A fine-tuned balance between cholesterol uptake and excretion by the body is pivotal to maintain health and to remain free from the deleterious consequences of cholesterol accumulation such as cardiovascular disease. The pathways involved in intracellular and extracellular cholesterol transport are a subject of intense investigation and are being unraveled in increasing detail. In addition, insight into the complex interactions between cholesterol and bile acid metabolism has increased considerably in the last couple of years. This review provides an overview of the mechanisms involved in cholesterol uptake and excretion, with a particular emphasis on the most recent progress in this field. Special attention is given to the transintestinal cholesterol excretion (TICE) pathway, which was recently demonstrated to have a remarkably high transport capacity and to be sensitive to pharmacological modulation.

Introduction
Although organisms like plants, bacteria, and fungi are able to live and reproduce without cholesterol, this hydrophobic molecule is indispensable for life in higher organisms because of its function as a vital structural component of cell membranes. Receptor-mediated signaling often depends on the presence of rigid membrane domains called lipid rafts that have a relatively high cholesterol density [1]. Furthermore, cholesterol is the precursor of several biologically important molecules, such as steroid hormones and bile acids (see Glossary and Box 1). The importance of cholesterol for human life and development is underscored by the severe consequences observed in children with inborn errors of cholesterol synthesis [2,3]. Multiple inborn errors in the cholesterol synthesis pathway have been described [4]. The best known example is Smith–Lemli–Opitz syndrome, resulting from a deficiency in 7-dehydrocholesterol reductase [5]. The enzymes and pathways involved in cholesterol synthesis, along with their regulation, exceed the scope of this review and are meticulously described elsewhere [6,7].

High plasma cholesterol concentrations affect about 40% of the global population [8]. Elevated circulating low-density lipoprotein cholesterol (LDL-c) represents the major independent risk factor for atherosclerotic cardiovascular disease (CVD) [9]. Over a lifetime, each single percent increase in circulating LDL-c raises CVD risk by 3% [10]. It is therefore of the utmost importance to keep plasma LDL-c levels within acceptable limits. Plasma cholesterol levels are the net result of processes controlling cellular cholesterol uptake and excretion. In this review, we will focus on the latest insights in the pathways engaged in cholesterol transport and also discuss a newly emerged role of the intestine in the control of whole-body cholesterol homeostasis, that is, the process called transintestinal cholesterol excretion (TICE) [11,12].

Highlights
Membrane fluidity appears important for the uptake of lipids, including cholesterol, in the intestine. Enzymes such as LPCAT3 may therefore represent therapeutic targets.

Emerging information on the mechanism of LDL internalization as well as intracellular cholesterol transport will lead to identification of novel genes involved in familial hyperlipidemia.

TICE is active in humans. Recent data indicate that this process can be induced in humans, which opens opportunities for therapeutic stimulation of this pathway to lower plasma cholesterol levels.

Bile acid synthesis not only represents an important catalytic pathway for cholesterol removal, but also the composition of the bile acid pool impacts cholesterol uptake and active excretion by the intestine.

The farnesoid X receptor (FXR) mediates cholesterol metabolism, not only by regulating bile acid synthesis but also by impacting intestinal cholesterol secretion.
Cholesterol Absorption

Cholesterol intake from dietary sources in Western societies is about 0.3–0.4 g/day, but highly depends on dietary habits. From an average Western diet, about 55% of consumed cholesterol is derived from meat and fish, whereas egg consumption (25%) and dairy products (20%) also considerably contribute [13]. It has been estimated that improving dietary routine can reduce LDL-c up to 25–30% [14]. Considering the 3% increase in CVD risk for every percent elevation of LDL-c levels, the impact of dietary habits on the risk cardiovascular events is therefore considerable.

On average, about 50% of cholesterol that is consumed enters the body, although fractional cholesterol absorption varies substantially between individuals (20–80%) [15,16]. Of note, about 75% of cholesterol that is absorbed from the intestine is derived from endogenous production and has been secreted into the intestine with the bile. Daily biliary cholesterol secretion in humans is about 1 g/day [17,18], which is about threefold more than the amount of cholesterol entering the intestine directly from the diet. This means that cholesterol absorption inhibitors can, potentially, reduce the total amount of cholesterol that is absorbed from the intestine to a greater extent than can be achieved by limiting dietary cholesterol intake.

