High-density lipoprotein metabolism and reverse cholesterol transport: strategies for raising HDL cholesterol

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Abstract
A key to effective treatment of cardiovascular disease is to understand the body’s complex lipoprotein transport system. Reverse cholesterol transport (RCT) is the process of cholesterol movement from the extrahepatic tissues back to the liver. Lipoproteins containing apoA-I [high-density lipoprotein (HDL)] are key mediators in RCT, whereas non-high-density lipoproteins (non-HDL, lipoproteins containing apoB) are involved in the lipid delivery pathway. HDL particles are heterogeneous; they differ in proportion of proteins and lipids, size, shape, and charge. HDL heterogeneity is the result of the activity of several factors that assemble and remodel HDL particles in plasma: ATP-binding cassette transporter A1 (ABCA1), lecithin cholesterol acyltransferase (LCAT), cholesteryl ester transfer protein (CETP), hepatic lipase (HL), phospholipid transfer protein (PLTP), endothelial lipase (EL), and scavenger receptor class B type I (SR-BI). The RCT pathway consists of the following steps: 1. Cholesterol efflux from peripheral tissues to plasma, 2. LCAT-mediated esterification of cholesterol and remodeling of HDL particles, 3. direct pathway of HDL cholesterol delivery to the liver, and 4. indirect pathway of HDL cholesterol delivery to the liver via CETP-mediated transfer There are several established strategies for raising HDL cholesterol in humans, such as lifestyle changes; use of drugs including fibrates, statins, and niacin; and new therapeutic approaches. The therapeutic approaches include CETP inhibition, peroxisome proliferator-activated receptor (PPAR) agonists, synthetic farnesoid X receptor agonists, and gene therapy. Results of clinical trials should be awaited before further clinical management of atherosclerotic cardiovascular disease. (Anatol J Cardiol 2017; 18: 149-54)

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Introduction
A key to effective treatment of cardiovascular disease is to understand the body’s complex lipoprotein transport system (1–5). Apolipoproteins (apo) are responsible for the transport of triacylglycerols (TG), phospholipids, cholesterol, and cholesteryl esters between organs (6). In muscles, the fatty acids are oxidized for energy, whereas in the adipose tissues, they are re-esterified for storage as TG. Cholesterol can be used as a cell membrane structure, for steroid hormone synthesis, or finally converted into bile acids (1). Reverse cholesterol transport (RCT) is the process of cholesterol movement from the extrahepatic tissues back to the liver (7, 8). Lipoproteins containing apoA-I (HDL) are key mediators in RCT (9–11), whereas non-high-density lipoproteins (non-HDL, lipoproteins containing apoB) are involved in the lipid delivery pathway. There are two types of apoB-containing lipoproteins: apoB-48 and apoB-100 that are produced in the intestine and liver, respectively. With attachment to the proteoglycans in the capillary endothelium, lipoprotein remodeling process begins (12–14). In the capillaries of muscles and adipose tissues, lipoprotein lipase (LPL), activated by apoC-II, converts TG to fatty acids and glycerol. As fatty acids exit the lipoproteins, they become smaller and smaller remnants. In remodeling of apoB-100 lineage, hepatic lipase (HL) transforms remnant low density lipoproteins (LDL) particles to LDL.

LDL may be taken up by peripheral cells for its cholesterol content or may become the target for uptake by the arterial wall macrophages. Excess apoB-containing particles can invade the arterial wall, become oxidized, and be taken up by macrophage scavenger receptors, creating the foam cells that lead to atheroma.

HDL particles
HDL particles are heterogeneous (15–21). They differ in proteins, lipids, size, shape and charge. HDL can vary considerably in its protein content. The major proteins are apoA-I and apoA-II (22). HDL particles contain a hydrophobic core of cholesterol es-
HDL particles have different proportions of different lipids: TG, cholesterol esters (CE), free cholesterol (FC), and phospholipids (PL). Some HDL particles are lipid poor or lipid-free.

They range in size from 7.5 nm to 15 nm; they can range from size of albumin to size of LDL. They differ in shape. Many people have spherical HDL particles and some have discoidal HDL particles.

Density ranges from 1.063 g/mL to 1.25 g/mL. According to density, HDL can be divided in two subfractions: HDL$_3$ (small and dense particles) and HDL$_2$ (large and less-dense particles) (15–21). HDL particles have charge. Most of them have $\alpha$ electrophoretic migration, some of them have pre-$\beta$ migration, and some of them have pre-$\alpha$ migration. Lipid poor apoA-I and discoidal particles have pre-$\beta$ mobility, and spherical particles have $\alpha$ mobility on electrophoresis.

