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Review article

Cholesterol transfer at the plasma membrane

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HIGHLIGHTS

- Free cholesterol transfer from lipoprotein particles to the plasma membrane occurs in mammals.
- Amphiphilic lipid transfer occurs upon contact of lipoprotein particles to the plasma membrane.
- Both transfer mechanisms are driven by a concentration gradient.
- They are independent of the apolipoprotein/protein composition of the lipoprotein particle.
- Close proximity of lipoprotein particles to the membrane yields immediate transfer of cholesterol.

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ABSTRACT

Cholesterol homeostasis is of central importance for life. Therefore, cells have developed a divergent set of pathways to meet their cholesterol needs. In this review, we focus on the direct transfer of cholesterol from lipoprotein particles to the cell membrane. More molecular details on the transfer of lipoprotein-derived lipids were gained by recent studies using phospholipid bilayers. While amphiphilic lipids are transferred right after contact of the lipoprotein particle with the membrane, the transfer of core lipids is restricted. Amphiphilic lipid transfer gains special importance in genetic diseases impairing lipoprotein metabolism like familial hypercholesterolemia. Taken together, these data indicate that there is a constant exchange of amphiphilic lipids between lipoprotein particles and the cell membrane.

1. Introduction

Cholesterol is a lipid of central importance for cell homeostasis. Among other functions, it is crucial for membrane structure and maintenance and serves as a building block for hormone and bile acid synthesis. About 80–90% of free cholesterol (i.e. cholesterol that has not been esterified) resides in the plasma membrane [1,2], where it is particularly abundant in detergent-resistant membrane fractions [3,4]. Membrane cholesterol fills three distinguishable pools, while assuring membrane morphology [5]. One relatively constant pool of cholesterol is essential for membrane integrity, while the other two are flexible in their proportions: cholesterol is either sequestered with sphingomyelin or accessible for transport from and to the endoplasmic reticulum (ER) [5,6]. The last pool is also accessible to cholesterol oxidase [7,8] and Perfringolysin O [9]. Cholesterol is enriched in areas with increased membrane rigidity and order [10–13]. These structural components may influence the cholesterol transfer capacity. It is still debated how cholesterol is distributed between the inner and outer leaflet; most studies support the notion that cholesterol is more abundant in the cytoplasmic leaflet than in the exofacial leaflet (for reviews see Refs. [14,15] and comment [16]). Supported lipid bilayers (SLBs) are widely used as a model system for these biological membranes and have been used previously to study lipoprotein adsorption and phospholipid transfer [17]. Taken together, cellular cholesterol is distributed in a non-uniform allocation among cell compartments and membranes and thus cholesterol levels are under tight control either via *de novo* cholesterol synthesis or cholesterol uptake. Cholesterol exchange between cells and body fluids like blood, bile and liquor is of central importance for higher organisms and therefore cells have developed a divergent set of transfer mechanisms. In general, lipoprotein particles play a central role in cholesterol transport and exchange in mammalians [9].

Lipoproteins are composed of a monolayer of amphiphilic lipids and cholesterol stabilized by embedded apolipoproteins and a core of cholesteryl esters (CE) and triglycerides. Lipoproteins can be classified

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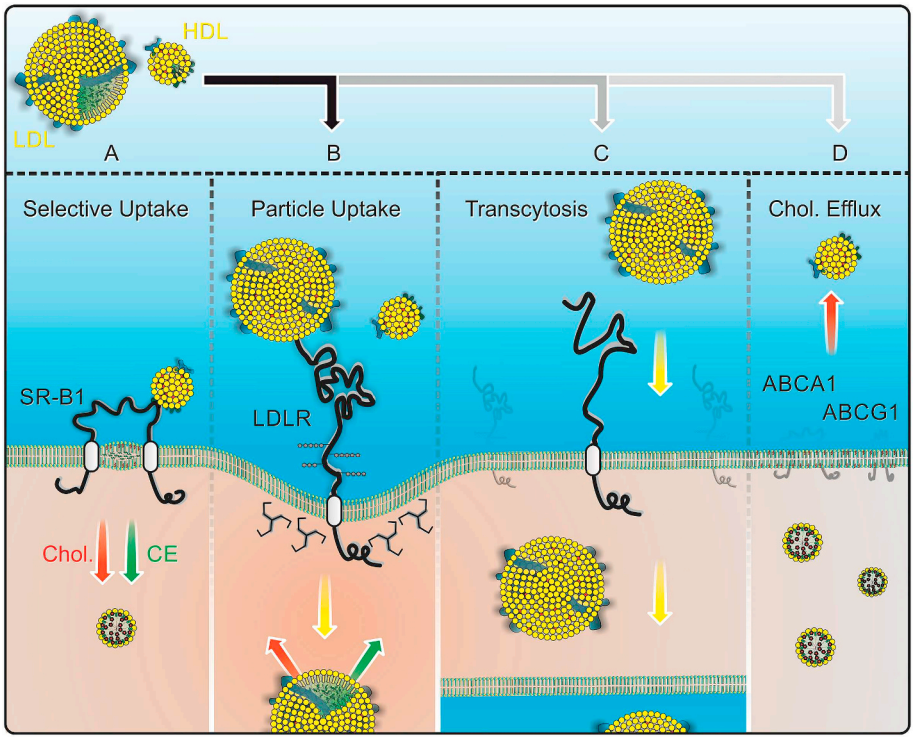

