014 *Nat. Rev. Drug Discov.* **13** 445
[8] Bilheimer D W, Eisenberg S and Levy R I 1972 *Biochim. Biophys. Acta* **260** 212
[9] Eisenberg S, Bilheimer D W and Levy R I 1972 *Biochim. Biophys. Acta* **280** 94
[10] Tatami R, Mabuchi H, Ueda K, Ueda R, Haba T, Kametani T, Ito S, Koizumi J, Ohta M and Miyamoto S 1981 *Circulation* **64** 1174
[11] Superko H R, Nejedly M and Garrett B 2002 *Prog. Cardiovasc. Nurs.* **17** 167
[12] Lusis A J 2000 *Nature* **407** 233
[13] Fielding C J and Fielding P 1995 *J. Lipid Res.* **36** 211
[14] Schaefer E J, Anthanont P and Asztalos B F 2014 *Curr. Opin. Lipidol.* **25** 194
[15] Parks J S, Chung S and Shelness G S 2012 *Curr. Opin. Lipidol.* **23** 196
[16] Rousset X, Vaisman B, Amar M, Sethi A A and Remaley A T 2009 *Curr. Opin. Endocrinol. Diabetes Obes.* **16** 163
[17] Hoekstra M, Van Berkel T J and Van Eck M 2010 *World J. Gastroenterol.* **16** 5916
[18] Martinez L O, Jacquet S, Esteve J P, Rolland C, Cabezón E, Champagne E, Pineau T, Georgeaud V, Walker J E and Tercé F 2003 *Nature* **421** 75
[19] Barter P J, Brewer H B, Chapman M J, Hennekens C H, Rader D J and Tall A R 2003 *Arterioscl. Throm. Vas.* **23** 160

