Although cholesterol is necessary to maintain cellular integrity and is an essential precursor for steroid hormones and bile acids, an elevated concentration of circulating cholesterol is a strong risk factor for cardiovascular diseases (CVDs), which accounts for ≈1 of every 3 deaths in the United States. In recognition of National Cholesterol Month, this article highlights recent studies published in ATVB focused on the regulation of hepatic cholesterol metabolism and reverse cholesterol transport (RCT), the pathophysiological effect of hypercholesterolemia, and therapeutic strategies to counteract hypercholesterolemia and its associated CVD.

**Hepatic Cholesterol Metabolism**

The liver plays a central role in whole-body cholesterol homeostasis, thus perturbations in hepatic cholesterol metabolism can result in hypercholesterolemia. 3-Hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) is the rate-limiting enzyme in cholesterol synthesis and is a target of the low-density lipoprotein (LDL)-lowering drugs statins. However, mice with liver-specific deficiency of HMGR developed hepatic steatosis, despite mildly decreased hepatic cholesterol levels, which was attributable to compensatory increases in triglyceride and fatty acid synthesis. The regulation of hepatic cholesterol synthesis is also regulated by the uptake of different lipoprotein classes. In hamster hepatocytes, cholesterol synthesis was not affected by LDL uptake but was significantly reduced by uptake of other lipoprotein particles.

The secretion of hepatic apolipoprotein B (apoB)–containing lipoproteins and the clearance of circulating apoB-containing lipoproteins through hepatic lipoprotein receptors are essential to maintain hepatic and whole-body cholesterol homeostasis. It was found that a large portion of LDL cholesterol (LDL-C) internalized by hepatocytes was secreted in very-low density lipoprotein (VLDL) or high-density lipoprotein (HDL) without reaching equilibrium with the regulatory pool. In a rat hepatoma cell line, an adaptor protein, kelch-like protein 12, was colocalized with apoB100, facilitating the endoplasmic reticulum exit and secretion of apoB100-VLDL particles. Kelch-like protein 12 knockdown, therefore, reduced secretion of the most lipided apoB100-VLDL species and led to the accumulation of apoB100 in the endoplasmic reticulum. Hepatic lipoprotein receptors are responsible for triglyceride-rich lipoprotein clearance. In mice, heparin sulfate proteoglycan receptors (syndecan-1) and LDL receptor (LDLR) dominated clearance under fasting conditions and postprandial conditions, but LDLR-related protein-1 contributed significantly when LDLR was absent. The expression of Ldlr was regulated by heterogeneous nuclear ribonucleoprotein D, an mRNA binding protein that promoted Ldlr mRNA decay via 3′ untranslated region in mouse primary hepatocytes. Knockdown of heterogeneous nuclear ribonucleoprotein D, therefore, increased Ldlr mRNA expression, but the potential of heterogeneous nuclear ribonucleoprotein as a therapeutic target for LDL-C lowering is an open question.

**Dysregulation of Cholesterol Homeostasis**

Atherosclerosis is driven by cholesterol accumulation within monocytes/macrophages causing increased inflammation and cytotoxicity in the artery wall. The secreted protein profile of acetylated-LDL–loaded murine peritoneal macrophages was linked to 3 functional modules: lipid metabolism, lysosomal biology, and complement activation, implicating that sterol accumulation in macrophages has previously unknown contributions to the regulation of complement activation and proteolysis. In human THP-1 monocytes, unesterified cholesterol induced the production of microvesicles, which serve as a platform for the transport of danger signals. Exposure of mouse aortic explant to the microvesicles provoked the recruitment of leukocytes or monocytes, but the effects were blunted by pretreatment of microvesicles with a physiological concentration of HDL. Unesterified cholesterol-mediated cytotoxicity was further illustrated in mice with global knock-out of acyl-coenzyme A:cholesterol acyltransferase (ACAT) 1. ACAT1 converts cellular cholesterol to cholesteryl esters (CE); however, ACAT1 deficiency caused enlarged lesion size and progression of atherosclerosis, as well as increased hematopoietic progenitor cell proliferation and leukocytosis.