Fig. 1. Major pathways of lipid cargo-transfer between lipoprotein particles and cells.
Receptor-mediated interaction of high- and low-density lipoprotein (HDL, LDL) particles yields either selective uptake of cholesterol (Chol.) and cholesteryl ester (CE) via the scavenger receptor class B type 1 (SR-B1) (A), particle uptake via the low-density lipoprotein receptor (LDLR) located in clathrin-coated pits (B) or lipoprotein particle endocytosis and subsequent transcytosis (C). Cellular cholesterol efflux to external acceptors occurs via ABCA1 and ABCG1 (D).

according to their increasing density, namely chylomicrons, very-low density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL). So far, lipoprotein binding to the plasma membrane and subsequent cargo transfer has mainly been thought to occur via receptor interaction. Four main pathways have been described: (A) selective cholesteryl ester/lipid uptake via Scavenger Receptor class B type 1 (SR-B1) without particle degradation [18,19], (B) receptor-mediated uptake and degradation of apolipoprotein B/E (apo B/E) containing lipoprotein particles via the Low-Density Lipoprotein Receptor (LDLR) [20–22] and other family members [23,24], (C) lipoprotein endocytosis and transcytosis [25–28] and (D) cholesterol efflux to external acceptors [29] (Fig. 1). Due to their importance, these pathways are very redundant – the loss of function of LDLR or SR-B1 does not lead to immediate intrauterine death. However, patients lacking functional LDLR suffer from early onset of atherosclerosis [20,30]. Similarly, mice with a double knock-out of LDLR and SR-B1 are viable, but develop a severe phenotype including occlusive coronary artery atherosclerosis and myocardial infarction when fed a high fat diet [31–34]. These observations show that a redundant set of pathways keeps the flux of membrane cholesterol active and thus the intra-cellular cholesterol level in balance, even if one of the major pathways is non-functional.

Primarily, cholesterol is transferred to cells by endocytosis of LDL particles after their binding to LDL receptors. The majority of these receptors are located in the plasma membrane of hepatocytes, although all other tissues and cells express the LDL receptor [20,35,36]. LDL receptors bind LDL particles specifically and with high affinity. After binding and subsequent endocytosis via clathrin-coated pits, the LDL particle is released from its receptor within early endosomes [36] due to a low pH value in the endosomal lumen. The receptor is afterwards recycled back to the plasma membrane. Degradation of LDL particles, which includes hydrolysis of its CEs by acid lipase, takes place in late endosomes. This process is called *LDL-receptor-mediated endocytosis* [35]. The cholesterol backbone leaves the late endosomes/lysosomes and moves to other organelles, primarily the ER mediated by Niemann Pick Type C (NPC) 1 and 2 protein and to the plasma membrane (PM) [5]. In addition, the Lysosomal Integral Membrane Protein-2 (LIMP-2/SCARB2), the closest homologue to SR-B1, was described recently to transport cholesterol out of the lysosome - at least in states where the NPC pathway is not operative [37].