[20] Qiu X, Mistry A, Ammirati M J, Chrunyk B A, Clark R W, Cong Y, Culp J S, Danley D E, Freeman T B and Geoghegan K F 2007 *Nat. Struct. Mol. Biol.* **14** 106
[21] Miller G J and Miller N E 1975 *Ann. Int. Med.* **1** 16
[22] Badimon J J, Badimon L and Fuster V 1990 *J. Clin. Invest.* **85** 1234
[23] Rubin E M, Krauss R M, Spangler E A, Verstuyft J G and Clift S M 1991 *Nature* **353** 265
[24] Tangirala R K, Tsukamoto K, Chun S H, Usher D, Puré E and Rader D J 1999 *Circulation* **100** 1816
[25] Rader D J and Tall A R 2012 *Nat. Med.* **18** 1344
[26] Rosenson R S, Brewer H B, Ansell B, Barter P, Chapman M J, Heinecke J W, Kontush A, Tall A R and Webb N R 2013 *Circulation* **128** 1256
[27] Rader D J, Alexander E T, Weibel G L, Billheimer J and Rothblat G H 2009 *J. Lipid Res.* **50** S189
[28] Lewis G F and Rader D J 2005 *Circul. Res.* **96** 1221
[29] Rye K A, Bursill C A, Lambert G, Tabet F and Barter P J 2009 *J. Lipid Res.* **50** Suppl S195
[30] Zannis V I, Chroni A and Krieger M 2006 *J. Mol. Med.* **84** 276
[31] Goldstein J L and Brown M S 1977 *Ann. Rev. Biochem.* **46** 897
[32] Vainio S and Ikonen E 2003 *Ann. Med.* **35** 146
[33] Born G V 1994 *Basic Res. Cardiol.* **89** Suppl(1): 103
[34] Proctor S D, Vine D F and Mamo J C 2002 *Curr. Opin. Lipidol.* **13** 461
[35] Glass C K and Witztum J L 2001 *Cell* **104** 503
[36] Kruth H 2001 *Front. Biosci.* **6** D429
[37] Qiao J H, Tripathi J, Mishra N K, Cai Y, Tripathi S, Wang X P, Imes S, Fishbein M C, Clinton S K and Libby P 1997 *Am. J. Pathol.* **150** 1687
[38] Ross R 1976 *N. Engl. J. Med.* **295** 420
[39] Liu L K, Lee H J, Shih Y W, Chyau C C and Wang C J 2008 *J. Food Sci.* **73**
[40] Barter P J, Brewer H B, Chapman M J, Hennekens C H, Rader D J and Tall A R 2003 *Arterioscl. Throm. Vas.* **23** 160
[41] Barter P J and Jones M E 1980 *J. Lipid Res.* **21** 238
[42] Ihm J, Quinn D M, Busch S J, Chataing B and Harmony J A 1982 *J. Lipid Res.* **23** 1328
[43] Tall A 1995 *Ann. Rev. Biochem.* **64** 235
[44] Morehouse L A, Sugarman E D, Bourassa P A, Sand T M, Zimetti F, Gao F, Rothblat G H and Milici A J 2007 *J. Lipid Res.* **48** 1263
[45] Rennings A J and Stalenhoef A 2008 *Expert Opin. Inv. Drug.* **17** 1589
[46] Xie L, Li J, Xie L and Bourne P E 2009 *Plos Comput. Biol.* **5** e1000387
[47] Clark R W, Ruggeri R B, Cunningham D and Bamberger M J 2006 *J. Lipid Res.* **47** 537
[48] Ranalletta M, Bierilo K K, Chen Y, Milot D, Chen Q, Tung E, Houde C, Elowe N H, Garcia-Calvo M, Porter G, Eveland S, Frantz-Wattley B, Kavana M, Addona G, Sinclair P, Sparrow C, O'Neill E A, Koblan K S, Sitlani A, Hubbard B and Fisher T S 2010 *J. Lipid Res.* **51** 2739
[49] Nicholls S J, Lincoff A M, Barter P J, Brewer H B, Fox K A A, Gibson C M, Grainger C, Menon V, Montalescot G, Rader D, Tall A R, McErlean E, Riesmeyer J, Vangerow B, Ruotolo G, Weerakkody G J and Nissen S E 2015 *Am. Heart J.* **170** 1061
[50] Borhani D W, Rogers D P, Engler J A and Brouillette C G 1997 *Proc. Natl. Acad. Sci.* **94** 12291
[51] Brouillette C G, Anantharamaiah G, Engler J A and Borhani D W 2001 *Biochim. Biophys. Acta* **1531** 4
[52] Rogers D P, Roberts L M, Lebowitz J, Engler J A and Brouillette C G 1998 *Biochemistry-Us* **37** 945
[53] Brouillette C G, Dong W J, Yang Z W, Ray M J, Protasevich I I, Cheung H C and Engler J A 2005 *Biochemistry-Us* **44** 16413
[54] Borhani D W, Engler J A and Brouillette C G 1999 *Acta Crystallogr. Sect. D. Biol. Crystallogr.* **55** 1578
[55] Ajees A A, Anantharamaiah G, Mishra V K, Hussain M M and Murthy H K 2006 *Proc. Natl. Acad. Sci. USA* **103** 2126
[56] Tanaka M, Koyama M, Dhanasekaran P, Nguyen D, Nickel M, Lund-Katz S, Saito H and Phillips M C 2008 *Biochemistry-Us* **47** 2172
[57] Silva R G D, Hilliard G M, Fang J, Macha S and Davidson W S 2005 *Biochemistry-Us* **44** 2759
[58] Davidson W S and Thompson T B 2007 *J. Biol. Chem.* **282** 22249
[59] Gursky O and Atkinson D 1996 *Proc. Natl. Acad. Sci. USA* **93** 2991
[60] Mei X and Atkinson D 2011 *J. Biol. Chem.* **286** 38570
[61] Chetty P S, Mayne L, Lund-Katz S, Stranz D, Englander S W and Phillips M C 2009 *Proc. Natl. Acad. Sci.* **106** 19005
