Regulation of Intestinal Cholesterol Absorption

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Key Words

bile salt, transporter, chylomicron, nutrition, sitosterol

Abstract

The identification of defective structures in the ATP-binding cassette (ABC) transporters ABCG5 and ABCG8 in patients with sitosterolemia suggests that these two proteins are an apical sterol export pump promoting active efflux of cholesterol and plant sterols from enterocytes back into the intestinal lumen for excretion. The newly identified Niemann-Pick C1–like 1 (NPC1L1) protein is also expressed at the apical membrane of enterocytes and plays a crucial role in the ezetimibe-sensitive cholesterol absorption pathway. These findings indicate that cholesterol absorption is a multistep process that is regulated by multiple genes at the enterocyte level and that the efficiency of cholesterol absorption may be determined by the net effect between influx and efflux of intraluminal cholesterol molecules crossing the brush border membrane of the enterocyte. Combination therapy using cholesterol absorption (NPC1L1) inhibitor (ezetimibe) and 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitors (statins) provides a powerful novel strategy for the prevention and treatment of hypercholesterolemia.

INTRODUCTION

Bile acids: the end products of cholesterol degradation in the liver that are the major constituents of bile and that are essential for the absorption of cholesterol from the small intestine

Plant sterol (phytosterol): sterols present in plants

Stenol: sterols with one or more nuclear double bonds

ACAT: acyl-CoA:cholesterol acyltransferase

ATP-binding cassette (ABC) transporters: a superfamily of highly conserved proteins involved in the membrane transport of a variety of substrates to various compartments

Sterol: any unsaponifiable steroid alcohol with an aliphatic side chain of 8–10 carbon atoms and a hydroxyl group at C3

Because increased plasma cholesterol levels are one of the most important risk factors for coronary heart disease, the National Cholesterol Education Program Adult Treatment Panel III guidelines and the results of the Heart Protection Study have provided a stronger rationale to treat high-risk patients to a low-density lipoprotein (LDL) cholesterol goal of <100 mg dl^{-1} (1). In particular, individuals at substantial risk for atherosclerosis or patients with cardiovascular diseases should meet defined targets for LDL cholesterol levels. The setting of these targets has greatly increased the number of individuals who need cholesterol-lowering therapy.

The cholesterol carried in LDL is derived principally from de novo synthesis and absorption from the diet. In humans, there is a significant and positive correlation between the level of plasma LDL cholesterol and the efficiency of intestinal cholesterol absorption (2). Thus, the restriction of dietary calories, cholesterol, and saturated fat has been recommended as the primary initial therapeutic intervention for the treatment of patients with dyslipidemia (3, 4). Despite significant restrictions in dietary intake, the reduction of dietary cholesterol is often not associated with a significant decrease in circulating LDL cholesterol levels. Therefore, pharmacological modulation of cholesterol absorption is potentially an effective way of lowering plasma LDL cholesterol levels in the general population.

Because intestinal cholesterol absorption is a complex process that involves multiple interrelated sequential degradative and synthetic pathways, it provides multiple therapeutic targets in the management of patients with hypercholesterolemia. For example, the bile acid sequestrants (e.g., cholestyramine, resins, and colestipol) reduce cholesterol absorption primarily via interruption of the enterohepatic circulation of bile acids and may result in a secondary increase in hepatic LDL receptor activity (5, 6). Dietary plant sterols at a dose of 2 g per day have been recommended as an adjunctive lifestyle treatment for hypercholesterolemia (7). Recent clinical studies have found that plant sterol treatment induces an approximately 8–14% decrease in plasma LDL cholesterol levels in subjects with mild or moderate hypercholesterolemia (8). Specific lipase inhibitors such as orlistat may also suppress cholesterol absorption by blocking the degradative process within the gastrointestinal lumen (9, 10), which results in a decreased solubility of cholesterol during the critical stage of intestinal diffusion. The intestinal acyl-CoA:cholesterol acyltransferase (ACAT) inhibitors (11) and cholesterol ester transfer protein inhibitors (12) are currently being tested in clinical trials, and the potential to alter ATP-binding cassette (ABC) transporter activity in the intestine is also being investigated. Moreover, the discovery and development of ezetimibe (13, 14)—a novel, selective, and potent inhibitor that effectively blocks intestinal absorption of dietary and biliary cholesterol—open a new door to the treatment of hypercholesterolemia (15–18). Ezetimibe, which can be administered either alone or in combination with statins, is a safe and efficacious treatment for hypercholesterolemia and potentially enables more patients to reach recommended LDL cholesterol goals.

Pharmacological inhibitors of cholesterol absorption should ideally be targeted for use in individuals who demonstrate quantitatively enhanced cholesterol transport from the intestinal tract to the circulation. In particular, two recently identified intestinal sterol transporters provide further insights in the regulation of intestinal cholesterol absorption. Mutations in the genes encoding either ABCG5 or ABCG8 result in sitosterolemia (19, 20), which is characterized by increased intestinal absorption and diminished biliary secretion of plant sterols, inducing a significant increase in plasma concentrations of plant sterols (21). These findings suggest that

ABCG5 and ABCG8 work as sterol efflux pumps in the small intestine and liver. The discovery of ezetimibe greatly helped to reveal the role of the Niemann-Pick C1–like 1 (NPC1L1) protein in the intestinal uptake of cholesterol and plant sterols (22, 23). As shown in **Figure 1**, intestinal cholesterol absorption is a multistep process that is regulated by multiple genes in the enterocyte. This review highlights the recent progress in elucidating the genetic mechanisms of intestinal cholesterol absorption, the molecular biology of intestinal sterol transporters, and the pharmacological approaches by which plant sterols and ezetimibe inhibit the absorption process.

Niemann-Pick C1–like 1 (NPC1L1) protein: has 50% amino acid homology to NPC1, functions in intracellular cholesterol trafficking, and is defective in the cholesterol storage disorder, i.e., the Niemann-Pick type C disease

Sitosterolemia: a rare autosomal-recessive inherited disease induced by mutations of either the *ABCG5* or *ABCG8* gene and characterized by increased plasma plant sterol levels, xanthomas, and premature onset of atherosclerosis