Besides LDL receptor mediated endocytosis, another process termed *selective cholesteryl ester uptake* delivers CE - predominantly from HDL - to the cells without the uptake and lysosomal degradation of the particle itself [38,39]. This high-capacity, physiologically regulated lipid delivery system operates in steroidogenic tissues and the liver, in which the cholesterol backbone is utilized for steroidogenesis or bile acid synthesis, respectively [40]. The cholesterol backbone derived by hydrolysis of CE or from the free form is transported via plasma membrane and ER contact sites facilitated by ASTER B proteins [41–43] which are linking/clipping the two membranes. The direct transfer of cholesterol from the lipoprotein particle to the plasma membrane has not received much attention in the last decade. However, it was analyzed and discussed in the decades before the turn of the century.

This review focuses on the first steps of direct free cholesterol transfer from lipoproteins to cells namely docking to the cell membrane and subsequent transfer of free cholesterol from lipoprotein particles to the plasma membrane. For direct lipid transfer, cholesterol has to leave the membrane environment and pass the so-called unstirred water layer [44]. In this process, tethering of lipoprotein particles to the plasma membrane provides the spatial vicinity required for cholesterol transfer (Fig. 1).

1.1. HDL-derived free and esterified cholesterol are taken up via different pathways

Several lines of evidence suggest that lipoprotein-derived free cholesterol is taken up rapidly by cells and that its intracellular transport differs from that of lipoprotein-derived cholesteryl ester. Wüstner et al. [45] demonstrated in polarized human HepG2 cells that HDL-derived free cholesterol arrived earlier at the apical membrane and the biliary canaliculi than the protein moiety of HDL particles at the Endocytic Recycling Compartment (ERC). Furthermore, it has been described that cholesterol and cholesteryl ester derived from HDL particles are handled differently by rat liver [46,47]. While HDL-derived ^{3}H-cholesterol

was observed in the bile of rats within 30 min, ^{3}H-cholesteryl oleate was delayed [46,48]. In addition, Wüstner et al. [49] showed that some HDL-associated cholesterol reached the biliary canaliculi as a constituent of HDL particles, whereas most of HDL-derived sterols were released from the HDL particle and transferred to biliary canaliculi via a non-vesicular transport mechanism. Hepatic lipase induced the accumulation of free cholesterol derived from HDL particles in a Fu5AH rat hepatoma cell line and the transfer of free cholesterol was preferred 10 times compared to esterified cholesterol and 25 times compared to sphingomyelin [50]. In a rat model, the liver rapidly took up HDL-derived free cholesterol and thus removed about 60% from the blood within the first 10 min, whereas only about 1% was taken up by the spleen and the lung – even smaller amounts of radioactivity were found in other tissues [46]. These data argue for a rapid removal of free cholesterol from HDL particles by hepatic tissue - with an involvement of hepatic lipase which might increase binding of the remodeled HDL particles to proteoglycans [51]. In extra-hepatic tissues much less direct cholesterol transfer appears to occur and selective CE uptake seems to be preferred. Similarly, Rajan & Menon [52] showed that rat luteal cells used free cholesterol directly derived from HDL particles for progestin synthesis. Utilization of this free cholesterol was about threefold higher than would be expected from its concentration in the HDL particle. These data strongly suggest that free cholesterol derived from HDL is quickly distributed to cells for synthesis of bile acids or hormones by direct transfer whereas the transfer of esterified cholesterol proceed slower and requires further interaction with receptors and possibly additional proteins.

1.2. Direct transfer of free cholesterol from HDL is an important source of cholesterol in human liver

In humans, the role of HDL particles in lipoprotein metabolism differs considerably from rodents: while mice do not express Cholesteryl Ester Transfer Protein (CETP) and thus HDL is their major lipoprotein particle, this is not the case in humans. Mice do not develop atherosclerosis even when severely challenged with a high fat diet – at least in part because their reverse cholesterol transport via HDL particles seems to be very efficient. Nevertheless, studies in humans with a bile fistula showed [53] that similar to rodents, HDL-derived free cholesterol is more rapidly incorporated into biliary cholesterol than that derived from LDL particles [54]. This suggests that the human liver selectively utilizes and secretes free cholesterol derived from lipoproteins into the bile [54,55]. Goodman, Noble & Dell [56] measured long-term turnover of plasma cholesterol by using it as tracer. They concluded in their computational analysis using a three-pool model that free cholesterol of the plasma and the liver are in a rapid equilibrium - indicating a direct exchange of free cholesterol. Using multi-compartmental analysis and two different tracers (the precursor for newly synthesized cholesterol mevalonic acid and cholesterol itself), Schwartz et al. [57] reported that about 20–30% of the cholesterol input into bile is derived from newly synthesized cholesterol while most of the remainder originates from lipoprotein-derived free cholesterol. In line, the same author [58] assessed the role of cholesteryl, ester: irreversible ester transfer was found from VLDL, IDL and LDL but little originated from HDL particles. These data suggest that selective cholesteryl ester uptake and holo HDL particle uptake are minor pathways for cholesterol delivery to the bile. These *in vivo* results are not in complete agreement with studies using an *in silico* kinetic model predicting plasma cholesterol concentration in humans [59]. In this model, free cholesterol transferred from the liver to HDL particles would be predicted to exceed hepatic uptake of free cholesterol and cholesteryl esters from HDL particles. However, even in this prediction model still a considerable transfer of free cholesterol from HDL particles to the liver is observed. Summarized, these studies suggest a crucial role of free cholesterol transfer as a supply for hepatic cholesterol pools. In this process, the first step is direct transfer of cholesterol from a lipoprotein particle as demonstrated in our studies using phospholipid bilayers as model system [60–62].