The first step in cholesterol absorption is its solubilization in the proximal intestinal lumen. Because of its hydrophobic nature, cholesterol is virtually insoluble in water. To allow absorption, cholesterol and other hydrophobic nutrients first need to be solubilized. This is mediated by the bile that is produced by the liver. Upon ingestion of a meal, bile that is stored in the gallbladder is expelled into the common bile duct, and flows into the intestine where it enters through the sphincter of Oddi. Bile contains millimolar concentrations of bile acids, phospholipids, and cholesterol in the form of mixed micelles, which act as an emulsifier and solubilize cholesterol as well as other lipids. Bile formation and secretion of its constituents is discussed in more detail later. In the absence of bile acids, as is the case in

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**Box 1. Bile Acids – More Than Intestinal Soaps**

The immediate products of the bile acid (BA) synthetic pathways in humans, that is, primary BAs, are cholic (3α,7α,12α-trihydroxy-5β-cholanic acid) and chenodeoxycholic acids (3α,7α-dihydroxy-5β-cholanic acid). The steps leading to formation of primary BAs include the hydroxylation of cholesterol at the C7 position of the steroid ring structure or at the C24, C25, or C26 positions at the side chain, subsequent further modification of the steroid ring structure, followed by side-chain shortening. In particular, the hydroxylation step catalyzed by the microsomal enzyme cholesterol 7α-hydroxylase (CYP7A1), catalyzing the first step of the so-called “classical pathway” of BA biosynthesis that yields 7α-hydroxycholesterol, is considered to be of great regulatory importance and the activity of this enzyme is subject to complex modes of control, amongst others, by the BA-activated nuclear receptor FXR. Side-chain hydroxylated cholesterol molecules like 24-hydroxycholesterol, 25-hydroxycholesterol, and 27-hydroxycholesterol also can serve as substrates for BA synthesis. For further conversion into BAs, these oxysterols undergo 7α-hydroxylation by the microsomal cytochrome P450 enzyme CYP39A1 or CYP7B1. The 7α-hydroxy intermediates that are thus formed can be converted into 3-oxo,Δ5 intermediates by microsomal 3β-hydroxy-Δ5-Δ7-steroid oxidoreductase (HSD3B7). The product of HSD3B7 activity, in turn, can take two routes: if it interacts with microsomal sterol 12α-hydroxylase (CYP8B1), the end product will be cholic acid. Chenodeoxycholic acid will be formed when 12α-hydroxylation does not occur. The activity of CYP8B1 thus determines the ratio in which the primary BAs are formed and, thereby, the physicochemical and biological properties of the BA pool. In rodents, alternative hydroxylation reactions give rise to differently structured chenodeoxycholic acid-derived primary BAs, particularly the very hydrophilic α- and β-muricholic acids (3α,6β,7α-trihydroxy-5β-cholanic acid and 3α,6β,7β-trihydroxy-5β-cholanic acid, respectively). Recently, it was demonstrated that Cyp2c70 is likely responsible for this rodent-specific pathway. In humans as well as rodents, the diversity of the BA pool is increased by the actions of intestinal bacteria, giving rise to so-called secondary BA species such as deoxycholic (3α,12α-dihydroxy-5β-cholanic acid) and lithocholic (3α-hydroxy-5β-cholanic acid) acids. This has major physiological consequences because the different BA species show great variability in their ability to activate the BA-activated receptors FXR and TGR5 that both modulate various aspects of whole-body metabolism. As a rule of thumb – the greater the hydrophilicity, the weaker the activating capacity. The muricholates, for instance, act as FXR antagonists rather than as agonists like the more hydrophobic species.

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severely cholestatic patients and in circumstances in which complete bile diversion has been performed, cholesterol absorption is essentially absent. The absorption of other lipids (e.g., unsaturated fatty acids) remains, however, preserved to a certain extent [19]. When bile enters the proximal small intestine, it is diluted by pancreatic and gastric juices causing rearrangement of the micelles and an improved capacity to take up dietary lipids [20]. The mixed micelles then carry the cholesterol through the unstirred water layer [21] to the brush border membrane of the intestinal epithelium. The physicochemical properties of the mixed micelles impact cholesterol absorption. Micelles containing more hydrophobic bile acid species can solubilize cholesterol more efficiently and thereby promote cholesterol absorption. However, the efficiency of delivery of cholesterol to the enterocytic membrane is not directly related to the amount of cholesterol solubilized in the micelles [22]. Phytosterols resemble cholesterol molecules and can inhibit cholesterol absorption not only by competing for receptor-mediated uptake (see below), but also by replacing cholesterol from the mixed micelles and thereby reducing its solubilization [23].

