Current Concept of Reverse cholesterol transport and novel strategy for atheroprotection

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Abstract

Elevated levels of low-density lipoprotein cholesterol (LDL-C) increase the risk of coronary heart diseases (CHD), but pharmacologic therapy to decrease LDL-C significantly reduces the risk of cardiovascular events. Despite the effectiveness of statin therapy, there still remains significant residual cardiovascular risk. A low plasma concentration of high-density lipoprotein cholesterol (HDL-C) is associated with increased risk of CHD. Moreover, recent studies have shown that HDL-C levels during statin treatment remain an independent predictor of the risk of cardiovascular events. However, recent clinical trials aimed at reducing CHD risk by raising HDL-C levels have not shown a satisfactory results and it is becoming evident that a functional HDL-C is a more desirable target than simply elevating its level. This review summarizes the function of HDL-C in reverse cholesterol transfer from peripheral tissue and discusses the latest HDL-targeting therapeutic strategies including the manipulation of microRNAs.

ABBREVIATIONS

low-density lipoprotein cholesterol (LDL-C)

coronary heart disease (CHD)

high-density lipoprotein cholesterol (HDL-C)

reverse cholesterol transfer (RCT)

cholesteryl ester transfer protein (CETP)

cholesteryl ester (CE)

apolipoprotein A-I (apoA-I)

scavenger receptor class B type I (SR-BI)

ATP-binding cassette transporter (ABC)

triglyceride (TG)

TG-rich lipoproteins (TRL)

very low-density lipoprotein (VLDL)

lipoprotein lipase (LPL)

lecitin-cholesterol acyltransferase (LCAT)

hepatic lipase (HL)

endothelial lipase (EL)

liver X-receptor (LXR)

peroxisome proliferator-activated receptors (PPARs)

farnesoid X receptor (FXR)

microRNAs (miRs)

sterol regulatory element binding protein 1 (SREBP1)

I. INTRODUCTION

Although the lowering of low-density lipoprotein cholesterol (LDL-C) with the use of statins has revolutionized the treatment of atherosclerotic cardiovascular disease, statins can only reduce the risk of cardiovascular events by up to 30-40% depending on the disease status and the amount of statin used, which still leaves a large burden of residual disease risk [1-3]. Recent studies have shown that high-density lipoprotein cholesterol (HDL-C) levels during statin treatment remain an independent predictor of the risk of cardiovascular events in patients who have been treated with statins to lower LDL-C levels [4]. Clinical and epidemiologic studies have consistently shown that there is an inverse relationship between the HDL-C concentration and cardiovascular risk. In fact, a 1 mg/dl (0.026 mM) increment in HDL-C levels was associated with a significant decrease in the risk of coronary heart disease (CHD) of 2% in men and 3% in women [5].

These data led to the conclusion that current treatment strategies for reducing CHD risk are insufficient to prevent most CHD events, and the development of therapies that raise HDL-C is necessary to reduce cardiovascular diseases in the midst of the "statin era" in the management of atherosclerosis. However, convincing epidemiologic data linking HDL-C and CHD risk has not yet translated into solid evidence. There are no clinical trials that support linearity between HDL-C increases and CHD risk reduction. The recent negative results obtained by the ILLUMINATE [6] and AIM-HIGH trials [7] suggest that HDL-C increases by the strategies tested may not always be beneficial for the reduction of CHD risk. The metabolic heterogeneity of HDL particles may underlie

the inconsistency between epidemiologic studies and experimental approaches in clinical trials of lipid treatments. HDL comprises many kinds of lipoproteins, lipids, proteins and nucleotides that influence the functions and metabolism of the particles, and there are substantial differences in their size and density [8]. Thus, several reports already suggested that subpopulations of HDL have more or less anti-atherogenic potential [9]. All of these results have led to a quest to find new strategies to reduce CHD events by improving the quality and quantity of HDL-C. This review summarizes the function of HDL-C in reverse cholesterol transfer (RCT) from peripheral tissue (Fig.1) and discusses recent discovery of the function of microRNAs (miRs) in HDL metabolism.

