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EDITORIAL

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The lipid flux rheostat: implications of lipid trafficking pathways

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High triglyceride/low HDL syndromes are established predictors of coronary heart disease (CHD), and primary genetic defects associated with HDL deficiency states have been attributed to the ATP binding cassette transporter A1 (ABCA1) [1–3], apolipoprotein A-I (apoA-I), lecithin cholesteryl acyl transferase (LCAT), and Niemann–Pick C proteins 1 and 2 (NPC1, NPC2) [4]. Additional candidate genes with genetic variants affecting plasma HDL cholesterol levels include hepatic lipase and the genes of the apolipoprotein AI/CIII/AIV/AV gene cluster [5]. Moreover, relatively common DNA sequence variants in ABCA1 and apoE were recently identified to contribute to the variation in plasma levels of HDL cholesterol [6, 7].

Based on these interesting findings from genetic studies and to further elucidate the cellular and molecular mechanisms of lipid trafficking, the editor of *Journal of Molecular Medicine* invited us to coedit two review articles, which present highly interesting overviews on the role of ABCA1, NPC-proteins, SR-BI, and apolipoproteins in cellular lipid transport and HDL metabolism. Boadu and Francis [8] excellently summarize current information on the regulation and molecular functions of ABCA1, NPC-1, and NPC-2 in cellular lipid homeostasis. Moreover, Zannis, Chroni, and Krieger [9] review the current status of HDL biogenesis, focusing on the molecular interactions of apoAI with ABCA1 and SR-BI.

The interconnection of NPC1 and ABCA1 pathways in lipid efflux and the formation of HDL have been addressed successfully by studying mutant cells from Tangier disease and Niemann–Pick C disease (reviewed by Boadu and Francis [8]). Based on the common localization of ABCA1 and NPC1 in vesicular compartments of late endosomes and on the aberrant NPC1 protein levels in Tangier disease fibroblast cells, ABCA1 function seems to be involved in correct NPC1 processing [10]. On the other hand, NPC1 fibroblasts lack ABCA1 mRNA, and protein upregulation have impaired lipid efflux, and NPC1 patients have low HDL cholesterol levels, implicating impaired ABCA1 regulation in NPC1 deficiency [11]. Interesting but contradictory data on the role of NPC1 in ABCA1-related macrophage lipid metabolism have been provided by Feng et al. [12, 13] using the cholesterol trafficking inhibitor U18666 and cultured mouse peritoneal macrophages from NPC1 heterozygous animals. Free cholesterol loading of macrophages resulted in a dysfunction of apoA-I-dependent and ABCA1-mediated cholesterol and phospholipid efflux due to enhanced degradation of the ABCA1 protein. Lipid efflux and ABCA1 protein levels were restored in partial NPC1 deficient cells and cells treated with the NPC1 inhibitor U18666. Furthermore, necrosis of atherosclerotic lesions and macrophage apoptosis were significantly reduced in NPC1 heterozygous animals, implicating that NPC1-mediated lipid trafficking might also accelerate lesion progression [12, 13]. Whether these findings also translate to the human system awaits further confirmation in primary human cells.

The biogenesis of HDL is critically linked to the ABCA1-mediated transfer of phospholipids and cholesterol to lipid-poor apoA-I and preβ-HDL particles. A direct interaction of apoA-I and ABCA1 could be identified with the use of biochemical assays with ABCA1 and apoA-I containing natural mutations and targeted amino acid changes in important regions of both molecules. In a two-step model (reviewed by Zannis et al. [9]), a tight complex between ABCA1 and apoA-I is formed initially, which is then transformed into a productive complex resulting in the lipidation of apoA-I. The central helices 2–7 (amino acids 50–185) and the C-terminal region of apoA-I, comprising amino acids 220–231, are required for cross-linking to ABCA1, stimulated lipid efflux, and the formation of discoidal and later spherical HDL particles. These data correlate to findings from class A amphipathic helical peptides, such as the apoA-I mimetic D-4F, which is able to form preβ-HDL, increase paraoxonase activity, and fulfill

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anti-inflammatory activity [14]. Interestingly, other apolipoproteins including apoE, ApoA-II, apoA-V, apoC-I, and apoC-II, as well as the serum amyloid A protein, are capable of promoting ABCA1-dependent lipid efflux, which raises the possibility that HDL particle formation requires further interactions of ABCA1 with other proteins [15, 16].