Once solubilized and transported through the unstirred water layer, uptake by enterocytes lining the intestine represents the second step of cholesterol absorption. Niemann–Pick type C1-like 1 (NPC1L1) is responsible for uptake of cholesterol from the intestinal lumen into the enterocytes. NPC1L1 is expressed in the small intestine and liver in humans, but in mice and rats its expression is essentially restricted to the small intestine [24]. This sterol transporter has 13 transmembrane domains [25], and is the target of the widely used cholesterol absorption inhibitor ezetimibe [26]. Inactivating mutations in the NPC1L1 gene are associated with reduced cholesterol absorption. Subjects carrying a heterozygous null allele show a modest decrease in LDL-c, on average only 0.31 mmol/l, but a decrease in CVD risk by 54% [27], which delineates the steep relation between plasma LDL-c levels and risk for CVD. These observations are thus in line with the dogma that a subtle, lifelong reduction in plasma cholesterol levels markedly lowers cardiovascular risk. The possibility that an increase of turnover of the body’s cholesterol pool, as will be induced by inactivation NPC1L1, also contributes to the cardioprotective effect is intriguing and awaits further experimental evidence. In line with the observations made in the carriers of inactivating mutations of NPC1L1, ezetimibe was shown to induce additional LDL-c lowering and to have a beneficial effect on cardiovascular outcomes when added to statin therapy in the IMPROVE-IT trial [28]. The mechanism by which NPC1L1 mediates cholesterol absorption is still not fully understood. Recently, it was reported that the clathrin adaptor Numb interacts with the cytoplasmic C terminus of NPC1L1 to facilitate internalization of the transporter along with the cholesterol bound to it [29]. Disruption of the NPC1L1–Numb interaction reduced cholesterol absorption. However, the concept that internalization of NPC1L1 is a prerequisite for cholesterol absorption to occur has recently been challenged. Ezetimibe was shown to have no effect on NPC1L1 internalization, whereas small-molecule inhibitors of endocytosis failed to inhibit cholesterol uptake from taurocholate micelles in cultured rat hepatoma cells stably transfected with GFP-tagged NPC1L1 [30]. However, the GFP-tag fused to NPC1L1 as well as the cell type used may have impacted the results. Therefore, more research will be required to elucidate the exact mechanism of action of NPC1L1-mediated cholesterol absorption. Recently, it has become evident that the fluidity of the brush border membrane is an important determinant of fat as well as cholesterol absorption. In mice, intestine-specific deletion of the gene encoding the enzyme lysophosphatidylcholine acyltransferase 3 (Lpcat3), which produces sn-2 polyunsaturated phosphatidylcholines and thereby alters membrane characteristics, resulted in decreased cholesterol absorption [31]. Decreased expression of Npc1l1, however, was also reported in mice lacking Lpcat3 expression in the intestine [32], which likely contributed to the observed phenotype.

Glossary

**Bile acids:** amphaphilic molecules that are synthesized from cholesterol by the liver and aid the absorption of lipid-soluble nutrients in the intestine but also serve as important signaling molecules.

**Biliary cholesterol secretion:** cholesterol that is secreted by the liver into the bile and transported to the intestine where it is either reabsorbed or leaves the body in the feces.

**Cholesterol turnover:** the rate at which cholesterol present in a certain compartment or in the whole body is replaced by cholesterol from other sources.

**Farnesoid X receptor (FXR):** a nuclear receptor that is activated by bile acids and plays important roles in the regulation of bile acid synthesis, but also has impacts on cholesterol, lipid, and glucose metabolism.

**Mixed micelles:** aggregates composed of bile acids, phospholipids, and cholesterol in aqueous solution, which solubilize hydrophobic molecules in bile and in the intestinal lumen to facilitate their absorption.

**Neutral sterol excretion:** excretion of cholesterol and its bacterial derivatives into the feces.

**Niemann-Pick C1-Like 1 (NPC1L1):** the transporter that is active at the luminal side of enterocytes and mediates the uptake of cholesterol by the intestine.

**Transintestinal cholesterol excretion (TICE):** a cholesterol excretion pathway mediating transport of cholesterol from the blood across the intestinal wall into the lumen, resulting in removal of cholesterol from the body.