II. CURRENT LIPID MANAGEMENT THERAPY AND HDL-C

Management of dyslipidemia in patients with cardiovascular disease should be started with statin therapy. In patients with low HDL-C, statins are very effective in reducing the absolute cardiovascular event rate. However, it has been pointed that the residual risk of CHD events is still high. It is known that statins can increase HDL-C levels by 5-10%. Although the mechanisms of increasing HDL-C are still unclear, it is suggested that statins can stimulate ABCA1 expression in the liver through a liver-specific promoter element [10]. Statins also appear to reduce the rates of endogenous cholesteryl ester transfer protein (CETP)-mediated cholesteryl ester (CE) transfer from HDL by decreasing the quantity of apo B lipoproteins available to accept CE from HDL. Moreover, some statins are known to enhance hepatic apolipoprotein A-I (apoA-I) production, which increases the production of nascent HDL-C [11].

Other pharmacologic reagents for the treatment of dyslipidemia including niacin, fibrates, and PPAR agonists have the potential to raise HDL-C levels. Among them, niacin, or nicotinic acid, which is a soluble B vitamin, is known as the most effective drug approved for raising HDL-C levels. In clinical trials, it has been shown to lower LDL-C levels by 10-20%, triglyceride by 20- 40%, and Lp(a) by 10- 30%, as well as raise HDL-C levels by 15- 30% [12]. Niacin appears to increase HDL-C levels by decreasing the hepatic uptake of apo AI, thereby delaying catabolism [13]. Although niacin is widely used in the management of dyslipidemia, side-effects can affect compliance to therapy. Its potential side-effects are flushing, hepatotoxicity, hyperuricemia, uppergastrointestinal distress, and hyperglycemia [14]. In comparison with immediate-release niacin, extended-release formulations result in reduced flushing

symptoms. Thus, extended-release niacin has been used in recent clinical trials. The AIM-HIGH trial was designed to examine whether extended-release niacin added to simvastatin to raise low levels of HDL-C is superior to simvastatin alone in reducing residual CHD risk. However, among patients with atherosclerotic cardiovascular disease and LDL cholesterol levels of less than 70 mg per deciliter (1.81 mmol per liter), there was no incremental clinical benefit from the addition of niacin to statin therapy during a 36-month follow-up period, despite significant improvements in HDL-C and triglyceride levels [7]. These results suggest that with the well-controlled statin therapy, there seem to be no beneficial effects of additional treatments with niacin. However, benefits by niacin might be observed in higher-risk cardiac patients or in those whose LDL cholesterol levels are not intensively controlled by statins, and a further prospective study will be required.

III. EVOLUTION OF THE RCT CONCEPT

HDL-C has been proposed to have several anti-atherosclerotic properties, including the ability to mediate macrophage cholesterol efflux, antioxidant capacity, anti-inflammatory properties, nitric oxide-promoting activity, and an ability to transport proteins with their own intrinsic biological activities. HDL particles are critical acceptors of cholesterol from lipid-laden macrophages and thereby play an important role in the maintenance of net cholesterol balance in the arterial wall and in the reduction of proinflammatory responses by lipid rich macrophages. The idea of RCT, which refers to the overall flux of cholesterol from the periphery to the liver and its fecal excretion, was first proposed nearly 50 years ago [15, 16]. From the earliest

proposed concept for HDL-mediated cholesterol efflux, the concentration of the cholesterol content in HDL particles has been considered as key to the efficiency of the 'RCT' process. However, the use of static measurements of HDL-C is an inadequate approach for the characterization of RCT, because RCT involves active flux and passive transport mechanisms from macrophage foam cells, HDL-modifying enzymes and transporters, and hepatic cholesterol receptors.

The RCT concept is also sometimes used to refer to specific steps or specific cell types in this process, thus it causes confusion sometimes. Macrophage-derived cholesterol represents one of the components of cholesterol transport by HDL particles. Therefore, cholesterol efflux from macrophage foam cells is referred to as macrophage-specific RCT. Even though cholesterol efflux from lipid-laden macrophages contributes a relatively minor amount to the cholesterol content of HDL particles, macrophage-specific RCT is thought to be the major function of lipid-modifying therapies.