Dysregulation of cholesterol homeostasis affects not only the artery wall but also the other tissue/organ systems. Deficiency in the HDL receptor, scavenger receptor class B type I, caused increased plasma cholesterol and abnormal...
HDL levels, as well as increased susceptibility to deep vein thrombosis, in mice.10 Human maternal supraphysiological hypercholesterolemia was associated with reduced umbilical vein dilation, potentially increasing the risk for an adverse fetal outcome.11 Moreover, mice lacking the sterol transporters ATP-binding cassette (ABC) transporters G5/G8 were more susceptible to hepatic insulin resistance and loss of glycemic control when challenged with a high-fat diet.12

Reverse Cholesterol Transport
RCT is a process whereby cholesterol from peripheral tissues is effluxed to lipoprotein acceptors, such as HDL, trafficked through the circulatory system, generally internalized by the liver, secreted into the bile as the parent molecule or bile acid, and ultimately excreted into the feces.13 Although not much was known about how cholesterol loaded onto HDL leaves atherosclerotic plaques to make its way to the plasma, recent convincing data have pointed out that lymphatic vessels are quantitatively the major route for cholesterol mobilization to plasma after macrophage RCT.14

Macrophage Cholesterol Efflux and HDL Functionality
The first step of RCT is mobilization of cholesterol from foam cells, which requires hydrolysis of CE and efflux of cholesterol by ABCA1 and ABCG1 to HDL.15 In a recent study to identify proteins that regulate cholesterol trafficking through lipid droplets, Goo et al16 revealed that lipid droplet–associated hydrolase, a protein highly expressed in macrophage-rich area in both mouse and human atherosclerotic lesion, is a potential target to stimulate RCT from atherosclerotic lesions. As detailed, lipid droplet–associated hydrolase upregulation increased not only CE hydrolysis but also cholesterol efflux from lipid laden-macrophages.

Recent clinical studies have aimed to reduce atherosclerotic CVD by increasing HDL and consequently stimulating RCT. Unfortunately, the outcomes of these studies suggest that raising HDL per se may not prevent atherosclerosis. Khera et al17 showed that cholesterol efflux capacity of HDL was a better predictor of atherosclerotic burden than HDL concentration. In contrast, Li et al18 showed that higher cholesterol efflux to apoB-depleted serum was paradoxically associated with increased prospective risk for myocardial infarction, stroke, and death. The latter results suggest that cholesterol efflux capacity may not be associated with coronary events resulting from acute plaque rupture but may be instead related with total atherosclerotic plaque burden.19

Consistent with the idea that HDL function is more important than HDL concentration in relationship to RCT, Bi et al20 found that mice with hepatocyte-specific deletion of ABCA1 had a ≈50% reduction in HDL concentration but no change in RCT. These results indicate that in the absence of hepatic production of nascent HDL, the intestine and other ABCA1-expressing tissues synthesize enough HDL to maintain RCT. In addition, Hung et al21 reported that in spite of having >10-fold less HDL-C, Apoa1−/− versus Apo1 transgenic mice only had 2.2-fold less RCT. The majority of macrophage-derived cholesterol in the blood of Apoa1−/− mice was associated with red blood cells that contributed significantly to RCT. In contrast, red blood cells had a lesser role in RCT in wild-type with normal HDL concentration. The results from this study indicate that not only HDL but also red blood cells participate in RCT, and the contribution of red blood cells to RCT may be more pronounced in individuals with low HDL concentration or anemia.

What other factors can modulate cholesterol efflux capacity? CE transfer protein, which transfers CE from HDL to VLDL and LDL in humans, has been recently described as promoting plasma efflux capacity through ABCA1 in women.22 Furthermore, the same group identified a group of single nucleotide polymorphisms that significantly modulate the capacity of plasma to mediate cholesterol efflux from human macrophages.23 The authors conclude that this genetic determination of plasma cholesterol efflux capacity, which is sex specific, is a better predictor of macrophage cholesterol removal when compared with plasma HDL-C levels.

Lecithin-cholesterol acyltransferase converts plasma cholesterol into CE and plays a central role in determining HDL concentration. However, the beneficial effect of lecithin-cholesterol acyltransferase in atheroprotection has been questioned recently.24,25 In a study conducted in a Japanese population, Tanaka et al26 reported that increased lecithin-cholesterol acyltransferase activity measured as serum cholesterol esterification rate is a risk factor for coronary heart disease and sudden death.

Enterohepatic Components of RCT
After efflux to HDL and the conversion of free cholesterol (FC) to CE by lecithin-cholesterol acyltransferase, HDL-CE can be internalized into hepatocytes by scavenger receptor class B type I–mediated selective uptake. Unlike HDL-FC, HDL-CE must be hydrolyzed to FC before it can be secreted into bile or converted into bile acids. Zhao et al27 reported that hepatic overexpression of the CE hydrolase carboxylesterase-3 increased the excretion of macrophage-derived cholesterol as bile acid. The same group then showed that hepatocyte-specific deletion of carboxylesterase-3 increased atherosclerosis development in Ldlr−/− mice and reduced fecal excretion of HDL-CE in the form of FC and bile acid.28 The results indicate that therapies that increase hepatic CE hydrolase expression or function could stimulate RCT and consequently reduce atherosclerosis.