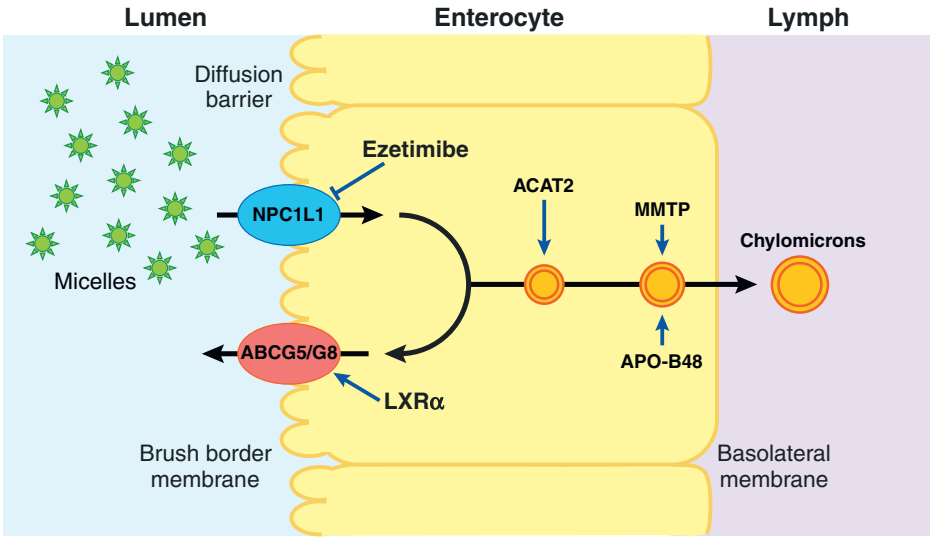

Figure 1

Within the intestinal lumen, the micellar solubilization of sterols allows them to move through the diffusion barrier overlying the surface of the absorptive cells. In the absence of bile acids, individual sterol molecules must diffuse across the diffusion barrier overlying the brush border of the enterocyte. Hence, uptake of the sterols is largely diffusion limited. In the presence of bile acids, large amounts of the sterol molecules are delivered to the aqueous-membrane interface so that the uptake rate is greatly increased. The principal mechanism whereby hydrophilic bile acids inhibit cholesterol absorption appears to be via diminution of intraluminal micellar cholesterol solubilization. Plant sterols and plant stanols have a higher affinity to mixed micelles than does cholesterol, and they thereby displace cholesterol from these micelles and reduce cholesterol absorption. The Niemann-Pick C1–like 1 (NPC1L1) protein, a newly identified sterol influx transporter, is located at the apical membrane of the enterocyte, which may actively facilitate the uptake of cholesterol by promoting the passage of sterols across the brush border membrane of the enterocyte. Most likely, ezetimibe reduces cholesterol absorption by inhibiting the activity of intestinal NPC1L1. In contrast, ABCG5 and ABCG8 promote active efflux of cholesterol and plant sterols from the enterocyte into the intestinal lumen for excretion. Liver X receptor α (LXRα) may be essential for the upregulation of the *ABCG5* and *ABCG8* genes in response to high dietary cholesterol. The combined regulatory effects of NPC1L1, ABCG5, and ABCG8 may play a critical role in modulating the amount of cholesterol that reaches the lymph from the intestinal lumen. In addition, several proteins involved in other steps in the absorption process, e.g., acyl-CoA:cholesterol acyltransferase isoform 2 (ACAT2), apolipoprotein B48 (APO-B48), and microsomal triglyceride transfer protein (MTTP), involve esterification of cholesterol and its incorporation into nascent chylomicrons that are subsequently secreted into the lymph. These intracellular events may also exert major influences on cholesterol absorption. Therefore, intestinal cholesterol absorption is a multistep process that is regulated by multiple genes. Modified from Reference 93, with permission.

DISTINGUISHING INTESTINAL CHOLESTEROL ABSORPTION AND INTESTINAL CHOLESTEROL UPTAKE

Conceptually, intestinal absorption of cholesterol has to be distinguished from its uptake by the enterocyte. Intestinal absorption of cholesterol is most accurately defined as the transfer of intraluminal cholesterol into intestinal or thoracic duct lymph. Intestinal uptake of cholesterol refers to its entry from the lumen into intestinal absorptive cells. As can be inferred from these definitions, intestinal cholesterol absorption is a multistep process that is regulated by multiple genes, and any factors that change the transportation of cholesterol from the intestinal lumen to the lymph can influence the efficiency of intestinal cholesterol absorption.

PHYSICAL CHEMISTRY AND PHYSIOLOGY OF INTESTINAL CHOLESTEROL ABSORPTION

Micelles: small globular polymolecular aggregates of bile acids that form by apposition of their hydrophobic surfaces in an aqueous solution and that incorporate cholesterol and phospholipids, enhancing their solubility

Cholesterol that enters the small intestinal lumen to be absorbed by the enterocytes derives mainly from three sources: diet, bile, and intestinal epithelial sloughing. The average intake of cholesterol in the Western diet is approximately 300–500 mg per day. Bile is estimated to contribute nearly 800–1200 mg of cholesterol per day to the intraluminal pool. A third source of intraluminal cholesterol comes from the turnover of intestinal mucosal epithelium, which provides roughly 300 mg of cholesterol per day. Although the entire length of the small intestine can absorb cholesterol from the lumen, the major sites of absorption are in the upper part of the small intestine, i.e., the duodenum and proximal jejunum. Thus, because intestinal sloughing occurs throughout the intestinal tract and cholesterol absorption seems to be confined to the very proximal small intestine, the intestinal sloughing pool may not contribute greatly to cholesterol absorption.

Cholesterol absorption begins in the stomach, where dietary ingredients are mixed with lingual and gastric enzymes, resulting in partial fat digestion by preduodenal lipases and emulsification by peristalsis. The stomach also regulates the delivery of gastric chyme to the duodenum, where it is mixed with bile and pancreatic juice. The major lipases and proteins secreted by the pancreas into the intestinal lumen in response to a meal include carboxyl ester lipase (CEL), pancreatic triglyceride lipase, and the Group 1B phospholipase A_2, as well as pancreatic lipase–related protein-1 and -2. Only unesterified cholesterol can be incorporated into bile acid micelles and transported to the brush border of enterocyte, so an extremely important step is de-esterification of intestinal cholesteryl esters. Additionally, because the pool of unesterified cholesterol (mainly biliary source) in the intestinal lumen is relatively much larger than the esterified dietary pool of cholesterol, inhibition or loss of some of the pancreatic lipolytic enzyme activities is not likely to be effective in reducing cholesterol absorption. These observations may partly explain why targeted disruption of the *CEL* gene has only a slight inhibitory effect on intestinal cholesterol absorption in mice (24, 25). Interestingly, the lack of triglyceride hydrolytic activity in the intestinal lumen significantly reduces dietary cholesterol absorption but does not influence triglyceride digestion or fat absorption in pancreatic triglyceride lipase knockout mice (26). The regulatory effects of the Group 1B phospholipase A_2 as well as of pancreatic lipase–related protein-1 and -2 on intestinal cholesterol absorption have not yet been defined.

In general, most of the cholesterol in food exists in the unesterified form—<15% of cholesterol is in the form of cholesteryl esters—and dietary cholesterol ingestion is often associated with fat consumption. Dietary cholesterol appearing in the intestinal lumen is usually mixed with triglycerides and phospholipids in the form of lipid emulsion. Digestion of the phospholipids and triglycerides in the surface and core, respectively, of the lipid emulsion particles is necessary to liberate the dietary cholesterol to phospholipid vesicles and bile acid micelles for its transport to the brush border of enterocyte for absorption.

Because some lipolytic products (e.g., cholesterol) are poorly soluble in a pure aqueous environment, they must depend on the solubilizing properties of bile acids. Luminal bile acids are derived from hepatic secretion and reabsorbed from the intestinal lumen back to the liver in a process termed the enterohepatic circulation of bile acids. The detergency of bile acids is obligatory for intestinal cholesterol uptake through micellar solubilization of the intraluminal sterol. Bile acids are a biological amphipathic detergent, and their monomers can aggregate spontaneously to form simple micelles when their concentrations exceed the critical micellar concentration. Simple micelles (3 nm in diameter) are small, thermodynamically stable aggregates that can solubilize a minimal amount of cholesterol. In contrast, phospholipids, monoacylglycerides, and free fatty acids are readily soluble.