IV. MECHANISM AND REGULATION OF RCT

The first important step in the RCT pathway is the removal of excess cholesterol from macrophage-derived foam cells present in the atherosclerotic plaque. Cholesterol can be effluxed from the macrophage only in the unesterified or free form, but not as CE. Free cholesterol can leave the macrophage by different pathways, which either might be transporter-independent or dependent on cholesterol transporters such as (scavenger receptor class B type I (SR-BI), ATP-binding cassette transporter A1 (ABCA1), and ABCG1. Ablation of ABCA1 in macrophages decreased the flux of

cholesterol from foam cells into the serum as well as feces [17, 18]. The critical role that ABCA1 plays in determining plasma HDL-C levels is well established. Patients with Tangier disease, who are homozygous for mutations of ABCA1, have undetectable level of HDL-C and apoA-I, because of impaired lipidation and subsequent catabolism of lipid-poor apoA-I. In addition to ABCA1- and ABCG1-mediated efflux, cholesterol can be effluxed from machrophages to HDL in an SR-BI-dependent pathway. Another important player in cholesterol efflux and machophage-specific RCT is lipid-poor apoE produced by macrophages. A recent study revealed that in vivo macrophages-to-feces RCT is diminished in wild-type mice receiving macrophages that are deficient in apoE [19]. At normal cholesterol levels, aqueous diffusion is predominant, whereas in cholesterol-loaded macrophages, ABC transporters predominate for cholesterol removal. In human cholesterol-loaded THP-1 macrophages, the ABCA1 and SR-BI pathways are predominant, whereas ABCG1 does not contribute to efflux [20].

A second essential determinant of efficient cholesterol removal from macrophage foam cells is the amount of acceptors such as apoA-I and HDL. ApoA-I constitutes about 70% of the apolipoprotein content of HDL particles; as a result, plasma apoA-I concentrations correlate closely with plasma HDL-C. ApoA-I is secreted predominantly by the liver and intestine as lipid-poor apoA-I and nascent cholesterol-poor HDL particles. Nascent apoA-I acquires phospholipids and cholesterol via cellular efflux as well as by transfer of surface components of triglyceride (TG)-rich lipoproteins (TRL; chylomicrons and very low-density lipoprotein (VLDL)) during lipoprotein lipase (LPL)-mediated intravascular lipolysis of TRL. Overexpression of human apoA-I in mice resulted in more cholesterol being removed from macrophages and deposited in the feces via the RCT pathway leading strong support to the concept that raising HDL

levels protects against atherosclerosis through increasing RCT [21]. Dietary fat, alcohol, estrogen, androgens, thyroid hormone, retionids, glucocorticoids, fibrates, niacin and HMG-CoA reductase inhibitors are known to influence transcriptional induction of the apoA-I gene. Promotion of apoA-I induction has been an attractive therapeutic target for drug development and some of such reagents are undergoing clinical trials. It is known that the naturally occurring apoA-I mutant apoA-I Milano was more effective in stimulating macrophage RCT than wild-type apoA-I [22]. Administration of the apoA-I mimetic peptides D-4F, 5A, or ATI-5261 to mice all increased the transfer of macrophage-derived cholesterol to plasma and feces [23-25].

The association between HDL cholesterol levels and macrophage-specific RCT is less straightforward. There are still a substantial number of patients that experience complications of CHD despite exhibiting high HDL-C levels and there are individuals with low plasma HDL-C levels that do not develop clinically significant atherosclerosis. Because regulation and metabolism of HDL-C is complex and there are several subclasses with different functions in HDL-C, plasma HDL levels do not necessarily represent a reliable reflection of macrophage RCT rates. Therefore, HDL-C levels should be used with caution as a surrogate for predicting fluxes through the RCT pathway.