Another important protein in HDL remodeling is SR-BI, which was the first HDL receptor to be identified [17]. As a scavenger receptor, SR-BI has a variety of other ligands including LDL, VLDL, and serum amyloid A. SR-BI mediates the selective uptake of cholesteryl esters from HDL and LDL and, furthermore, catalyzes the bidirectional flux of cholesterol from and into cells [18]. Again, using in vitro mutagenesis and binding assays, direct binding of ligands to SR-BI and the formation of a productive complex have been proposed as requirements for SR-BI mediated lipid transport with some analogy to the ABCA1/apoA-I system. Physiologically, SR-BI is a major receptor on hepatocytes important for reverse cholesterol efflux and delivers cholesterol to steroidogenic tissues and other peripheral cells. Further in vivo studies in humans will be required to understand the full complexity of SR-BI functions, which have been put forward by in vitro experiments and animal models.

Merging these data with findings from other cellular systems, a unifying and general model we term the "lipid flux rheostat" can be generated. As depicted in Fig. 1, lipid uptake or loading mechanisms from modified LDL particles (enzymatically modified LDL, E-LDL and oxidized LDL, Ox-LDL), involving class A and cysteine-rich scavenger receptors and Fcγ-receptors, apoE-mediated internalization via LDL-receptor-related proteins (LRPs), and selective uptake of HDL-cholesteryl esters by SR-BI, are major pathways for sterol and phospholipid uptake, which simultaneously trigger intracellular lipid trafficking and efflux pathways.

Of unexpected but major importance is the identification of the ectopic β-chain of ATP synthase as an apoA-I receptor involved in sinking in type II phagocytosis of HDL holoparticles [19]. Although the ATP synthase β-subunit is mostly found in the inner mitochondrial membrane, recent studies have clearly shown a cell surface expression on endothelial cells, lymphocytes, and hepatocytes [20–22]. The α-chain and β-chain of ATP-synthase were also identified as high affinity binding sites for apoE-enriched HDL particles [23, 24]. These findings implicate a direct connection between ATP metabolism, energy homeostasis, and the lipid uptake and efflux capacity of the liver and peripheral cells.

CD13 (aminopeptidase N), which is highly expressed in leukocytes, is another important receptor for cholesterol uptake in the brush border membrane of enterocytes and is the molecular target for the cholesterol absorption inhibitor ezetimibe [25]. The Niemann–Pick C1-like 1 protein (NPC1L1) also binds ezetimibe and may act as further downstream molecule mediating vesicular trafficking of sterols from late endosomal compartments [26].

These lipid influx mechanisms clearly influence intracellular lipid metabolism including lipoprotein assembly, routing of lipoprotein holoparticles and remnant-particles and cholesterol efflux to HDL isoforms. Several laboratories have shown that a significant part of apoE endocytosed by cells via lipoprotein receptors escapes from degradation and is recycled. HDL and apoA-I both activate the secretion and reuptake of endogenous apoE, as well as lipid efflux, via HDL-like particles, supporting the hypothesis that apoE recycling is an antiatherogenic