Together with ionized and nonionized fatty acids, monoacylglycerides, and lysophospholipids, bile acids form mixed micelles, which significantly enhances the solubility of cholesterol. Mixed micelles (4–8 nm in diameter) are large, thermodynamically stable aggregates, and their sizes vary principally depending on the relative proportion of bile acids and phospholipids. They function as a concentrated reservoir and transport vehicle for cholesterol across the unstirred water layer toward the brush border of the small intestine to facilitate uptake of monomeric cholesterol by the enterocyte. Furthermore, excess lipids not dissolved in the micellar phase can be maintained as a stable emulsion by bile acids, phospholipids, monoacylglycerides, and fatty acids in the intestinal lumen.

During lipolysis, a liquid crystalline phase composed of multilamellar products of lipid digestion forms at the surface of the emulsion droplets. Vesicles are unilamellar spherical structures that contain phospholipids and cholesterol and few, if any, bile acids. Thus, vesicles (40–100 nm in diameter) are substantially larger than either simple or mixed micelles but much smaller than liquid crystals (500 nm in diameter) that are composed of multilamellar spherical structures. This liquid crystalline phase provides an accessible source of cholesterol and other lipids for continuous formation and modification of mixed micelles in the presence of bile acids. Within the intestinal lumen, the presence of hydrophilic bile acids may reduce solubility of cholesterol by inducing phase separation of the sterol from mixed micelles to a coexisting liquid crystalline vesicle phase (27). Most likely, hydrophilic bile acids facilitate incorporation of cholesterol molecules into a stable liquid crystalline/vesicle phase from which they are poorly absorbed by enterocytes. In contrast, hydrophobic bile acids markedly increase micellar cholesterol solubility (27, 28) and thereby augment cholesterol absorption. This suggests that the hydrophobic bile acids are more effective promoters of cholesterol absorption than are the hydrophilic bile acids.

Intestinal diffusion barrier: an unstirred water layer and a surface mucous coat on the apical membrane of the enterocyte

Before cholesterol molecules in the small intestinal lumen can interact with the newly identified intestinal sterol transporter NPC1L1 for uptake and subsequent transport across the brush border of the enterocyte, they must pass through a diffusion barrier that is located at the intestinal lumen–membrane interface, which may alter the kinetics of cholesterol absorption. Moreover, diffusion through the unstirred water layer is a relatively slow process for cholesterol that is virtually insoluble in aqueous systems. Therefore, the unstirred water layer, a series of water lamellae at the interface between the bulk water phase of the lumen and the apical membrane of the enterocyte, is considered an important barrier through which a cholesterol molecule in the bulk phase must pass to be absorbed. Additionally, the intestinal mucous coat has an important role as a diffusion-limiting barrier, as cholesterol molecules may be extensively bound to surface mucins prior to being transferred into the enterocyte. Physiological levels of the epithelial mucin encoded by the *MUC1* gene are necessary for normal intestinal uptake and absorption of cholesterol in mice (29). Because cholesterol absorption

Chylomicrons: the major transport vehicles of cholesterol and triglycerides from the intestine to the liver

MTTP: microsomal triglyceride transfer protein

HMG-CoA: 3-hydroxy-3-methylglutaryl-CoA

efficiency is reduced by 50% in MUC1-deficient mice, there may be alternative pathways for cholesterol absorption. Furthermore, uptake and absorption of cholesterol but not fatty acids are decreased in MUC1 knockout mice because the movement of big rigid molecules, such as cholesterol, across the cell membrane is different from that of smaller, less rigid, and space-occupying molecules, such as fatty acids. As the lipid-protein interaction and structural assembly of proteins may influence the kinetics of net cholesterol movement across the cell membrane of enterocyte, it is crucial to investigate how structural protein integrity or assembly at the cell membrane level is maintained during the intestinal absorption of cholesterol.

Another potential step for sorting/regulation is when the absorbed cholesterol molecules reach the endoplasmic reticulum, where an enzyme usually known as ACAT2 esterifies cholesterol (30). ACAT2 is highly specific for cholesterol and does not appreciably esterify plant sterols. Cholesteryl esters are then pumped by a key step in chylomicron biogenesis; microsomal triglyceride transfer protein (MTTP) transfers neutral lipids into nascent chylomicrons, allowing them to mature and exit the endoplasmic reticulum for eventual secretion as chylomicron particles into the lymph (31). During cholesterol absorption, there is little increase in the cholesterol content of the small intestinal wall, demonstrating that the absorbed cholesterol can be rapidly processed and exported from the enterocyte into the intestinal or thoracic duct lymph. Essentially all cholesterol molecules that move from the intestinal lumen into enterocytes are unesterified; however, cholesterol exported into intestinal lymph following a cholesterol-rich meal is approximately 70–80% esterified, suggesting that esterification is an important step for bulk entry into the nascent chylomicrons. Therefore, the enterocyte's cholesterol-esterifying activity may be an important regulator of intestinal cholesterol absorption because re-esterification of the absorbed cholesterol within the enterocyte enhances the diffusion gradient for intraluminal cholesterol entry into the cell. The inhibition of ACAT by pharmacological intervention significantly reduces transmucosal transport of cholesterol in rats (32, 33), and deletion of the *ACAT2* gene decreases intestinal cholesterol absorption in mice (34). Moreover, the inhibition of intestinal 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase by pharmacological treatment with statins also diminishes intestinal cholesterol absorption in humans (37) and other animals (35, 36).

In addition, intestinal cholesterol absorption is significantly inhibited in apolipoprotein-B48 knockout mice (38) and in "apolipoprotein-B100-only" mice that synthesize exclusively apolipoprotein-B100 (39) because of a failure in the assembly and/or delivery of chylomicrons into the intestinal lymph. Intestinal MTTP transfers neutral lipids into newly formed chylomicrons in the endoplasmic reticulum (40), and *MTTP* mutations cause abetalipoproteinemia in humans, which is characterized by severe steatorrhea, neurological symptoms, fatty liver, and very low plasma cholesterol levels (41). Targeted disruption of the *CEL* gene induces a significant decrease in the number of chylomicron particles produced by the enterocyte after a lipid meal; most of the intestinal lipoproteins produced by CEL knockout mice are VLDL-sized particles (24). The exact mechanism by which CEL participates in chylomicron assembly is currently unknown, but indirect evidence suggests that CEL has an important effect on intracellular lipid trafficking. Although intestinal apolipoproteins AI/CIII/AIV may play a role in the regulation of cholesterol absorption (42), the regulatory effects of these proteins remain to be defined. Nevertheless, all these observations on chylomicron assembly suggest that the later steps in the cholesterol absorption process are also critically important.

Finally, cholesterol and bile acids that escape intestinal reabsorption are excreted as fecal neutral and acidic sterols. This represents

the major route for sterol elimination from the body.