1.3. Direct transfer of HDL-derived free cholesterol to the plasma membrane requires tethering

While molecular details of direct transfer cholesterol from HDL particles to membranes are not fully known, some insights may be gained from the mechanisms mediating cellular cholesterol efflux. For cellular cholesterol efflux to HDL particles, two passive processes involving simple diffusion have been proposed in addition to cholesterol efflux via active transport mediated by ATP-binding cassette transporter 1 (ABCA1): an aqueous diffusion pathway and a facilitated diffusion mechanism mediated via SR-B1[29]. It is conceivable that these pathways are also active in the opposite direction mediating cholesterol flux from cholesterol-loaded HDL particles to the cell membrane. This would imply that close proximity of an HDL particle to the membrane leads to immediate transfer of cholesterol [61]. In tissue culture and *in vivo* experiments, this close proximity may be mainly mediated by HDL particle binding to SR-B1. Recently Marques et al. [63] reported that SR-B1, which is stable of several hours at the plasma membrane, multimerizes for efficient cholesterol transfer (see Ikonen & Kanerva [64]). Indeed, it has been shown that SR-B1 mediates bidirectional cholesterol transport [65–70]. In cholesterol depleted cells, the cholesterol transfer efficiency of SR-B1 depends only on the cholesterol content of the lipoprotein particle – irrespective of the nature of the particle [66,69,71]. Thus, this passive and bidirectional exchange depends only on the concentration gradient. In equilibrium, the passive cholesterol transfer to the membrane ceases [66] until a sink - represented for instance by bile acid synthesis - is present. In this respect, direct cholesterol transfer from the plasma membrane to the endoplasmic reticulum was seen recently, which is mediated by GRAMD1 or ASTER B proteins [41,42] acting as clips linking of the two respective membranes. Aster-B is required for delivery of HDL-derived cholesterol to adrenals [41]. This mechanism operative between lipid bilayers and cell membranes occurs also between intracellular membrane contact sites, such as endosomal ER and the plasma membrane or between mitochondria and endosomes: StART-like domains of Ysp2p and GramD1a-c mediate the transfer of dehydroergosterol (DHE) used as a cholesterol surrogate between membranes [42]. In addition, LIMP-2, the closest homologue of SR-B1, has been described recently to be involved in transport of cholesterol out of the lysosome [37]. Both, SR-B1 and CD36, another close homologue, also bind anionic phospholipid-liposomes [72]. Phosphatidylserine- and phosphatidylinositol-containing liposomes effectively competed LDL particle binding in receptor-overexpressing cells [73], suggesting that lipids alone are capable of binding to the previously described additional high affinity lipid bind site of SR-B1[73]. After binding, transfer of lipids may occur according to their concentration gradient [74].

Accordingly, we observed the transfer of HDL-derived amphiphilic lipids like DiI or BodipyFL–labeled cholesterol directly at the native plasma membrane (Fig. 3). Within a few minutes, observable amounts of amphiphilic lipids were transferred to the membrane whereas cholesteryl ester transfer was not detected [62]. Our observations indicate that amphiphilic lipids are transferred from the HDL particle right after membrane contact. In the native cell system, tethering of lipoprotein particles is mainly mediated by SR-B1 – an anchor function, which can be replaced by an artificial biotin–streptavidin linkage in a model system [61] (Fig. 2). These data suggest that SR-B1 is dispensable for the transfer process of amphipathic cargo itself and is mainly required for tethering the HDL particle close to the plasma membrane surface.