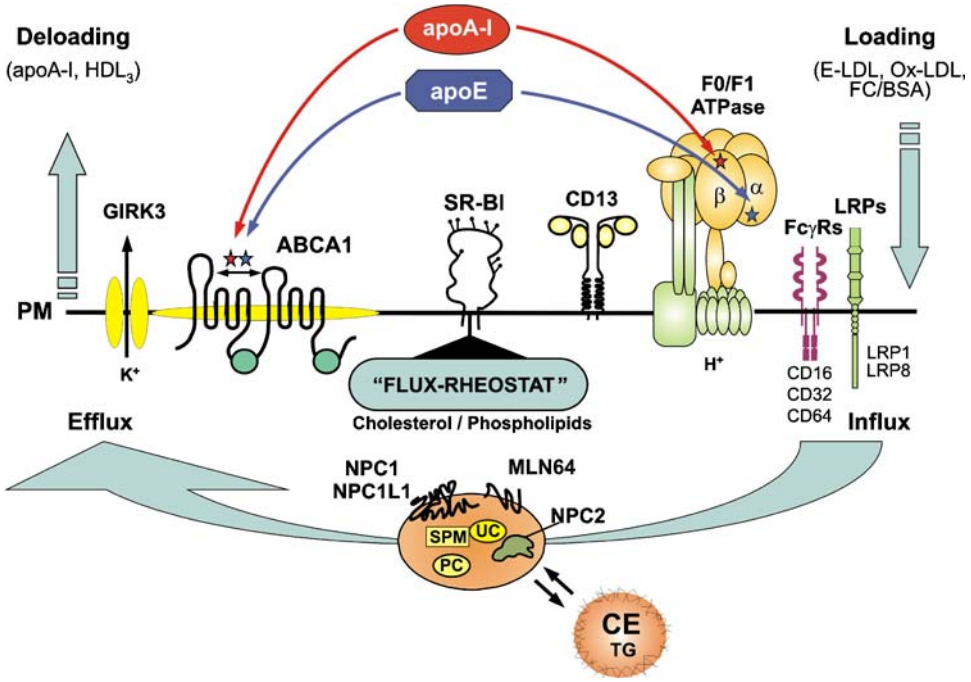

Fig. 1 The lipid flux rheostat controls lipid uptake and export mechanisms. *ABCA1* ATP-binding cassette transporter A1, *apoA-I* apolipoprotein A-I, *apoE* apolipoprotein E, *CD13* (aminopeptidase N), *CE* cholesteryl ester, *E-LDL* enzymatically modified LDL, *GIRK3* G-protein coupled inward rectifier 3, *LRP* low-density lipoprotein receptor-related protein, *MLN64* metastatic lymph node 64, *NPC1* Niemann–Pick C1 protein, *NPC1L1* Niemann–Pick C1-like 1 protein, *NPC2*, Niemann–Pick C2 protein, *PM* plasma membrane; *SPM* sphingomyelin, *SR-BI* scavenger receptor B1, *TG* triglyceride, *UC* unesterified cholesterol

mechanism [27–29]. Interestingly, apoE also mediates lipid–antigen uptake and presentation via the CD1 system, implicating a connection with immunological functions in phagocytes and dendritic cells [30]. Furthermore, apoE binds to specialized membrane microdomains rich in ceramide-containing particles and thereby prevents lipoprotein aggregation and potentially also atherosclerotic lesion initiation [31].

A direct link between apoE and ABCA1-mediated lipid efflux was recently provided by Hirsch-Reinshagen et al. [32], analyzing both astrocytes and microglia-cells from ABCA1-deficient mice. Significantly reduced lipid efflux from astrocytes of ABCA1-knockout mice was detected independent of the apoE isoform. However, only apoE2 and apoE3, but not apoE4, induced significant cholesterol efflux from microglia cells, which was also reduced in ABCA1-deficient microglia. The reduced cholesterol efflux to apoE corresponds to cellular lipid accumulation and a significant reduction in apoE levels in cultured astrocytes and microglia cells. Similar to the findings by Wahrle et al. [33], an important in vivo role of ABCA1 in brain apoE metabolism was demonstrated by reduced apoE levels in the brain of ABCA1-deficient mice. Interestingly, when individual brain regions from the ABCA1-deficient and wild-type mice were examined, reduction in apoE levels was seen most significantly in the hippocampus and striatum and least in the cerebellum. ApoJ levels were not affected by the absence of ABCA1 [32]. These findings clearly highlight the importance of apoE and ABCA1 interactions especially in macrophages, the brain, and in lipid homeostasis in general. In the future, the exact mechanisms showing how ABCA1, apoE, and apoA-I regulate the cellular lipid flux rheostat will be of major importance.

Taken together, the knowledge of lipid uptake, intracellular transport, and efflux mechanisms especially those related to apolipoproteins, ABCA1 and NPC1 has been growing substantially over the last years. Further milestones can be expected when combining data from knockout and transgenic animal models with experiments in primary cells from patients with monogenetic lipid disorders, using classical biochemical assays and novel genomic and lipidomic technologies such as DNA-microarrays and mass spectrometry analysis to monitor lipid gene expression and metabolic fluxes of intracellular lipid pools. This approach will enable researchers to manage the transition from single views of lipid metabolism to network research approaches required for understanding the complex pathways of lipid metabolism.

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