FACTORS INFLUENCING INTESTINAL CHOLESTEROL ABSORPTION EFFICIENCY

Any factor that changes the transportation of cholesterol from the intestinal lumen to the lymph can influence the efficiency of cholesterol absorption because intestinal cholesterol absorption is a multistep process. **Table 1** summarizes dietary, pharmacological, biliary, cellular, and luminal factors that may influence intestinal cholesterol absorption. When dietary conditions are controlled, biliary factors may exert a major influence on the efficiency of cholesterol absorption, any changes in which may partly explain interindividual and interstrain differences in cholesterol absorption efficiency in humans and other animals. For example, hepatic output and pool size of biliary bile acids are markedly reduced in mice with homozygous disruption of the cholesterol 7α-hydroxylase (*CYP7A1*) gene that encodes the key enzyme of the neutral pathway of bile acid synthesis (43). As a result, the mice absorb only trace amounts of cholesterol because of bile acid deficiency in bile. Similarly, upon deletion of the sterol 27-hydroxylase (*CYP27*) gene, which encodes the main enzyme of the alternative pathway of bile acid synthesis, the knockout mice display significantly reduced bile acid synthesis and pool size. Consequently, intestinal cholesterol absorption decreases from 54% to 4%, whereas fecal neutral sterol excretion increases 2.5-fold (44). However, in both knockout strains, cholesterol absorption is restored by feeding a diet containing cholic acid (43, 44).

These findings confirm that hepatic output and pool size of biliary bile acids play a critical role in intestinal cholesterol absorption by the regulation of intraluminal bile acid micellar concentrations. Hydrophilic and hydrophobic bile acid feeding studies in mice (27, 28) show that changes in the hydrophilic-hydrophobic balance of biliary bile acid pool influence cholesterol absorption. In an alloxan-induced mouse model of diabetes, percentages of cholesterol absorption are significantly increased. This is because the biosynthesis of hydrophilic tauro-β-muricholic acid in the liver is reduced and the biosynthesis of cholic acid is augmented so that the hydrophilic-hydrophobic index of bile acid pool is increased remarkably (45). These alterations in turn induce biliary cholesterol hypersecretion and cholesterol gallstone formation (45). Moreover, targeted deletion of the *ABCB4* gene encoding the canalicular phosphatidylcholine flippase of the hepatocyte abolishes biliary secretion of phospholipids, which significantly suppresses intestinal cholesterol absorption (46, 47). Studies of homozygous and heterozygous *ABCB4*-deficient mice suggest that physiological levels of biliary phospholipid outputs are necessary for normal intestinal cholesterol absorption (47). Disruption of the ileal bile acid transporter (IBAT) eliminates enterohepatic cycling of bile acids in mice (48). However, there is a mild modulation in cholesterol absorption efficiency under chow feeding conditions, which can be explained by the facts that cholesterol absorption occurs predominantly in the proximal intestine and that IBAT-mediated uptake of bile acids occurs primarily in the distal intestine. In addition, the bile acid pool size in the proximal intestine is not different between control and IBAT inhibitor–treated animals, primarily because of the compensatory increase in bile acid synthesis by the liver in the latter group of animals.

IBAT: ileal bile acid transporter

Several human and animal studies have found that changes in small intestinal transit rate influence the efficiency of cholesterol absorption. Mice with deletion of the cholecystokinin-1 receptor (*CCK-1R*) gene show a significant increase in intestinal cholesterol absorption, which correlates with a significantly slower small intestinal transit rate (49). This in turn induces biliary cholesterol hypersecretion and cholesterol

Table 1 Possible factors influencing intestinal cholesterol absorption[a,b]

Factors[c]	Effects on percent cholesterol absorption and type of study	Mouse Chr[d]	cM	Human ortholog	References
Dietary factors					
↑ Cholesterol	(–) Animal feeding studies				93
↑ Monounsaturated	↓ African green monkey feeding studies				140
↑ ω-3 polyunsaturated	↓ African green monkey feeding studies				140
↑ Fish oils	↓ Rat lymphatic transport studies				141
↑ Sphingomyelin	↓ Animal feeding studies				142
↑ Fiber	↓ Human and animal feeding studies				143
↑ Plant sterols (phytosterols)	↓ Human and animal feeding studies				123, 129
Pharmacological factors					
↑ Hydrophilic bile acids	↓ Human and animal feeding studies				27
↑ Hydrophobic bile acids	↑ Human and animal feeding studies				27, 28
↑ Ezetimibe	↓ Human and animal feeding studies				98, 131
↑ ACAT inhibitors	↓ Human and animal feeding studies				11, 32, 33
↑ Statins	↓ Human and animal feeding studies				35–37
↑ Bile acid sequestrants	↓ Human and animal feeding studies				5, 6
↑ Intestinal lipase inhibitors	↓ Human and animal feeding studies				9, 10
↑ Estrogen	↑ Animal feeding studies				55
Biliary factors					
↓ Biliary bile salt output	↓ Cholesterol 7α-hydroxylase (–/–) mice				43, 144
↓ Size of bile salt pool	↓ Cholesterol 7α-hydroxylase (–/–) mice				43, 144
↓ Biliary phospholipid output	↓ *Abcb4* (–/–) mice				46, 47
↑ Biliary cholesterol output	↑ Animal studies				52, 77
↑ Cholesterol content of bile	↑ Animal studies				45, 52, 77
↑ HI of bile salt pool	↑ Animal studies				27, 45
Cellular factors					
↓ ACAT2	↓ ACAT2 inhibitors and *Acat2* (–/–) mice	15	61.7	12q13.13	11, 32–34
↓ HMG-CoA reductase	↓ HMG-CoA reductase inhibitors in human and mouse studies	13	49.0	5q13.3-q14	35–37
↓ ABCA1	↓↑ *Abca1* (–/–) mice[e]	4	23.1	9q31.1	116–118
↓ ABCG5 and ABCG8	↑ *Abcg5/g8* (–/–) mice and *Abcg5/g8* transgenic mice; and sitosterolemia	17	54.5	2p21	19–21, 78–80, 89
↓ NPC1L1	↓ *Npc1l1* (–/–) mice and ezetimibe feeding studies	11	ND	7p13	22, 23, 98, 131
Aminopeptidase N	To be identified	7	ND	15q25-q26	
↓ SR-BI	(–) ↓ *Sr-b1* (–/–) mice and *Sr-b1* transgenic mice[e]	5	68.0	12q24.31	113–115
↓ IBAT	(–) *Ibat* (–/–) mice	8	20	13q33	48
↓ Caveolin 1	(–) Caveolin 1 (–/–) mice	6	A2[f]	7q31.1	145
Caveolin 2	To be identified	6	A2	7q31.1	
MTTP	To be identified	3	66.2	4q24	
SCP2	To be identified	4	52.0	1p32	
OSBP	To be identified	19	7.0	11q12-q13	

(*Continued*)

Table 1 (*Continued*)