**Unstirred water layer:** the layer that does not mix with the bulk fluid phase in the small intestine, but separates it from the luminal membrane of the enterocytes.
After uptake into enterocytes, cholesterol can either be secreted back into the lumen by the ATP-binding cassette subfamily G member 5 (ABCG5)/G8 transporter or can leave the cell at the basolateral side associated with chylomicrons or high-density lipoprotein (HDL) particles. The majority of cholesterol secreted with chylomicrons is first esterified by acetyl-CoA acetyltransferase 2 (ACAT2) in the enterocytic endoplasmic reticulum (ER) [33,34]. Chylomycin assembly and secretion require apolipoprotein B (APOB) and the microsomal triglyceride transfer protein (MTTP) to form the structural basis and to mediate lipidation of the particle being assembled, respectively [35]. Chylomicrons are assembled in a two-step process [36,37]. In the first step, dense APOB48 phospholipid-rich particles are produced. In the ER lumen, these particles rapidly acquire triglycerides from lipid droplets to form prechylomicron transport vesicles [38]. These vesicles then acquire vesicular transport proteins, including Coat protein complex II (COP II) proteins, bud from the ER, and fuse with the membranes of the Golgi apparatus [38]. In the Golgi, the prechylomicrons are subjected to further maturation and are eventually secreted into the lymphatic fenestrae. Given the key function that MTTP fulfills in the lipidation of dense APOB48 particles, it is not surprising that intestine-specific deletion of Mttp was shown to reduce cholesterol absorption in mice [39]. Carboxylesterase 1 (CES1) is an enzyme involved in triglyceride as well as cholesteryl ester hydrolysis [40]. Ablation of Ces1g (encoding CES1 in mice) resulted in increased chylomycin production, indicating a role for CES1 in this process. Transmembrane 6 superfamily member 2 (TM6SF2) has been implicated in very-low-density lipoprotein (VLDL) production by the liver [41], and a loss-of-function variant was associated with hepatic steatosis [42]. Interestingly, highest expression of TM6SF2 is found in the small intestine [43,44]. Carriers of a loss-of-function mutation in TM6SF2, identified in the Amish Complex Disease Research Program, exhibited lower postprandial serum triglycerides following an oral high-fat challenge [43]. In addition, appearance of orally administered radiolabeled oleic acid and cholesterol in the serum was delayed in Tm6sf2-deficient mice [44]. Finally, knockdown of TM6SF2 in Caco-2 cells resulted in reduced secretion of radiolabeled triglycerides into the culture medium in pulse-chase studies using [1H]oleate [43]. These results indicate that TM6SF2 is also involved in chylomicron production and thereby contributes to cholesterol secretion into the lymph. In addition to excretion with chylomicrons, part of the absorbed cholesterol is exported from the enterocytes with HDL particles. This mainly takes place in the form of free cholesterol and is mediated by the cholesterol and phospholipid transporter ABCA1 [38]. Intestine-selective deficiency of Abca1 reduced fractional cholesterol absorption considerably [45,46]. Cholesterol absorption was virtually absent in mice lacking expression of Abca1 and Mttp in the intestine [46]. In vitro studies suggest that the amount of cholesterol secreted within chylomicrons increases upon higher availability of fatty acids, while the amount of cholesterol secreted with HDL is not sensitive to the influx of fatty acids into the enterocytes [47]. Thereby, high dietary fat intake may give rise to increased levels of remnant cholesterol, which is thought to contribute to atherosclerosis development [48].