Several plasma proteins, enzymes, and transfer proteins are involved in HDL remodeling and metabolism, which results in HDL heterogeneity (17). Every component of HDL separately enters the plasma, and HDL particle is assembled in the plasma.

**Protective properties of HDL**

- Free apoA-I, pre-$\beta$ HDL, and $\alpha$-HDL can acquire cholesterol from peripheral cells and transfer it to the liver for excretion (23).
- HDL has antioxidant and anti-inflammatory properties: it can inhibit the oxidation of LDL and the expression of endothelial adhesion molecules (24, 25).
- HDL has antithrombotic properties.
- HDL can improve endothelial function, promote the repair of endothelial cells and promote angiogenesis (26).
- HDL can improve glycemic control (27).

**Assembling and remodeling of HDL in plasma**

There are several factors that are involved in the process of HDL remodeling and metabolism:

- Apolipoprotein A-I (apoA-I)
- ATP-binding cassette transporter A1 (ABCA1)
- Lecithin cholesterol acyltransferase (LCAT)
- Cholesteryl ester transfer protein (CETP)
- HL
- Phospholipid transfer protein (PLTP)
- Endothelial lipase (EL)
- Scavenger receptor class B type I (SR-BI)

**Apolipoprotein A-I**

ApoA-I is synthesized in the liver and intestine. After synthesis, it is secreted in the plasma. Intestinal apoA-I is incorporated into the chylomicrons and as soon as the chylomicrons are broken down by LPL, apoA-I falls off from the surface. Free apoA-I has a high affinity for cholesterol and it soon becomes lipidated (28–30).

**ATP-binding membrane cassette transport protein A1**

Lipid poor apoA-1 is released into the plasma from the liver or intestine. It interacts with cell membranes in the plasma throughout the body. ABCA1 translocates both FC and PL from the cell membrane on the lipid-free apoA-1 (31). With a complex rearrangement, it converts apoA-I into a discoidal particle with two apoA-I molecules (32). This discoidal particle contains cholesterol and PL.

**Lecithin cholesterol acyltransferase**

LCT is highly reactive with discoidal HDL particles (33). It plays a role in the process of transesterification. It catalyzes the transfer of fatty acids from lecithin to FC, resulting in the formation of cholesteryl ester and lysophosphatidylcholine. The best substrate for LCAT is HDL, particularly discoidal form of HDL. apoA-I is a potent activator, but it is not the only one. LCAT can also be activated by apoA-IV, apoE, and to some extent by apoC. It cannot be activated by apoA-II. The LCAT reaction is responsible for the majority of CE circulating in the plasma. About two-thirds of the plasma cholesterol (about 70%) is esterified with fatty acids.

Discoidal HDL can accept more cholesterol from peripheral cells (or other lipoproteins), and more cholesterol is further esterified by LCAT. CE are less soluble than TG, and they cannot circulate when exposed to water; hence, they migrate from the surface of the particle to its core. This migration causes transition from discoidal to spherical form of HDL particle (34, 35).

LCAT can increase the size of HDL in two ways. The first way is by combining two HDL particles, each having two apoA-I molecules, resulting in one big fusion product with four apoA-I molecules, which is very unstable. One apoA-I molecule falls off from this fusion product and a particle with three apoA-I proteins is formed.

There is another way of formation of a particle with three apoA-I molecules. A small HDL particle (with two apoA-I molecules) is reactive with LCAT, which esterifies cholesterol. This makes the particle bigger, and as it gets bigger, it needs more protein on the surface. LCAT can mediate incorporation of one apoA-I molecule into spherical HDL particle, resulting in the formation of an enlarged particle containing three apoA-I molecules.

These particles are not stable but are constantly remodeled in the plasma (34, 35).

**Cholesteryl ester transfer protein**

CETP is a lipid transfer protein involved in the exchange and transfer of lipids among different classes of lipoproteins (36, 37).
Cholesteryl esters are transferred from HDL to triacylglycerol-rich lipoproteins (VLDL, IDL, and LDL) in reciprocal exchange for triacylglycerols in other direction. This produces triacylglycerol-enriched HDL and cholesteryl-enriched LDL and VLDL.

The main sources of CETP in humans are the liver and adipose tissue. CETP-mediated transfer of lipids occurs in humans, primates, and rabbits. Species such as rats and mice lack CETP and are very resistant to the development of atherosclerosis.

Since the molecular size of TG is greater than that of CE, the HDL size is increased. TG are the best substrate for enzyme HL. HL breaks down TG and releases FFA that can be taken up by tissues. It makes the particles smaller because they lose TG. CETP plays a major role in the remodeling of HDL, particularly when acting in the presence of HL (36–38).

When HDL particle becomes smaller, it does not need much protein and some apoA-I can dissociate. ApoA-I can be reincorporated in other HDL particles or it can be filtered in the kidneys and excreted through urine.