Factors[c]	Effects on percent cholesterol absorption and type of study	Mouse Chr[d]	cM	Human ortholog	References
↓ APO-B48	↓ *ApoB48* (–/–) mice and "Apo-B100-only" mice	12	2.0	2p24-p23	38, 39
APO-AI	To be identified	9	27.0	11q23-q24	
↓ APO-AIV	(–) *Apo-AIV* (–/–) mice and *Apo-AIV* transgenic mice	9	27.0	11q23	146, 147
APO-CIII	To be identified	9	27.0	11q23.1-q23.2	
↑ Estrogen receptor α	↑ Animal studies	10	12.0	6q25.1	55
Estrogen receptor β	To be identified	12	33.0	14q23.2	
NR1H4 (FXR)	To be identified	10	50.0	12q23.1	
NR1H3 (LXRα)	To be identified	2	40.4	11p11.2	
NR1H2 (LXRβ)	To be identified	7	ND	19q13.3	
↑ NR2B1 (RXRα)	↓ RXR agonist and mouse study	2	17.0	9q34.3	116
↑ NR1C1 (PPARα)	↓ PPARα agonist and PPARα (–/–) mice	15	48.8	22q13.31	148
↑ NR1C2 (PPARδ)	↓ PPARδ agonist and mouse study	17	13.5	6p21.2-p21.1	149
NR1C3 (PPARγ)	To be identified	6	52.7	3p25	
Luminal factors					
↑ Small intestinal transit time	↑ Cck-1 receptor (–/–) mice				49
↑ Gastric emptying time	↑ Inbred strains of mice				150
↓ MUC1 mucin	↓ *Muc1* (–/–) mice	3	44.8	1q21	29
MUC2 mucin	To be identified	7	69.0	11p15.5	
MUC3 mucin	To be identified	5	75.0	7q22	
MUC4 mucin	To be identified	16	ND	3q29	
MUC5ac mucin	To be identified	7	69.0	11p15.5	
MUC5b mucin	To be identified	7	69.0	11p15.5	
MUC6 mucin	To be identified	7	69.0	11p15.5	
↓ Carboxyl ester lipase	(–) ↓ Carboxyl ester lipase (–/–) mice	2	16.0	9q34.3	24, 25
↓ Pancreatic triglyceride lipase	↓ Pancreatic triglyceride lipase (–/–) mice	19	29.0	10q26.1	26
Sphingomyelinase	To be identified	4	ND	8q12-q13	

[a]Table is modified from Reference 52, with permission.

[b]Abbreviations: ABC, ATP-binding cassette (transporter); ACAT2, acyl-CoA:cholesterol acyltransferase, isoform 2; APO, apolipoprotein; CCK, cholecystokinin; Chr, Chromosome; cM, centimorgan; FXR, farnesoid X receptor; HI, hydrophobicity index; HMG, 3-hydroxy-3-methyglutaryl; IBAT, ileal bile acid transporter; LXR, liver X receptor; MTTP, microsomal triglyceride transfer protein; MUC, mucin gene; ND, not determined; NPC1L1, Niemann-Pick C1 like 1 (protein); NR, nuclear receptor; OSBP, oxysterol-binding protein; p, short arm of the Chr; q, long arm of the Chr; RXR, retinoid X receptor; PPAR, peroxisomal proliferator activated receptor; SCP2, sterol carrier protein 2; SR-BI, scavenger receptor class B type 1.

[c]↑ represents increase, ↓ decrease, and (–) no effect.

[d]Map position is based on conserved homology between mouse and human genomes and assigned indirectly from localization in other species. Information on homologous regions was retrieved from the mouse/human homology databases maintained at the Jackson Laboratory (**http://www.informatics.jax.org/searches/marker_form.shtml**) and the National Center for Biotechnology Information (**http://www.ncbi.nlm.nih.gov/HomoloGene**).

[e]Contradictory results were reported by different groups (see text for details).

[f]As inferred from conserved map locations in mouse and human genomes, the mouse gene may be localized on proximal Chr 2 band A2.

gallstone formation (49). In contrast, guinea pigs resistant to systemic effects of dietary cholesterol display shorter small intestinal transit times than do hypercholesterolemic guinea pigs (50). Furthermore, acceleration of small intestine transit induced by pharmacological intervention is consistently associated with decreased cholesterol absorption in humans (51). However, it is surprising that there are essentially similar small intestinal transit times, lengths, and weights among low-, middle-, and high-cholesterol-absorbing inbred mouse strains (52). These findings suggest that under normal physiological conditions, luminal factors apparently do not account for the major differences in the efficiency of intestinal cholesterol absorption in diverse healthy inbred strains of mice.

In addition, there are well-known gender differences in the efficiency of cholesterol absorption in humans and other animals (53–56). Estrogen significantly increases hepatic output of biliary lipids and bile acid–dependent bile flow rate (57–59). These biliary factors markedly promote cholesterol absorption in animals and humans, especially in those exposed to high levels of estrogen. In addition, estrogen likely regulates expression of the sterol transporter genes in the intestine via the estrogen receptor pathway (55). Furthermore, the efficiency of intestinal cholesterol absorption increases markedly with age (53–55), suggesting that cholesterol absorption is modified by aging. Aging significantly increases secretion rate of biliary lipids and cholesterol content of bile as well as the size and hydrophobicity index of the bile acid pool (53, 60–62). These biliary factors together may have a major effect on increased efficiency of cholesterol absorption with age (53–55). It is imperative to explore whether aging per se enhances intestinal cholesterol absorption by the mechanism whereby *Longevity* (aging) genes may influence expression of the intestinal sterol transporter genes.

GENETIC ANALYSIS OF INTESTINAL CHOLESTEROL ABSORPTION

Epidemiological investigations and animal studies show that there are significant interindividual differences and interstrain variations in intestinal cholesterol absorption efficiency in primates (63, 64), including humans (2, 65–68), as well as in inbred strains of mice (52, 69–72), rats (73), and rabbits (74, 75). These observations strongly suggest that intestinal cholesterol absorption is regulated by multiple genes, as diet, the key environmental factor, is controlled in these studies. The question arises, however, as to which cellular step(s) in the intestinal absorption of cholesterol might be inherently different. As examined by a fecal dual-isotope ratio method in probands with very high and low plasma cholestanol levels (a plant sterol that correlates with cholesterol absorption) and their siblings (76), siblings of the higher-absorbing probands display significantly higher cholesterol absorption efficiency ($49 \pm 2\%$) than do siblings of the lower-absorbing probands ($37 \pm 3\%$). Furthermore, there are significant differences in cholesterol absorption efficiency measured by plasma, fecal, and lymphatic methods among 12 inbred strains of mice: <25% in AKR/J, C3H/J, and A/J strains; 25–30% in SJL/J, DBA/2J, BALB/cJ, SWR/J, and SM/J strains; and 31–40% in C57L/J, C57BL/6J, FVB/J, and 129/SvJ strains (52). In particular, when dietary factors are controlled by feeding a normal rodent chow diet containing trace (<0.02%) amounts of cholesterol, the efficiency of cholesterol absorption in the C57L/J strain with intact enterohepatic circulation of bile acids is significantly higher than in the AKR/J strain, as measured by four independent methods, i.e., the plasma and the fecal dual-isotope ratio methods, the lymphatic transport of cholesterol, and the mass balance method (52, 77). When these studies were repeated in mice with chronic biliary fistulae but in the setting of duodenal

infusion of taurocholate and egg yolk lecithin, the marked differences in the efficiency of intestinal cholesterol absorption still persist between these two mouse strains (52, 77). Furthermore, cholesterol absorption in (AKR/J × C57L/J)F_1 progeny mimics that in the higher-absorbing parental strain C57L/J, suggesting that high cholesterol absorption is a dominant trait in mice (52). These systematic studies suggest that the genetic factors at the enterocyte level are crucial in determining the variations of intestinal cholesterol absorption efficiency (52).