1.4. Direct uptake of free cholesterol from apoB-containing lipoproteins follows the same pathway as HDL-derived free cholesterol

Of all serum lipoprotein particles, LDL particles are richest in

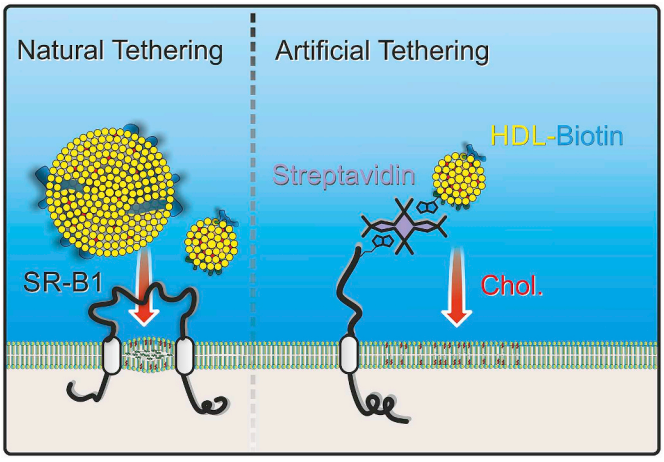

Fig. 2. Tethering of lipoprotein particles and subsequent cargo transfer.
Receptor-mediated interaction of high- and low-density lipoprotein (HDL, LDL) particles yields physiological tethering and thus transfer of at least free cholesterol (Chol.) and amphiphilic lipids to the plasma membrane via scavenger receptor class B type 1 (SR-B1). Artificial tethering via e.g. biotinylated plasma membrane proteins and HDL particles linked via streptavidin imitates biological tethering and also results in cholesterol transfer [61].

cholesterol and contribute over half of all plasma lipoprotein particles. LDL particles have an average diameter of 20–22 nm [75,76] and contain about 600 molecules of free cholesterol, which are located in the lipid monolayer at the particle's surface [77]. A single apoB-100 molecule, one of the largest monomeric proteins known, compensates for the structural and compositional changes during the particle's life cycle [76]. LDL-derived cholesterol is delivered to cells by endocytosis of LDL particles mainly via binding to LDL receptors. Besides LDL particles, further apoB containing lipoproteins, such as VLDL and chylomicrons, are predominantly catabolized via LDL receptor-mediated endocytosis.

Studies in humans with familial hypercholesterolemia (FH) and on Watanabe rabbits with heritable hyperlipidaemia – both have defective LDLR and therefore impaired LDL particle uptake via receptor-mediated endocytosis – have demonstrated that even under these conditions LDL particle catabolism still occurs in liver cells [78]. Individuals with FH metabolize even more LDL particles than healthy individuals despite of their defective LDLR - a consequence of their elevated LDL particle concentration [79]. Comparing fibroblast cells from healthy individuals and from FH patients, Fielding & Fielding [80,81] showed that these cells selectively transfer free cholesterol from LDL particles independent of receptor-mediated endocytosis. Interestingly, this transfer process, like receptor-mediated endocytosis, occurred at clathrin-coated pits [81]. The cellular level of free cholesterol was increased by around 15% and excessive cholesterol was transported to caveolae which in turn triggered cholesterol efflux to HDL particles [82]. This transfer of LDL-derived cholesterol occurred via a high-capacity, but low-affinity pathway. It was hypothesized that transfer of free cholesterol to the outer leaflet of coated pits might help to trigger the budding of endocytic vesicles. Similarly, data from Fong & Angel [83] demonstrated that adipocytes show a preference for transfer of free cholesterol from LDL particles which might reflect an exchange of free cholesterol between LDL particles and the plasma membrane. In addition, Slotte et al. [84] observed an increase in cholesterol of about 40% after incubation