The role of CETP in the development of atherosclerosis is still controversial. Some studies indicate that CETP is atherogenic, as CETP decreases the level of HDL and transfers cholesterol to other lipoproteins. In contrast, other studies indicate that CETP has no effect on atherosclerosis development as it plays an important role in RCT by promoting the transport of CE to the liver by LDL or VLDL (36, 37).

### Hepatic lipase

HL is a lypolitic enzyme primarily synthesized and secreted by the liver. It can be found in the steroid hormone-producing glands (adrenal glands and ovaries). This enzyme is involved in the lipoprotein metabolism, particularly in HDL remodeling and metabolism. HL has triglyceride and phospholipase activities. It is involved in the production of HDL particles from HDL particles (38).

### Phospholipid transfer protein

PLTP efficiently catalyzes the transfer of different lipids, including phospholipids, diacylglycerol, cerebrosides, and lipopolysaccharides between lipoproteins. PLTP can cause conversion of HDL particles; it promotes the fusion of two HDL particles to form a very large unstable HDL. During HDL remodeling, lipid poor apoA-I is generated and it acts as suitable and efficient cholesterol acceptor. The physiological or pathological role of PLTP is still unknown (39).

### Endothelial lipase

EL primarily hydrolyzes phospholipids in HDL and shows relatively little triglyceride lipase activity (40). Its role in HDL remodeling remains to be determined.

### Scavenger receptor class B type I

SR-BI is a cell membrane protein found in many tissues (liver, steroid hormone-producing tissues, monocytes, and macrophages). It also has an important role in HDL metabolism and remodeling action.

SR-BI promotes the selective uptake of HDL cholesterol and cholesteryl esters in the liver. When HDL binds to SR-BI receptors, it loses CE into the liver, dissociates from the liver, and circulates as a smaller particle (41).

### Reverse cholesterol transport

RCT is the transport of cholesterol from peripheral cells, including macrophages in the arterial wall, back to the liver for further metabolism and excretion.

### RCT comprises several steps:

#### Cholesterol efflux from peripheral tissues to plasma

Lipid-free apoA-I is synthesized in the liver or intestine and secreted in the plasma. In bloodstream, it interacts with PL and forms nascent pre-β-HDL. The ABCA1 transporter operates as a harvester of FC in the cell and delivers it to the cell membrane. The efflux of cellular cholesterol onto lipid poor apoA-I creates discoidal HDL. The ABCA1 transporter transfers cholesterol from the macrophages to HDL.

#### LCAT-mediated esterification of cholesterol and remodeling of HDL particles

LCAT esterifies FC on the surface of HDL particles. The discoidal particle is converted into a spherical particle. This particle is able to acquire more cholesterol via other pathways, such as diffusion, SR-BI, and ABCG1 transporter (which prefers big spherical particles, not discoidal or lipid-free particles). All forms of HDL particles, from lipid-free to large spherical HDL particles, have the ability to take cholesterol out of the cell. HDL participates in the collection of cholesterol from both caveolae and lipid rafts within the cell membrane (42). In these ways, HDL is involved in cholesterol movement (efflux) from the macrophages.

#### Direct pathway of HDL cholesterol delivery to the liver

HDL cholesterol can be delivered directly to the liver via SR-BI for further metabolism, which is called as cholesterol transport in most species.

#### Indirect pathway of HDL cholesterol delivery to the liver via CETP-mediated transfer

Alternatively, in some species, like humans, rabbits, and monkeys, cholesteryl ester present on HDL can be exchanged for triglycerides by CETP and transferred to VLDL and LDL. This CETP-mediated transfer results in an increased cholesterol concentration of apoB-containing lipoproteins and triglyceride accumulation in HDL. HDL triglycerides can be hydrolyzed by HL,
Converting them back to smaller HDL particles (HDL	extsubscript{3}). HDL	extsubscript{2} particles can be delivered back to the liver via SR-BI. Cholesterol-enriched lipoproteins deliver cholesterol to the liver via the LDL receptor for further metabolism and biliary excretion (Fig. 1).

**Experimental and therapeutic strategies for raising HDL cholesterol**

Rats and mice, naturally deficient in CETP, are resistant to the development of atherosclerosis. Expression of CETP gene in transgenic mice induces atherosclerosis (43). Rabbits have high levels of CETP and are naturally susceptible to atherosclerosis. CETP inhibition in rabbits leads to reduction of atherosclerotic lesions (44). In humans, CETP gene polymorphisms associated with lower CETP mass and/or lower activity are associated with increased levels of HDL, decreased levels of LDL, and reduced cardiovascular risk (45). Lifestyle changes (reduction of body weight, increased physical activity, and smoking cessation) are well-known strategies for raising HDL levels. There are several drugs, including fibrates, stains, and niacin, that have been shown to raise HDL.