With quantitative trait locus (QTL) mapping techniques, genetic loci that determine cholesterol absorption efficiency can be identified by genome-wide linkage studies in experimental crosses of inbred mouse strains. In backcrossing progeny of (AKR/J × DBA/2J)F_1 to the lower-absorbing DBA/2J parental strain (72), a QTL that influences cholesterol absorption efficiency is detected on chromosome 2 at 64 cM with a significant LOD score of 3.5 (designated cholesterol absorption gene locus 1, *Chab1*), and a second suggestive QTL (*Chab2*) is on chromosome 10 at 24 cM with a LOD score of 1.9. Three additional loci are identified in 21 different DBA/2J × AKR/J recombinant inbred strains with LOD scores between 1.6 and 2.0: *Chab3* on chromosome 6 at 51 cM, *Chab4* on chromosome 15 at 58 cM, and *Chab5* on chromosome 19 at 16 cM. In contrast, when (129P3/J × SJL/J)F_1 progeny are backcrossed to the higher-absorbing 129P3/J parental strain, QTL analysis reveals a locus named *Chab6* on chromosome 1 at 57 cM with a LOD score of 2.1 and a second locus named *Chab7* on chromosome 5 at 57 cM with a LOD score of 3.3. However, the individual genes underlying each of these cholesterol absorption QTLs remain to be identified. **Figure 2** shows physiological relevant genes as well as quantitative trait loci (QTL) and their candidate genes on chromosomes of the mouse genome, which may be involved in the regulation of intestinal cholesterol absorption.

IDENTIFICATION OF INTESTINAL STEROL TRANSPORTERS AND THEIR MOLECULAR BIOLOGY

Although accumulated evidence has clearly established the importance of cholesterol transfer to bile acid micelles before the transport of cholesterol to the brush border membrane of enterocytes for absorption, the mechanism by which the cholesterol molecules in micelles are taken up across the brush border membrane independently of bile acid uptake is still under extensive investigation. The long-standing hypothesis suggests that cholesterol absorption is an energy-independent, simple passive diffusion process in which micellar cholesterol is in equilibrium with monomolecular cholesterol in solution and the monomeric cholesterol is absorbed to the brush border membrane down a concentration gradient. The intestinal sterol uptake and transport process has been assumed to be controlled mainly by two enzymes: ACAT2, which enhances intracellular cholesterol esterification, and MTTP, which is responsible for intestinal chylomicron assembly.

However, much new evidence supports the notion that a transporter-facilitated mechanism is involved in cholesterol uptake by the enterocyte. First, interindividual differences and interstrain variations in the efficiency of intestinal cholesterol absorption occur in humans and animals, suggesting that intestinal cholesterol absorption is regulated by multiple genes. Second, patients with sitosterolemia mainly display excess plant sterol absorption, indicating that they have lost the ability to discriminate between plant sterols and cholesterol (21, 78–80). Third, structurally related plant sterols such as sitosterol and campesterol, which differ from cholesterol only in the degree of saturation of the sterol nucleus or in the nature of the side chain at C24, are less efficiently absorbed than cholesterol (81, 82). Fourth, intestinal cholesterol absorption can be specifically inhibited by cholesterol absorption inhibitors such as

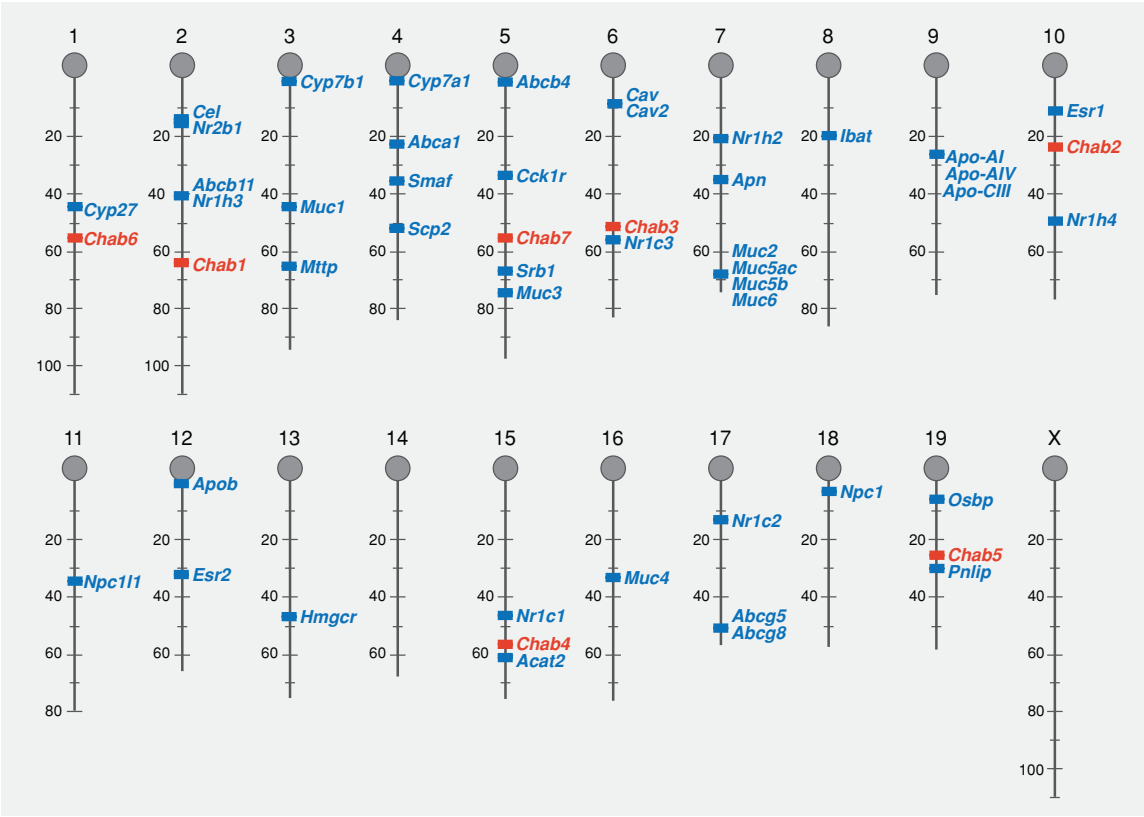

Figure 2

Composite map of the genes for intestinal sterol transporters and lipid metabolism, and of quantitative trait loci (QTLs) for cholesterol absorption (*Chab*) genes, as well as of candidate genes for the regulation of cholesterol absorption on chromosomes representing the entire mouse genome. A vertical line represents each chromosome, with the centromere at the top; genetic distances from the centromere (*horizontal lines*) are indicated to the left of the chromosomes in centimorgans (cM). Chromosomes are drawn to scale, based on the estimated cM position of the most distally mapped locus taken from Mouse Genome Database. The locations of the genes for intestinal sterol transporters and lipid metabolism as well as of candidate genes for the regulation of cholesterol absorption are represented by horizontal blue lines. QTLs (*Chab* genes) are indicated by horizontal red lines with the gene symbols to the right. Abbreviations of genes, followed by the protein encoded: *Acat*, acyl-CoA:cholesterol transferase; *Apn*, aminopeptidase N; *Apo*, apolipoprotein; *Cav*, Caveolin; *Cck1r*, cholecystokinin 1 receptor; *Cel*, carboxyl ester lipase; *Cyp7a1*, cholesterol 7α-hydroxylase; *Cyp7b1*, oxysterol 7α-hydroxylase; *Cyp27*, sterol 27α-hydroxylase; *Esr1*, estrogen receptor α; *Esr2*, estrogen receptor β; *Hmgcr*, HMG-CoA reductase; *Ibat*, ileal bile acid transporter; *Muc*, mucin; *Npc1*, Niemann-Pick C1 (protein); *Nr*, nuclear receptor; *Osbp*, oxysterol-binding protein; *Pnlip*, pancreatic triglyceride lipase; *Scp*, sterol carrier protein; *Smaf*, sphingomyelinase; *Srb*, scavenger receptor class B.