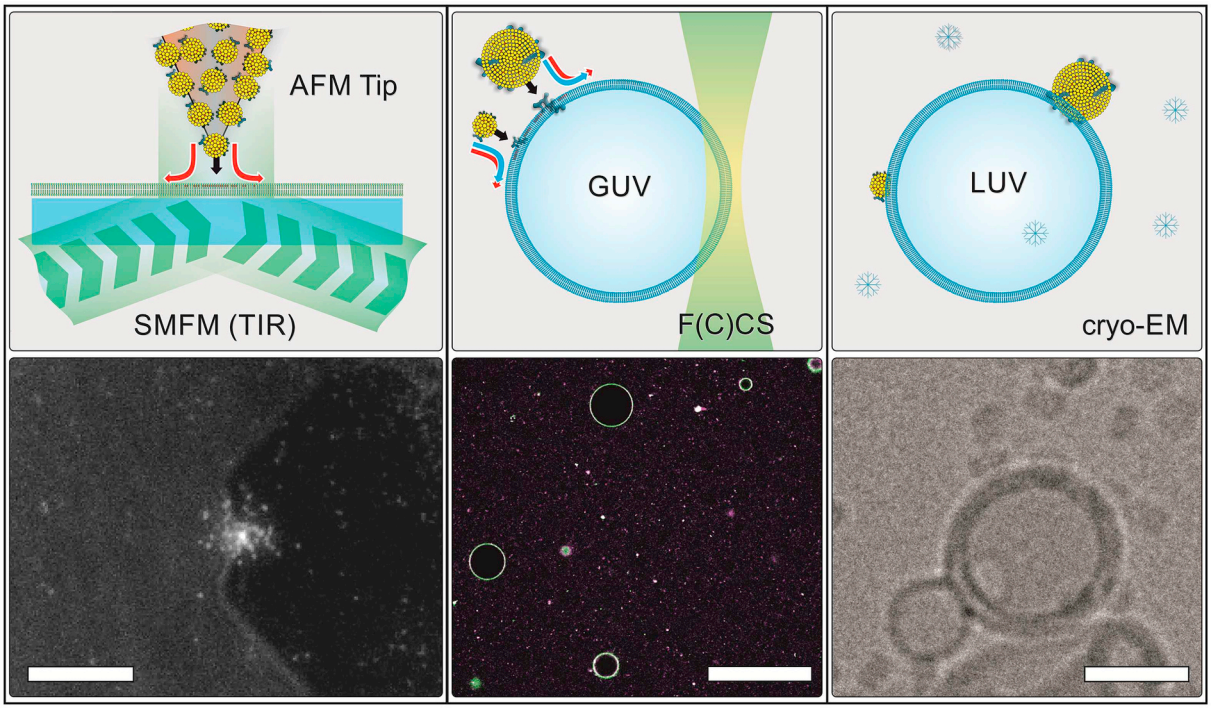

Fig. 3. Biophysical techniques used to observe the cargo transfer process.
(Left, upper panel) Schematic representation of the combination of atomic force and single-molecule-sensitive fluorescence microscopy (AFM & SMFM) using an AFM cantilever tip functionalized with lipoprotein particles. This combination allows the observation of the cholesterol transfer from lipoprotein particles to a supported lipid bilayer in total internal reflection (TIR) mode. (Lower panel) Combined brightfield/fluorescence image visualizing the AFM cantilever (right image half) functionalized with LDL and the radial propagation of fluorescently labeled cholesterol (BodipyFL) from the contact point (center). Scale bar = 10 µm. (Center, upper panel) Observation of the interaction and subsequent fusion of lipoprotein particles with giant unilamellar vesicles (GUVs) using fluorescence (cross) correlation spectroscopy. (Lower panel) Combined confocal image of the apolipoprotein- (Atto647N-labeled, magenta) and cholesterol-signal (BodipyFL-labeled, green) of LDL particles incubated with GUVs. Scale bar = 100 µm. (Right, upper panel) Schematic representation of the visualization of fused lipoprotein particles with large multilammelar vesicles (LUVs) using cryo-electron microscopy (cryo-EM). (Lower panel) Cryo-EM image showing the fusion of a LDL particle (left corner) with an LUV. Scale bar = 33 nm.

of aortic smooth muscle cells with LDL particles, which was independent of particle uptake. Furthermore, SR-B1 was found to bind VLDL, β-VLDL and chylomicron particles, followed by lipid transfer and uptake [85–91]. Thus, direct cholesterol transfer from apoB-containing lipoprotein particles occurs in addition to receptor-mediated endocytosis at the plasma membrane.