[62] Chetty P S, Mayne L, Kan Z Y, Lund-Katz S, Englander S W and Phillips M C 2012 *Proc. Natl. Acad. Sci.* **109** 11687
[63] Jonas A, Kézdy K E and Wald J H 1989 *J. Biol. Chem.* **264** 4818
[64] Jonas A, Zorich N L, Kézdy K E and Trick W E 1987 *J. Biol. Chem.* **262** 3969
[65] Jonas A, Wald J H, Toohill K L, Krul E S and Kézdy K E 1990 *J. Biol. Chem.* **265** 22123
[66] Segrest J P, Jones M K, Klon A E, Sheldahl C J, Hellinger M, De Loof H and Harvey S C 1999 *J. Biol. Chem.* **274** 31755
[67] Koppaka V, Silvestro L, Engler J A, Brouillette C G and Axelsen P H 1999 *J. Biol. Chem.* **274** 14541
[68] Mishra V K, Anantharamaiah G, Segrest J P, Palgunachari M N, Chaddha M, Sham S S and Krishna N R 2006 *J. Biol. Chem.* **281** 6511
[69] Bhat S, Sorci-Thomas M G, Tuladhar R, Samuel M P and Thomas M J 2007 *Biochemistry-Us* **46** 7811
[70] Martin D D, Budamagunta M S, Ryan R O, Voss J C and Oda M N 2006 *J. Biol. Chem.* **281** 20418
[71] Wu Z, Wagner M A, Zheng L, Parks J S, Shy J M, Smith J D, Gogonea V and Hazen S L 2007 *Nat. Struct. Mol. Biol.* **14** 861
[72] Chetty P S, Nguyen D, Nickel M, Lund-Katz S, Mayne L, Englander S W and Phillips M C 2013 *J. Lipid Res.* **54** 1589
[73] Wu Z, Gogonea V, Lee X, Wagner M A, Li X M, Huang Y, Undurti A, May R P, Haertlein M and Moulin M 2009 *J. Biol. Chem.* **284** 36605
[74] Jones M K, Zhang L, Catte A, Li L, Oda M N, Ren G and Segrest J P 2010 *J. Biol. Chem.* **285** 41161
[75] Zhang L, Song J, Cavigiolio G, Ishida B Y, Zhang S, Kane J P, Weisgraber K H, Oda M N, Rye K A and Pownall H J 2011 *J. Lipid Res.* **52** 175
[76] Murray S C, Gillard B K, Ludtke S J and Pownall H J 2016 *Biophys. J.* **110** 810
[77] Bibow S, Polyhach Y, Eichmann C, Chi C N, Kowal J, Albiez S, McLeod R A, Stahlberg H, Jeschke G and Güntert P 2017 *Nat. Struct. Mol. Biol.* **24** 187
[78] Segrest J, Jones M, Catte A, Manchekar M, Datta G, Zhang L, Zhang R, Li L, Patterson J and Palgunachari M 2015 *Structure* **23** 1214
[79] Silva R G D, Huang R, Morris J, Fang J, Gracheva E O, Ren G, Kontush A, Jerome W G, Rye K A and Davidson W S 2008 *Proc. Natl. Acad. Sci.* **105** 12176
[80] Gursky O 2013 *J. Mol. Biol.* **425** 1
[81] Wu Z, Gogonea V, Lee X, May R P, Pipich V, Wagner M A, Undurti A, Tallant T C, Baleanu-Gogonea C and Charlton F 2011 *J. Biol. Chem.* **286** 12495
[82] Huang R, Silva R G D, Jerome W G, Kontush A, Chapman M J, Curtiss L K, Hodges T J and Davidson W S 2011 *Nat. Struct. Mol. Biol.* **18** 416
[83] Segrest J P, Jones M K and Catte A 2013 *J. Lipid Res.* **54** 2718
[84] Prassl R and Laggner P 2008 *Eur. Biophys. J.* **38** 145
[85] Chapman M J, Guérin M and Bruckert E 1998 *Eur. Heart J.* **19** A24
[86] Prassl R 2011 *J. Lipid Res.* **52** 187
[87] Galeano N F, Alhaideri M, Keyserman F, Rumsey S C and Deckelbaum R J 1998 *J. Lipid Res.* **39** 1263
[88] Lundkatz S, Laplaud P M, Phillips M C and Chapman M J 1998 *Biochemistry* **37** 12867
[89] Deckelbaum R J, Shipley G G, Small D M, Lees R S and George P K 1975 *Science* **190** 392
[90] Laggner P and Müller K W 1978 *Q. Rev. Biophys.* **11** 371
[91] Laggner P, Kostner G M, Degovics G and Worcester D L 1984 *Proc. Natl. Acad. Sci. USA* **81** 4389
[92] Pownall H J, Shepherd J, Mantulin W W, Sklar L A and Gotto A M Jr 1980 *Atherosclerosis* **36** 299
[93] Pregetter M, Prassl R, Schuster B, Kriechbaum M, Nigon F, Chapman J and Laggner P 1999 *J. Biol. Chem.* **274**
[94] Baumstark M W, Kreutz W, Berg A, Frey I and Keul J 1990 *Biochim. Biophys. Acta* **1037** 48
[95] Spin J M and Atkinson D 1995 *Biophys. J.* **68** 2115
[96] Orlova E V, Sherman M B, Chiu W, Mowri H, Smith L C and Gotto A M 1999 *Proc. Natl. Acad. Sci. USA* **96** 8420
[97] Sherman M B, Orlova E V, Decker G L, Chiu W and Pownall H J 2003 *Biochemistry* **42** 14988
[98] Segrest J P, Jones M K, De L H and Dashti N 2001 *J. Lipid Res.* **42** 1346
[99] Kumar V, Butcher S J, K Ö, Engelhardt P, Heikkonen J, Kaski K, Ala-Korpela M and Kovanen P T 2011 *PLoS One* **6** e18841
[100] Vauhkonen M and Somerharju P 1989 *Biochim. Biophys. Acta* **984** 81