2-azetidinones (83). Finally, transport of cholesterol from mixed micelles or small unilamellar vesicles to brush border membrane vesicles follows second-order kinetics and is sensitive to proteases (84, 85).

Major progress in the search for the intestinal sterol transporters came from the discovery that mutations in the genes encoding human ABCG5 and ABCG8 transporters cause sitosterolemia. Patel et al. (86) first

mapped sitosterolemia to a special gene locus on the human chromosome 2p21, between *D2S2294* and *D2S2298*. Using a microarray analysis of cDNAs from intestines and livers of mice treated with a liver X receptor (LXR) agonist, as well as a positional cloning approach, two groups of scientists (19, 20) independently identified the two adjacent genes *ABCG5* and *ABCG8* encoding transporters expressed in the liver and intestine. Unlike other ABC transporter genes that encode proteins with 12 transmembrane domains, *ABCG5* and *ABCG8* each encode a protein with 6 transmembrane domains, and the heterodimerization of the resulting two proteins to form a 12-transmembrane protein complex is required for transport activity (87).

Patients with sitosterolemia absorb 20–30% of dietary sitosterol intake, in contrast to the typical <5% sitosterol absorption rate in normal individuals (21, 78–80). Interestingly, sitosterolemic patients also absorb a greater fraction of dietary cholesterol and excrete less cholesterol into the bile as compared with normal subjects, resulting in hypercholesterolemia. Indeed, the results from the studies of transgenic and knockout mice as well as in vitro systems (88–90) show that ABCG5 and ABCG8 are localized in the apical brush border membrane of enterocytes and the canalicular membrane of hepatocytes. These transporters may represent an efficient efflux pump system for both cholesterol and plant sterols in the small intestine and liver and may transport sterols out of the cell either back into the intestinal lumen or into the bile, thereby regulating the intestinal absorption and biliary secretion of cholesterol and plant sterols (88–91). Also, several polymorphisms in the *ABCG5* and *ABCG8* genes that may moderately influence plasma sterol levels have been identified (92). These findings explain, in part, why cholesterol absorption is a selective process, in which plant sterols and other noncholesterol sterols are absorbed poorly or not at all. The structure-function relationship between (*a*) cholesterol and plant sterols such as sitosterol, campesterol, and stigmasterol and (*b*) shellfish sterols should be further studied. More recently, researchers found that in inbred strains of mice, there is a negative correlation between the efficiency of cholesterol absorption and the expression levels of ABCG5 and ABCG8 in the jejunum and ileum but not in the duodenum (93). This suggests that under normal physiological conditions, the jejunal and ileal ABCG5 and ABCG8 play a major regulatory role in modulating the amount of cholesterol that is absorbed from the intestine (93).

LXR: liver X receptor

As found in LXRα und LXRβ knockout mice, LXRs are essential for diet-induced upregulation of the *ABCG5* and *ABCG8* genes (94). Also, studies in ABCG5 and ABCG8 double-knockout mice show that stimulation of cholesterol excretion by the nonsteroidal synthetic LXR agonist T0901317 requires intact ABCG5 and ABCG8 transporters (94). These studies suggest that mRNA expression for the *ABCG5* and *ABCG8* genes is activated by dietary cholesterol via LXRs. The observation that incubation with mixed micelles enriched with sitostanol, the Δ^5-saturated homolog of sitosterol, led to an increase in ABCA1 expression, which is also LXR mediated, in Caco-2 cells raises the question of whether plant sterols themselves or plant sterol derivatives have the capability to activate LXRs. LXRs are activated by endogenous oxysterols such as 22(*R*)-hydroxycholesterol, 24(*S*),25-epoxycholesterol, and 24*S*- and 27-hydroxycholesterol. Whereas plant sterols themselves do not exert any relevant LXR activation, several plant sterol derivatives show similar LXR activation capacities as does 24(*S*),25-epoxycholesterol (95). Despite these observations, it is imperative to investigate whether plant sterols, under conditions of high dietary plant sterol feeding, upregulate expression levels of the *ABCG5* and *ABCG8* genes via the LXR pathway. An upregulation of these transporters may promote transport of plant sterols from enterocytes back into the intestinal lumen and function as a gatekeeper to avoid a rapid increase in plasma levels of plant sterols.

SR-BI: scavenger receptor class B type I

The discovery of ezetimibe as the specific and potent inhibitor of intestinal cholesterol absorption has focused attention on a putative sterol influx transporter that may be a target for ezetimibe. The inhibition of cholesterol absorption by ezetimibe is not mediated via changes in either the size or composition of the intestinal bile acid pool or in the mRNA expression levels of *ABCG5*, *ABCG8*, *ABCA1*, and scavenger receptor class B type I (*SR-BI*). Rather, the mechanism of inhibition may involve disruption of the uptake of luminal sterol across the brush border membrane (96). Furthermore, ezetimibe neither inhibits pancreatic lipolytic enzyme activities in the intestinal lumen nor affects bile acid micelle solubilization of cholesterol (97). [^{3}H]-labeled ezetimibe is localized to the brush border membrane of the enterocyte (98) and appears to inhibit directly the activity of the putative brush border membrane transporter(s) that actively mediates the uptake of cholesterol. Using a genomic-bioinformatics approach, Altmann and coworkers (22, 23) identified transcripts containing expression patterns and structural characteristics anticipated in cholesterol transporters (e.g., sterol-sensing and transmembrane domains, extracellular signal peptides), and these researchers established NPC1L1 as a strong candidate for the ezetimibe-sensitive cholesterol transporter. Moreover, ezetimibe-treated mice and mice with targeted inactivation of the *NPC1L1* gene display similar dietary cholesterol absorption characteristics (22), suggesting that NPC1L1 is the ezetimibe-inhibitable cholesterol transporter. The NPC1L1 protein has 50% amino acid homology to NPC1, which is defective in the cholesterol storage disease Niemann-Pick type C and functions in intracellular cholesterol trafficking (99). However, in contrast to *NPC1*, which is expressed in many tissues, *NPC1L1* is expressed predominantly in the intestine, with peak expression in the proximal jejunum (22, 23), which parallels the efficiency of cholesterol absorption along the gastrocolic axis. Subfractionation of the brush border membrane suggests that NPC1L1 is associated with the apical membrane fraction of enterocytes. A rat homolog of the human *NPC1L1* gene has been found to be the only gene that encodes a protein that contains an extracellular signal peptide, transmembrane sequences, N-linked glycosylation sites, and a sterol-sensing domain (100). More recently, investigators discovered that the binding affinities of ezetimibe and several key analogs to recombinant NPC1L1 are virtually identical to those observed for native enterocyte membranes and that ezetimibe no longer binds to membranes from NPC1L1 knockout mice, suggesting that NPC1L1 is the direct molecular target of ezetimibe (101, 102).