In plasma, lipids are transferred between lipoprotein classes by spontaneous or protein-mediated transfer. Free cholesterol transfer occurs in a process driven by collision and aqueous diffusion [92,93]. In this regard, Phospholipid Transfer Protein (PLTP) was described to mediate transfer of free cholesterol [94] in addition to its role in HDL particle remodeling and phospholipid transfer. This process may increase free cholesterol in HDL particles. However, neither deletion nor overexpression of PLTP altered the excretion of macrophage-derived cholesterol in an *in vivo* reverse cholesterol transport (RCT) assay [95]. Thus, the biological significance of this effect remains to be established.

1.5. Red blood cells directly exchange free cholesterol with lipoproteins and tissues

Lipoprotein particles play a central role in free cholesterol exchange between blood cells and surrounding plasma [93]. The group of Daniel J. Rader extended their studies on reverse cholesterol transport to humans applying as tracer ^{3}H-cholesterol nanoparticles with albumin as a carrier [96]. This tracer first rapidly disappeared from plasma and then rapidly reappeared at a linear range for a couple of hours. This suggests that cholesterol taken up by the retroendothelial system is not esterified but transferred to lipoprotein particles and red blood cells (RBCs). Indeed, the process of lipid transfer between membranes is active in blood cells: RBCs are rich in cholesterol and act as exceptionally potent acceptors for cholesterol, as shown using cholesterol-loaded mouse peritoneal macrophages [97]. In humans, RBCs account for about 45% of the blood volume and their cholesterol concentration is comparable to that of circulating lipoproteins [98]. Phospholipids represent about 60–70% of total RBC lipids, while about 25% are free cholesterol. The RBC plasma membrane exhibit a bidirectional exchange of free cholesterol with lipoprotein particles with kinetics indicating transfer via aqueous diffusion [93]. RBCs acquire their cholesterol from plasma with a first order kinetic, indicating its primary dependence on the cholesterol gradient [93]. In line, RBC membrane constituents from Tangier patients – an inborn metabolic disease with near absent HDL-C levels – have a low cholesterol content [99–101]. Hung et al. [102] showed that apoAI-deficient mice carry most of their cholesterol in RBCs. When these mice are anemic, macrophage reverse cholesterol transport decreases by 35% - indicating a dynamic RBC cholesterol pool which is able to facilitate RCT from macrophages to the liver at low HDL levels [102]. Taken together, these data indicate a constant and direct exchange of free cholesterol between lipoprotein particles and RBCs and show that RBCs significantly contribute to RCT at least in states of altered lipoprotein metabolism.

1.6. Lipoprotein-mediated free cholesterol transfer to artificial biomembranes

One major challenge of quantitatively studying functional interactions between proteins and lipid membranes is the multitude of components involved. Due to the high complexity of living organisms, it is difficult to establish a direct functional connection between individual components. This encourages the use of defined experimental conditions such as artificial membranes. Previous studies [17,103] showed that apolipoprotein A–I interacts strongly with artificial membranes in dependence of its protein conformation and the membrane composition/heterogeneity. Moreover, HDL and LDL particles are able to remove lipids from and deposit lipids to artificial bilayers. Recently, we demonstrated the direct interaction of HDL and LDL particles with simple artificial membranes. The lipid transfer followed immediately after the interaction [60–62]. The rate of cholesterol transfer between lipid vesicles has been reported to be dependent on the surface curvature [104] and to decrease with increasing size of the donor vesicles. Moreover, the head groups of membrane phospholipids have been shown to influence the transfer rate [105]. To obtain a comprehensive view on the transfer process of lipoprotein-derived cholesterol, we combined several complementary biophysical measurement (Fig. 3). Our results show that lipoprotein particles incorporate into lipid membranes without receptor interaction just upon contact and transfer their cargo [60]. Thus, we suggest that the “high-capacity, low affinity pathway” for cholesterol transfer is represented by the plasma membrane itself. Indeed, passive transfer-distribution of free cholesterol between dehydroergosterol (DHE - a fluorescent cholesterol analogue used as marker for free cholesterol) containing liposomes and lipoprotein particles, albumin and erythrocytes was observed and its kinetics were modeled [106]. Taken together, the studies mentioned above indicate that on purely artificial membranes amphiphilic lipids can be transmitted via direct membrane-lipoprotein contact and that the transfer rate might be influenced by the physical properties of the membrane itself.

2. Conclusion

In conclusion, lipoprotein particles transfer their cargo upon contact with the plasma membrane due to a concentration gradient. In addition, direct transfer of free cholesterol from lipoprotein particles seems to be largely independent of the apolipoprotein/protein composition.

Declaration of competing interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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