**Hepatic Uptake of Cholesterol**

Intestine-derived cholesterol that enters the bloodstream in chylomicrons or HDL is mainly taken up by the hepatocytes in the liver. Steps involved in the hydrolysis of chylomicron-associated triglycerides, leading to the formation of chylomicron remnants that eventually end up in the liver, are reviewed elsewhere [49]. Hepatocytes are highly polarized cells with a basolateral membrane and an apical or canalicular membrane with specific transporters localized to either domain. Uptake of cholesterol from circulating lipoproteins takes place at the basolateral side of the hepatocytes. Chylomicron remnants and (V)LDL particles are mainly taken up via low-density lipoprotein receptor-related protein 1 (LRP1) and LDL receptor (LDLR) [50], whereas scavenger receptor class B type 1 (SR-BI; SCARB1) mediates the uptake of free cholesterol and cholesterol esters from HDL particles [51]. The pathways involved in
intracellular cholesterol traffic are gradually being defined. When LDL bound to its receptor is internalized via clathrin-coated pits, it enters the early endosomal pathway. The lipoprotein then dissociates from the receptor and proceeds to the lysosome where lysosomal lipase A releases the free cholesterol from its ester bound form. The majority of the LDL receptor molecules avoid lysosomal degradation and recycle back to the plasma membrane [52]. Recycling of the LDL receptor is subject to complex regulation. Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a protein secreted by hepatocytes that regulates LDLR cell surface expression by binding to the receptor and targeting it for lysosomal degradation [53]. Sortilin, encoded by the hypercholesterolemia-risk gene SORT1, was recently demonstrated to function as a high-affinity sorting receptor for PCSK9 that facilitates its secretion from hepatocytes [54]. LDL receptor recycling is also regulated by the COMMD/CCDC22/CCDC93 (CCC) complex [55], which was recently demonstrated to interact with the retromer and Wiskott–Aldrich syndrome protein and SCAR homolog (WASH) complexes that are involved in endosomal cargo sorting [56,57]. In parallel to LDLR recycling, cholesterol is released from lysosomes and moves to the plasma membrane. Brown et al. [58] have elucidated that free cholesterol released in the lysosomes is bound to Niemann–Pick protein 2 (NPC2) and subsequently presented to Niemann–Pick protein 1 (NPC1). The steroid is then flipped across the lysosomal membrane and further transported via a complex mechanism involving STAR-related lipid transfer domain-3 (STAR-D3) and oxysterol-binding protein-related proteins (see [59] for recent review). A second emerging possible mechanism by which cholesterol may move through the cell is by direct contact between lysosome, peroxisome, and mitochondrion. Proteins present at the contact sites are postulated to regulate lipid trafficking and cholesterol exchange between organelles in this case [59].

Cholesterol Secretion by the Liver

Under normal conditions, secretion of cholesterol into the bile is a major pathway for removal of cholesterol from the body in humans. Cholesterol is secreted into the bile at the canalicular membrane. Although the canalicular space is relatively small compared with the size of the surrounding hepatocytes, the canalicular membrane comprises about 13% of the total plasma membrane surface owing to the presence of extensive microvilli [60,61]. This membrane domain harbors the phospholipid; bile salt; and sterol transporters ABCB4 (MDR2/3), ABCB11 (BSEP), and ABCG5/G8, which aid the secretion of phospholipids, bile acids, and sterols, respectively, into the bile [62,63]. However, as outlined earlier, it remains to be elucidated how cholesterol reaches the pericanalicular area of the hepatocyte. Furthermore, in this case, cholesterol-binding proteins or direct contact between ER and the canalicular membrane could be involved. Secretion from the canalicular membrane is better delineated. The process is driven by bile salts transported into the canalicular space between hepatocytes by ABCB11. Water follows osmotically, leading to bile acid micelle formation. These micelles, in turn, obtain phosphatidylcholine via ABCB4, forming mixed micelles that have an increased affinity for cholesterol, which is then acquired either directly from the ABCG5/G8 heterodimer or extracted from cholesterol-rich domains in the canalicular membrane. SR-B1 may also mediate secretion of cholesterol into the bile [64], although to a minor extent. The fluidity of the canalicular membrane plays an important role in determining the efficacy of the biliary secretion process. The phosphatidylserine flipase ATP8B1 is thought to regulate the presence of cholesterol in the outer leaflet of the canalicular membrane by maintaining the asymmetry of phospholipids [63,65]. As mentioned earlier, LPCAT3 influences membrane fluidity and cholesterol absorption in the intestine [31], but it remains to be investigated whether this enzyme also affects biliary cholesterol secretion. ABCG5/G8 at the canalicular membrane controls a major part of the rate of biliary cholesterol secretion. The exact mechanism via which this transporter exports cholesterol from the cell has not yet been fully elucidated. The crystal structure of ABCG5/
G8 has only recently been reported and a possible entry site for cholesterol in the transporter was identified [66]. Expression of ABCG5/G8 can be induced by the liver X receptor (LXR; NR1H3), which is activated by oxysterols and thereby acts as an intracellular sensor that serves to control cholesterol excretion. An increased cholesterol content in the hepatocyte will lead to elevated oxysterol levels and will thereby, via LXR activation, enhance ABCG5/G8-mediated secretion of cholesterol into the bile. After temporary storage in the gallbladder, the bile enters the intestine where the cholesterol can be either reabsorbed via the mechanisms described in the previous section or excreted from the body into the feces. The micelles present in bile can only solubilize a limited amount of cholesterol. Consequently, when bile becomes supersaturated with cholesterol, the sterol crystalizes and gallstones develop. Human bile is already rather saturated with cholesterol. Therefore, the potential of stimulating cholesterol secretion into the bile to enhance cholesterol removal from the body is limited. Gain-of-function mutations in ABCG5/G8 would be anticipated to increase the risk of cholesterol gallstone disease. Common genetic variants of ABCG5/G8 that are associated with low LDL-c and reduced risk for myocardial infarction were indeed associated with increased risk for gallstone disease [67].