Lecitin-cholesterol acyltransferase (LCAT) plays an important role in the maturation of HDL-C. The activity of LCAT is critical to normal HDL metabolism. In humans, genetic LCAT deficiency syndromes are associated with markedly reduced HDL-C and apoA-I levels and rapid catabolism of apoA-I [26]. However, the importance of LCAT to the process of RCT has not been firmly established. In a recent report, there was no correlation between LCAT cholesterol esterification rates and the amount of

macrophage-derived cholesterol in feces [27].

Hepatic lipase (HL) and endothelial lipase (EL) are both negative regulators of HDL metabolism. HL- and EL- deficient mice as well as HL/EL double knockout mice have higher HDL-C than controls but also decreased uptake of HDL-derived cholesterol into the liver [28]. In these cases, not plasma HDL-C levels but the uptake of cholesterol into the liver appears to the rate limiting step for the macrophage RCT pathway.

CETP is a hydrophobic glycoprotein made by liver and adipose tissue and is important for human lipoprotein metabolism. It promotes the redistribution and equilibration of hydrophobic lipids packaged within the lipoprotein core between HDL and apo-B- containing lipoproteins. By facilitating the transfer of CEs from HDL to apoB-containing lipoproteins, CETP directs hepatic uptake of cholesterol to the LDL receptor, which is also an important route in the RCT pathway. In humans, radiolabeled HDL CE excreted into bile was transported to the liver almost entirely after transfer to apoB-containing lipoproteins. Moreover, there is increasing data that the functionality of HDL-Cs may be an important determinant of their anti-atherogenic effects. It is not yet clarified whether the large CE-rich HDL particles that accumulate as a result of CETP inhibition may serve as inhibitors for atherogenesis.

Macrophage RCT may also be influenced by specific apolipoproteins carried in the HDL particle. HDL-C of CAD patients carries distinct apolipoproteins and protein oxidation is thought to generate dysfunctional HDL-C. Increases in the levels of HDL-C that contains apolipoprotein C-III is reported to predict the increase in CAD risk.

After transport to the plasma compartment, the next step of RCT is the delivery of cholesterol from macrophages to the liver. SR-BI, a member of the scavenger receptor superfamily of proteins, is the key receptor responsible for the selective uptake of CEs

from HDL into the liver. Catabolism of all major HDL-C can occur by way of SR-BI, with the highest uptake constants for CE and free cholesterol. The importance of SR-BI, which is highly expressed in the liver, adrenal glands, and ovaries, for HDL metabolism has been readily demonstrated in genetically altered animals. Mice deficient in SR-BI have elevated levels of HDL-C but not of plasma apoA-I, consistent with a defect in selective uptake of HDL-C. Overexpression of SR-BI, on the other hand, results in reduced plasma concentrations of HDL-C and apoA-I, because of accelerated renal and hepatic clearance of cholesterol-depleted HDL-particles after interaction with SR-BI [29-31]. Thus, SR-BI has been recognized as a positive regulator of RCT [32]. The LDL receptor is also important in this step because it takes up CETP-mediated CEs from HDL in apoB-containing lipoproteins. An alternative mechanism by which HDL-C can be taken up into the liver is by holoparticle endocytosis [33].

Biliary secretion is the last step of the major route for RCT to eliminate cholesterol from the body. CEs are hydrolyzed by neutral cholesteryl ester hydrolase to generate free cholesterol and excreted into the bile. Bile acids are secreted by the bile salt export pump (or ABCB11). ABCG5 and ABCG8 are obligate heterodimers that mediate secretion of cholesterol and plant sterols into bile together with the cholesterol-binding protein Niemann-Pick C2 [34]. Of note, cholesterol is reabsorbed by the transport protein Niemann-Pick C1-like 1 in the intestine. It has been identified as the molecular target of the cholesterol absorption inhibitor ezetimibe [35].