[101] Ren G, Rudenko G, Ludtke S J, Deisenhofer J, Chiu W and Pownall H J 2010 *Proc. Natl. Acad. Sci. USA* **107** 1059

[102] Zhang L, Yan F, Zhang S L, Lei D S, Charles M A, Cavigiolio G, Oda M, Krauss R M, Weisgraber K H and Rye K A 2012 *Nat. Chem. Biol.* **8** 342

[103] Zhang L, Song J, Cavigiolio G, Ishida B Y, Zhang S L, Kane J P, Weisgraber K H, Oda M N, Rye K-A, Pownall H J and Ren G 2011 *J. Lipid Res.* **52** 175

[104] Zhang L, Song J, Newhouse Y, Zhang S L, Weisgraber K H and Ren G 2010 *J. Lipid Res.* **51** 1228

[105] Cilpakarhu G, Jauhiainen M and Riekkola M L 2015 *J. Lipid Res.* **56** 98

[106] Lei D S, Zhang X, Jiang S, Cai Z, Rames M J, Zhang L, Ren G and Zhang S L 2013 *Proteins: Struct. Funct. Bioinform.* **81** 415

[107] Lei D S, Rames M, Zhang X, Zhang L, Zhang S L and Ren G 2016 *J. Biol. Chem.* **291** 14034

[108] Lauer M E, Graff-Meyer A, Rufer A C, Maugeais C, Mark E V D, Matile H, D'Arcy B, Magg C, Ringler P and Müller S A 2016 *J. Struct. Biol.* **194** 191

[109] Zhang L, Yan F, Zhang S L, Lei D S, Charles M A, Cavigiolio G, Oda M, Krauss R M, Weisgraber K H, Rye K A, Pownall H J, Qiu X Y and Ren G 2012 *Nat. Chem. Biol.* **8** 342

[110] Zhang M, Charles R, Tong H, Zhang L, Patel M, Wang F, Rames M J, Ren A, Rye K A, Qiu X, Johns D G, Charles M A and Ren G 2015 *Sci. Rep.* **5** 8741