**Other approaches specifically targeting HDL:**

- Infusion of reconstituted HDL (rHDL)
  rHDL are complexes of PL and apoA-I. Their structure is very similar to the structure of nascent HDL particles secreted by the liver. They can accept cholesterol that is released from cells via ABCA1 (46).
- HDL delipidation
  With the process of selective delipidation, spherical HDL particles can be converted to smaller discoidal particles, which can accept cholesterol from macrophages (47).
- Development of apoA-I mimetic peptides
  They are synthetic peptides that mimic any potentially protective property of HDL particles (48).
- CETP inhibition as therapeutic strategy for treatment of cardiovascular disease (49, 50).

**CETP inhibitors**

Large randomized clinical trials with CETP inhibitors torcetrapib (ILLUMINATE, ClinicalTrials.gov ID NCT00134264), dalcetrapib (dal-OUTCOMES, ClinicalTrials.gov ID NCT00658515), and evacetrapib (ACCELERATE, ClinicalTrials.gov ID NCT01687998) failed to reduce atherosclerotic cardiovascular events. There are several possible explanations for the adverse events in the ILLUMINATE trial: CETP inhibition is proatherogenic, CETP inhibition produces dysfunctional HDL, and serious adverse events are not related to CETP (increased levels of serum sodium, bicarbonates, and aldosterone and decreased serum levels of potassium). The dal-OUTCOMES trial was stopped because there was no clinical efficacy. Possible explanations for the failure of dalcetrapib in reducing cardiovascular events are as follows: better protective properties of HDL were not seen with an increased HDL concentration and in patients who recently had an acute coronary syndrome event, CETP inhibition did not show effectiveness. The ACCELERATE trial was stopped because of insufficient efficacy of evacetrapib in the group of high-risk vascular patients, despite its impacts on cholesterol. The possible explanation why it failed is because the trial was too short to be able to detect benefit.

The REVEAL trial (ClinicalTrials.gov: NCT01252953) is an ongoing randomized trial investigating the effects of adding the CETP inhibitor anacetrapib to an effective LDL-lowering treatment with atorvastatin.

In November 2015 (after approximately 3 years’ median follow-up), the independent Data Monitoring Committee (DMC) considered whether there were reasons to recommend early stopping for efficacy and futility. The DMC recommended to the Steering Committee that the trial should continue without any modification. The estimated study completion date is January 2017.

**Future perspective targets**

**Peroxisome proliferator-activated receptor agonists**

Peroxisome proliferator-activated receptors (PPAR) are nuclear receptor proteins involved in the metabolism of lipids and glucose. These receptors influence the transcription of ABCA1 that facilitates cell-derived cholesterol efflux into nascent HDL. PPAR agonists (α, δ, and γ) are promising agents in future HDL raising strategies. Dual PPAR-α/γ agonists had positive effects on insulin sensitivity and dyslipidemia. But clinical trials were terminated due to increased ASCVD events. The potential of new selective PPAR agonists as therapeutic agents is still under investigation.

**Synthetic farnesoid X receptor agonists**

Farnesoid X receptors (FXRs) are nuclear receptors expressed primarily in the liver and intestines. It is shown that they interact with PPAR. They induce HDL-derived excretion of cholesterol from the liver into feces. Preclinical studies suggested antiatherosclerotic effects of FXR activation.
Gene therapy

Application of microRNA can increase ABCA1/ABCG1 expression which can be a new genetic therapy to raise HDL-C. Over expression of antisense miR-33 is shown to increase hepatic ABCA1, resulting in a 25% increase in HDL-C. Thus, antagonists of miR-33 appear to be a potential therapeutic target for the prevention or treatment of CVD.

Conclusions

HDL particles are assembled and remodeled in the plasma by a variety of enzymes and proteins. Particle heterogeneity is a result of their diversity in size, shape, charge, and composition.

HDL particles have many properties with the potential to protect against atherosclerosis; they play a pivotal role in RCT, a multistep process of cholesterol movement from the peripheral tissues to the hepatocytes.

HDL metabolism is influenced by genetic, metabolic, and environmental factors. Nonpharmacological approach that includes lifestyle changes (diet, reduction of body weight, regular aerobic exercise, smoking cessation, and moderate alcohol consumption) is a well-known strategy for raising HDL levels. Pharmacological therapy includes fibrates, stains, and niacin that have been shown to raise HDL. Future perspective targets include CETP inhibitors, PPAR agonists, synthetic FXR agonists, and gene therapy. Results of clinical trials should be awaited before further clinical management.

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