V. WHAT ARE THE FACTORS INFLUENCING STEPS IN THE RCT PATHWAY?

A major recognized functional property of HDL-C is to elicit cholesterol efflux and mediate RCT. Therefore, therapeutic strategies to specifically enhance RCT would be more beneficial than those that simply increase HDL-C levels. There are several factors and compounds that influence steps in the macrophage-specific RCT pathway.

Because synthetic liver X-receptor (LXR) agonists are known to induce the transcription of ABCA1 as well as other molecules involved in the RCT pathway, including apolipoprotein E [36], these compounds have been thought to raise HDL-C levels and reduce atherosclerosis. However, because of the fact that they induce fatty acid synthesis, these compounds have also been shown to cause liver steatosis, particularly in a mouse model of obesity [37-39]. Recently, partial LXR agonists that induce ABCA1 and ABCG1 but not fatty acid synthesis have been reported [40-42]. However, one of these compounds caused an increase in adverse neurologic events in individuals who received higher doses [40].

Peroxisome proliferator-activated receptors (PPARs) are transcription factors that, like LXRs, belong to the nuclear receptor family. Recent studies revealed that the potent PPARα agonist GW7647 increased macrophage RCT [43]. PPAR ligands have attracted interests in view of their potential use for the treatment of cardiovascular diseases.

Farnesoid X receptor (FXR) has been also implicated in the control of cholesterol metabolism. Activation of FXR, by treatment with the specific agonist GW4064 enhanced the transport of cholesterol from macrophages to feces [44]. However, additional experiments to prove their efficiency and safety are required for therapeutic use.

miRs are small, non-protein-coding RNAs that bind to specific mRNAs and inhibit translation or promote mRNA degradation. Recent reports, including ours, have

indicated that miR-33 controls cholesterol homeostasis [45-48]. miR-33 deficient mice were generated and the critical role for miR-33 in the regulation of ABCA1 expression and HDL biosynthesis was confirmed in vivo [48]. It has already reported that silencing miR-33a resulted in a reduction of atherosclerosis in mice [49]. We also have confirmed that HDL in miR-33 deficient mice has enhanced cholesterol efflux capacity.

In humans, not in rodents, sterol regulatory element binding protein 1 (SREBP1) and SREBP2 encode miR-33b and miR-33a, respectively [46]. It is well known that hypertriglycemia in metabolic syndrome is caused by the insulin-induced increase in SREBP1c mRNA and protein levels [50, 51]. Low HDL often accompanies with metabolic syndrome and it is possible that the reduction in HDL is caused by a decrease in ABCA1 through the increased production of miR-33b from the insulin-induced induction of SREBP1c. Although it is impossible to prove this hypothesis in mice models that lack miR-33b, antagonizing miR-33 could be a promising way to raise HDL levels when the transcription of both SREBPs is upregulated. Recently, it was shown that inhibition of miR-33a and -33b resulted in an increase in plasma HDL and reduction of VLDL triglycerides in non-human primates [52]. Thus, a combination of silencing of endogenous miR-33 and statins may be a useful therapeutic strategy for raising HDL and lowering LDL levels especially for metabolic syndrome patients.

VI. CONCLUSION AND FUTURE DIRECTIONS

In summary, the metabolism of HDL-C involves a complex relation of factors regulating the synthesis, intravascular remodeling, and catabolism of HDL-C. Recent investigations indicate that it is not enough to simply increase HDL-C level but to

increase its function and enhance RCT for the prevention of atherosclerosis and subsequent CHD. Recent findings including the function of miRNAs would clarify yet unknown physiological mechanisms in macrophage-specific RCT and provide new strategies to prevent atherosclerosis and CHD.

ACKNOWLEDGEMENTS

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Figure 1.

Schematic diagram of RCT.