**Active Cholesterol Excretion by the Intestine**

Only recently, a physiologically important role of the intestine in the excretion of cholesterol is being acknowledged. The first observations suggesting the existence of a TICE pathway were, however, already made about half a century ago. Simmonds and colleagues [68] performed intestinal perfusion experiments in humans to study the absorption of cholesterol from a micellar solution. While perfusing the proximal jejunum with a micellar solution containing 14C-cholesterol tracer, they observed a 40–60% decrease in specific activity of cholesterol between a proximal and a distal sampling site, implying that unlabeled cholesterol was actively secreted into the intestinal lumen. Decades later, when knockout mice became available, the importance of this pathway relative to biliary cholesterol secretion became apparent in a series of experiments from our laboratory. Genetic inhibition of cholesterol output into the bile only very modestly affected the excretion of cholesterol into the feces [69,70]. Intestinal perfusion experiments revealed that the proximal intestine mediates most of the total TICE flux [71]. Using an ex vivo approach in which mouse as well as human intestinal explants were mounted in Ussing chambers, Le May and coworkers [72] demonstrated that TICE is an active metabolic process that requires oxygen and can be inhibited by incubation of the tissues at 4°C. It was demonstrated that TICE contributes about 30% to total neutral sterol excretion in mice under control conditions [73]. Recent work from our group confirmed that TICE indeed is active in humans [18]. It was shown that about 35% of excreted neutral sterols were derived from cholesterol excreted via this pathway in healthy individuals. In mice, cholesterol removal via the TICE pathway can be strongly stimulated by various means. Activation of LXR has been demonstrated to increase TICE [69,73], but also activation of peroxisome proliferator-activated receptor-α [74] and farnesoid X receptor (FXR; NR1H4) [75] leads to augmented cholesterol removal via this pathway.

The mechanisms underlying TICE are not completely understood. The TICE pathway consists of four important steps: (i) the formation and packaging of cholesterol that serves as a substrate for TICE, most likely by the liver, and its secretion into the bloodstream, (ii) transport of this cholesterol to the enterocyte and its uptake into the cell at the basolateral side (see Box 2 for discussion), (iii) translocation of cholesterol from the basolateral to the apical side of the enterocyte, and finally (iv) excretion of cholesterol into the intestinal lumen. The final step, apical excretion of cholesterol into the intestinal lumen, is probably the part of the TICE pathway that is best understood. Various models have pointed out that ABCG5/G8 fulfills an important
Box 2. The Unsolved Conundrum: How Does the Intestine Acquire Cholesterol for Excretion via TICE?

In mice, mRNA and protein expression data pinpoint the liver as the most important source of cholesterol that is excreted via the TICE pathway. Upon stimulation of the TICE pathway by various means, enzymes involved in cholesterol synthesis are strongly upregulated in liver, whereas hardly any effects are observed in the intestine. Moreover, microarray data of mice in which TICE was strongly induced by FXR activation failed to show upregulation of the cholesterol synthesis pathway in the proximal small intestine, whereas it was the most prominently induced pathway in the liver of mice. The carrier utilized to transport cholesterol from the liver through the bloodstream to the proximal intestine remains to be identified. Liver-derived HDL has the right characteristics to serve as a carrier to fuel cholesterol into the TICE pathway, but results obtained in Abca1-deficient mice, that lack HDL, disproved such a function for these particles. The amount of neutral sterols excreted into the feces by these mice was found to be similar [82] or even higher [83] compared with wild-type littermates. Importantly, no effects on hepatic expression of enzymes involved in cholesterol synthesis were found in Abca1-deficient mice [82]. These data indicate that liver-derived HDL particles do not fulfill a significant function in transport of liver-derived cholesterol to the intestine for subsequent removal via TICE. Yet, Le May et al. [72] did show activity of HDL in TICE induced under in vitro conditions. A role for Psck9 and the LDL receptor pathway in basolateral uptake of cholesterol by the intestine has been suggested by these authors as well [72], implying a role for ApoB-containing lipoproteins in the delivery of cholesterol to the intestine for efflux via TICE. However, available data do not unambiguously identify the LDLR receptor as a major basolateral uptake transporter driving cholesterol secretion through the TICE pathway. Further research, including experiments using intestine-specific LDL receptor knockout mice, will be required to verify whether uptake of cholesterol by the LDL receptor indeed significantly impacts on TICE and which other basolateral transporters may play a role.