A driving force of RCT is thought to be biliary cholesterol secretion. This idea was supported by the recent work of Xie et al,29 which showed that NPC1L1 liver transgenic mice lacking intestinal Npc1L1 (L1LivOnly) had significant reductions in biliary cholesterol, cholesterol absorption, and RCT. Treatment of the L1LivOnly mice with the NPC1L1 inhibitor ezetimibe resulted in normalization of both biliary cholesterol concentration and RCT. These results indicate that biliary cholesterol secretion is a critical process for RCT. However, it is important to note that other groups have found that reducing biliary cholesterol had no effect on RCT.30,31

Fecal excretion is the primary route by which the body rids itself of excess cholesterol and is the last step in RCT. Although under normal conditions, it plays a lesser role than biliary cholesterol and bile acid secretion, transintestinal cholesterol efflux (TICE) can contribute to cholesterol excretion. TICE is...
a process whereby cholesterol in the blood is transported to the basolateral surface of enterocytes, trafficked across the cell, and effluxed into the lumen of the small intestine. Le May et al. showed that TICE was active in intestinal explants from both mice and humans and that both HDL and LDL could support TICE in this ex vivo system. Conditions that stimulated LDLR expression, such as Peg59 deficiency and statin treatment, increased TICE in mice undergoing intestinal perfusion. Unexpectedly, TICE was also increased in Ldlr<sup>−/−</sup> mice, therefore, indicating that multiple receptor systems can feed LDL-cholesterol into the TICE pathway. Finally, the authors showed that the cholesterol floppase Abcb1 participates in TICE by presumably facilitating the efflux of cholesterol from the apical surface of enterocytes into the intestinal lumen. Although this study elucidated some of the molecular players involved in the process, there is still much to learn about TICE in particular its relative contribution to cholesterol excretion in humans.

Therapeutic Interventions: Ongoing Studies

In light of recent findings on the mechanisms and the pathophysiological effect of cholesterol dysregulation, there are tremendous efforts to treat dyslipidemia and its associated CVD by exploiting novel therapeutic targets.

Ditichchenko et al. characterized a new formulation of recombinant HDL (CSL112), a disc-shaped particles bearing 2 molecules of human apoA-I and 110 molecules of phospholipid. With an optimized pharmacokinetic behavior, this new compound has been specifically constructed to enhance cholesterol efflux by ABCA1 in a rabbit model. Saeed et al. reported that LDN 193189, a compound designed to suppress hepatic hepcidin production and increase expression of a free iron exporter, ferroportin within macrophages, efficiently increased macrophage-specific expression of cholesterol efflux transporters, thus reducing atherosclerosis in Apoe<sup>−/−</sup> mice. Briand et al. reported that torcetrapib treatment did not stimulate RCT in hamsters with high-fat and high-fructose diet–induced dyslipidemia. However, coadministration of torcetrapib with berberine, a drug that increases LDLR expression, significantly enhanced RCT. The results, therefore, suggest that combined treatment of CE transfer protein inhibitors and a LDLR-elevating therapy, such as statins or PCSK9 antibodies, may be considered in patients with dyslipidemia to stimulate RCT.

Rong et al. recently assessed the effect of an ACAT inhibitor on pre-established, advanced lesions in Apoe<sup>−/−</sup> mice. Being aware of previous publications reporting the proatherogenic effect of ACAT inhibition, they showed that the cytotoxicity from FC accumulation in cells and tissues can be eliminated if the right dose is used because too much inhibition is likely to be undesirable. However, they stated that too little inhibition is likely to result in a lack of efficacy. Thus, in this model that reflects a more clinically relevant scenario of patients with dyslipidemia to stimulate RCT.

Summary

Great strides have been made toward understanding the mechanisms that maintain and disrupt cholesterol homeostasis. Over a century’s worth of research has led to the development of amazing drugs, such as statins, that potently reduce hypercholesterolemia and consequently decrease atherosclerosis development and CVD risk. In spite of the significant advances, it is clear from the research highlighted in this article that there is still much to learn about the effect of cholesterol on CVD. We think that current and future research on cholesterol homeostasis will steer us toward new targets and treatments that will further diminish CVD morbidity and mortality in the United States and around the world.

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References


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