LDL: low-density lipoprotein cholesterol, HDL: high-density lipoprotein cholesterol,

RCT: reverse cholesterol transfer, CETP: cholesteryl ester transfer protein (CETP), CE:

cholesteryl ester, ABCA1: ATP-binding cassette transporter A1, LCAT:

lecitin-cholesterol acyltransferase, LDLR: LDL-receptor, SR-BI: scavenger receptor

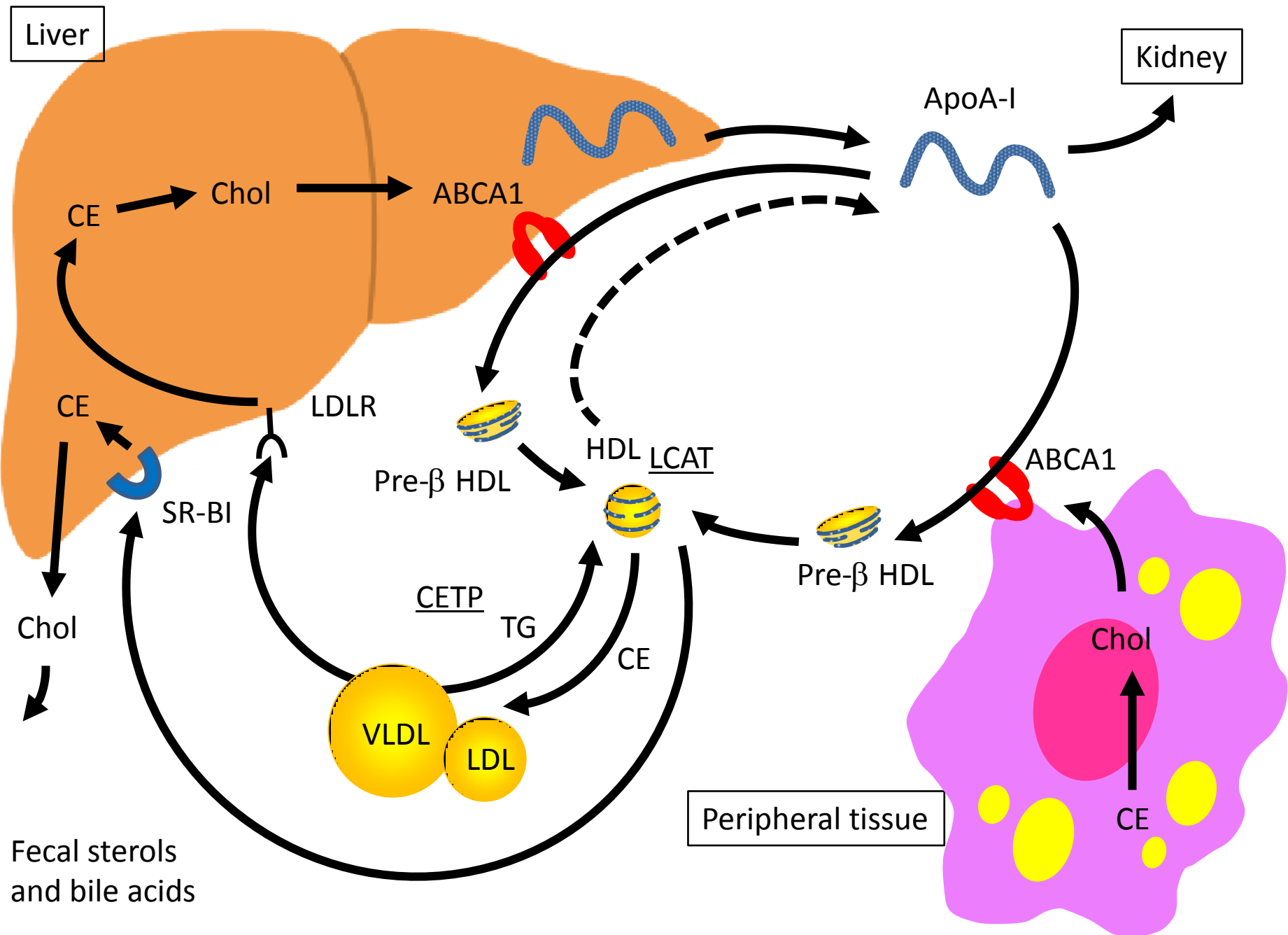class B type I, Apo A-I: apolipoprotein A-I

Figure 1