role in the TICE pathway by mediating excretion of cholesterol from the enterocytes into the intestinal lumen [73,75,76]. However, the flux of cholesterol through the TICE pathway is not completely inhibited in mice lacking ABCG5/G8 function in the intestine, indicating that other transporters must be active as well. In this respect, a role for ABCB1a/b has been proposed [72]. However, it remains to be demonstrated whether this transporter, like ABCG5/G8, is able to facilitate a high flux of cholesterol from the enterocyte into the intestinal lumen. Lack of functional ABCG5/G8 in the intestine was demonstrated to abolish the vast majority of TICE under stimulated conditions in multiple models [73,75,76]. Therapeutic stimulation of TICE will therefore in all likelihood require ABCG5/G8 activity. Ezetimibe is an inhibitor of cholesterol absorption and this activity was always regarded as the underlying mechanism for the increased neutral sterol excretion observed upon ezetimibe treatment. Interestingly, it has been demonstrated recently that ezetimibe increases neutral sterol excretion mainly by increasing TICE [18,77]. Most conceivably, a substantial part of the cholesterol secreted into the lumen of the intestine through TICE is normally taken up again by the enterocytes via NPC1L1. This essentially creates a cycle of repetitive cholesterol excretion and uptake by these cells, which may allow cells to quickly adapt to changing cholesterol concentrations in their environment and function to preserve intracellular cholesterol levels. Depending on the proportion of cholesterol excreted via TICE that is taken up again by the intestinal cells under normal conditions, inhibition of this reuptake may considerably increase the overall efficiency of cholesterol excretion via TICE. Successful attempts to quantify reabsorption of cholesterol secreted into the lumen via TICE have not yet been reported. However, judging from the strong stimulatory effect of ezetimibe on TICE, it can be anticipated that a considerable part of cholesterol that enters the intestinal lumen via TICE is reabsorbed by the intestine under normal conditions.

Bile Acids Drive Cholesterol Excretion

Bile acids contribute to control of cholesterol metabolism in several important ways. Bile acids are synthesized from cholesterol exclusively in the liver, conjugated to either glycine or taurine, and then actively secreted into bile to be stored in the gallbladder and subsequently discharged into the intestinal lumen upon ingestion of a meal. The multistep enzymatic conversion of the cholesterol molecule confers detergent-like properties to the bile acids that are important for
Figure 1. Stimulation of Transintestinal Cholesterol Excretion by Hydrophilic Bile Acids Combined with NPC1L1 Inhibition Results in a Strong Induction of Cholesterol Disposal. Under normal conditions, cholesterol cycles in and out of enterocytes, mainly mediated by NPC1L1 and ABCG5/G8, in a seemingly futile cycle that may serve to allow the enterocytes to quickly respond to changes in cholesterol availability. In mice, FXR activation leads to an increase in the abundance of hydrophilic bile acids entering the small intestine. These hydrophilic bile acids stimulate both cholesterol secretion into the bile and ABCG5/G8-mediated cholesterol efflux by the enterocytes. In addition, cholesterol (re)absorption is reduced under these conditions. Together, these mechanisms of action lead to enhanced cholesterol disposal in the feces. When ezetimibe is added additionally to block cholesterol absorption, enterocytic (re)cycling of cholesterol is interrupted but the increased ABCG5/G8-mediated outward flux is maintained, leading to a further amplification of cholesterol disposal. Therefore, these combined actions induce a turbo effect on cholesterol excretion. Abbreviations: ABCG5, ATP-binding cassette sub-family G member 5; ABCG8, ATP-binding cassette sub-family G member 8; Chol, cholesterol; FXR, Farnesoid X receptor; NPC1L1, Niemann-Pick type C1-like 1.

their physiological functions. Bile acids are effectively absorbed from the terminal ileum and cycle between intestine and liver in the so-called enterohepatic circulation. The magnitude of the flux of bile acids within the enterohepatic circulation is considerable. An average human bile acid pool is approximately 2 g and cycles approximately six to twelve times per day, which requires that the liver and intestine typically transport approximately 20 g of bile acids each day [78]. Highly effective transport systems in the liver and intestine have evolved to accommodate this flux. Only approximately 5% of the pool escapes absorption per cycle and is lost into the large intestine and finally into the feces: under steady-state conditions, this loss is compensated for by hepatic de novo synthesis to maintain bile acid pool size. Fecal bile acid loss, that is, hepatic synthesis rate, amounts up to the equivalent of 0.5–1.0 g cholesterol/day in healthy adult humans [79]. It thus exceeds average daily cholesterol intake (approximately 0.3 g/day) [13] and is in the same order of magnitude as estimates of whole-body cholesterol synthesis (about 0.5–1.0 g/day) [80,81]. These numbers underscore the relevance of bile acid synthesis in the maintenance of whole-body cholesterol homeostasis.

In addition to being major cholesterol metabolites, bile acids exert control on cholesterol turnover at multiple sites during their enterohepatic cycling. As described earlier, bile acids stimulate hepatobiliary cholesterol secretion by the liver and, in this way, contribute to control of
the disposal of hepatocyte-derived cholesterol into the intestinal lumen. The hydrophobicity of the bile acid pool, a key determinant of the ability to form mixed micelles and thus of its detergent capacity, appears to be of great functional importance. Very hydrophilic bile acids inhibit cholesterol absorption and, as we have recently shown, strongly stimulate the TICE pathway [75]. In mice, activation of the bile acid nuclear receptor FXR increased hydrophilicity of the bile acid pool [75], that is, increased the contribution of muricholic acids. When FXR activation was combined with simultaneous inhibition of cholesterol absorption by ezetimibe treatment, daily sterol excretion increased to an amount equivalent to 60% of the total body cholesterol content. The underlying mechanism needs to be further characterized but most conceivably involved stimulation of ABCG5/G8 activity by the hydrophilic bile acids, leading to an increased flux of cholesterol into the intestinal lumen and hence an induction of fecal neutral sterol loss (Figure 1). Thus, the picture emerges that the physicochemical characteristics of the bile acids secreted into the small intestine dictate the net movement of cholesterol molecules across the enterocytes and, thereby, critically regulate cholesterol (neutral sterol) loss into the feces. The feed-forward stimulatory effect of bile acids on cholesterol excretion provides a turbo boost to cholesterol excretion.

Concluding Remarks

The activity of cellular cholesterol uptake and excretion pathways, particularly in the liver and intestine, is crucial for maintenance of cholesterol homeostasis. Insight into the identity of these pathways and their regulatory mechanisms is slowly expanding. A plethora of processes controlling LDL receptor-mediated cholesterol uptake had been identified. The crucial transporter for intestinal cholesterol uptake is NPC1L1. Interestingly, inhibition of NPC1L1 induces the activity of the TICE pathway. The importance of TICE has now been confirmed by multiple independent research groups, but certain parts of this pathway remain to be further characterized (see Outstanding Questions). The TICE pathway has been shown, at least in mice, to have a very high transport capacity and its activity can be induced by pharmacological means. Therefore, the TICE pathway has a great utilization potential, which could be employed for the development of novel cholesterol lowering therapies.

References


Outstanding Questions

What is the exact mechanism by which NPC1L1 mediates intestinal cholesterol absorption? Does endocytosis play a role? How is the subsequent intracellular transport route of cholesterol determined? Understanding these mechanisms could pave the way for therapeutic manipulation of the import/export balance at the enterocytic lining of the intestine.

What is the mode of transport of cholesterol from liver toward the intestine for excretion via the TICE pathway? Identification of a specific donor particle for TICE may reveal new targets for future cholesterol lowering therapies.

What are the signals for communication between the liver and intestine that coordinate cholesterol uptake and excretion? Available data strongly suggest that the liver receives an as yet unidentified signal from the intestine when the TICE flux is high.

What is the mechanism via which hepatocellular cholesterol moves to the canalicular membrane?

What is the impact of the presence of mouse-specific muricholic acids on the translatability of results obtained in mouse experiments to the human situation? Cyp2c70 has been identified as the enzyme responsible for the production of muricholic acids: generation of Cyp2c70 knockout mice should therefore result in a mouse with a ‘humanized’ bile acid metabolism that is anticipated to improve extrapolation of experimental data to humans.
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