However, preliminary attempts to reconstitute cholesterol transport activity in nonenterocyte cells by overexpression of NPC1L1 have been unsuccessful; thus, additional proteins may be required to reconstitute a fully functional cholesterol transporter. In particular, these may include caveolin 1, which can form a heterocomplex with annexin 2 (and cyclophilins) in zebrafish and mouse intestines (103). A stable 55-kDa complex of annexin 2 and caveolin 1 seems involved in intracellular sterol trafficking. The resistance of the intestinal caveolin 1–annexin 2 heterocomplex to boiling in sodium dodecyl sulfate, reducing conditions, and ether extraction implies that a covalent interaction other than disulfide cross-linking is involved in formation of the complex (103). Moreover, incubation with ezetimibe leads to complete disruption of the caveolin 1–annexin 2 complex in the early state of zebrafish embryos. Pharmacological treatment of mice with ezetimibe disrupts the complex only in hypercholesterolemic mice, as induced by a high-cholesterol and high-fat diet or by LDL-receptor gene knockout (103), indicating that the caveolin 1 heterocomplexes may represent additional ezetimibe targets that regulate intestinal cholesterol transport. The immunoprecipitation experiments suggest that ezetimibe disrupts the caveolin 1–annexin 2 complex most likely by direct interaction with

the caveolin 1 protein. Nevertheless, the exact molecular mechanism by which NPC1L1 regulates cholesterol absorption remains to be defined.

Over the past decade, several groups have been searching for cholesterol transporters that are located at the apical brush border membrane of the enterocyte (104–107). Kramer and colleagues (108) investigated potential target structures of ezetimibe with the help of photoreactive derivatives of 2-azetidinones and a photoreactive cholesterol derivative photocholesterol, i.e., [3α-^{3}H]-6-azi-5α-cholestan-3β-ol. They identified an 80-kDa and a 145-kDa integral membrane protein as putative components of the intestinal cholesterol transporters (108). They found that the photoreactive analog of ezetimibe binds to another 145-kDa integral membrane protein. The 80-kDa cholesterol-binding protein does not interact with cholesterol absorption inhibitors and vice versa, and neither cholesterol nor plant sterols interfere with the 145-kDa molecular target for cholesterol absorption inhibitors (108). Interestingly, there is no competition in the binding of the 2-azetidinone at the 145-kDa protein with cholesterol. However, binding of photocholesterol to the 80-kDa cholesterol-binding protein may be inhibited in a concentration-dependent manner by cholesterol, sitosterol, and campesterol, whereas cholesterol absorption inhibitors, such as ezetimibe, have no influence on the binding of photocholesterol to the putative 80-kDa cholesterol-binding protein. Both proteins are different from the above-described candidate proteins for the intestinal cholesterol transporters (ABCA1, ABCG5, ABCG8, NPC1L1, and SR-BI). More recently, the 145-kDa ezetimibe-binding protein was purified by three different methods, and the protein sequencing reveals its identity with the membrane-bound ectoenzyme aminopeptidase N (APN) (109, 110). Because APN has a role in endocytotic processes, binding of ezetimibe to APN may block endocytosis of cholesterol-rich membrane microdomains, thereby inhibiting intestinal cholesterol absorption (109, 110). However, researchers have yet to pinpoint the exact mechanism whereby APN influences intestinal cholesterol absorption.

APN: aminopeptidase N

Immunoblotting data show that the SR-BI protein is expressed in brush border membrane preparations and in Caco-2 cells, and preincubation with an anti-SR-BI antibody partially inhibits cholesterol and cholesteryl ester uptake by brush border membrane vesicles and Caco-2 cells, in contrast to results in control incubations without antibody (111). These in vitro experiments suggest that SR-BI is a cholesterol transporter in the intestine and involved in the absorption of dietary cholesterol. The distribution of SR-BI along the gastrocolic axis and on the apical membrane of the enterocyte is also consistent with its participation in cholesterol absorption (112). However, targeted disruption of the *SR-BI* gene appears to have little effect on intestinal cholesterol absorption in mice (113–115). More importantly, the cholesterol absorption inhibitor ezetimibe, which labels SR-BI in the enterocyte, also inhibits cholesterol absorption in *SR-BI* knockout mice (114), suggesting that *SR-BI* cannot be the ezetimibe-sensitive target gene responsible for intestinal cholesterol absorption.

Some nuclear hormone receptor RXR and LXR agonists upregulate ABCA1 expression levels, with a concomitant decrease in cholesterol absorption (116), suggesting that the intestinal ABCA1 transporter serves to efflux cholesterol from the enterocyte back into the intestinal lumen for excretion. However, there are controversial results from the ABCA1 knockout mouse studies: One study shows only a marginal increase in cholesterol absorption (117), whereas another study shows decreased cholesterol absorption (118). Subsequent characterization of mice expressing no ABCA1 (i.e., *ABCA1*-deficient mice) (119) and of the Wisconsin hypoalpha mutant (WHAM) chicken with spontaneously occurring ABCA1 dysfunction (120, 121) revealed no impairment in percent cholesterol absorption, fecal neutral steroid excretion, or

biliary cholesterol secretion, even after challenge with a synthetic LXR agonist. Furthermore, in situ hybridization techniques revealed that ABCA1 is predominantly in cells present in the lamina propria in mice (94) and occasionally in the enterocyte in the primate (122), which is inconsistent with the view that it plays a major role in cholesterol absorption. ABCA1 may be involved in the transfer of cholesterol from enterocytes into lymph and/or to blood macrophages and promote efficient cholesterol efflux from enterocytes to plasma HDL, but additional studies are required to clarify these observations.

Besides ABCG5, ABCG8, and NPC1L1, other sterol transporters in the intestine may be involved in the regulation of intestinal cholesterol absorption, although these transporters have only been proposed and not identified. It will be important to investigate the molecular and genetic mechanisms underlying the dominant rate-limiting step/factor in intestinal cholesterol absorption.

INHIBITORS OF INTESTINAL CHOLESTEROL ABSORPTION

The use of cholesterol absorption inhibitors for treating hypercholesterolemia has a long history, and several classes of compounds have been established and developed. Here we focus discussion on plant sterols and plant stanols (phytosterols) and ezetimibe, as well as their inhibitory actions on intestinal cholesterol absorption, because they markedly lower plasma total and LDL cholesterol levels in humans.

Plant Sterols and Stanols (Phytosterols)

Plant sterols are naturally occurring sterols and structurally related to cholesterol. Their chemical structure is very similar to that of cholesterol, with a Δ^5 double bond and a 3β-hydroxyl group but with structural modifications of the side chain. Plant sterols have the same basic importance in plants as cholesterol in animals; i.e., they play a crucial role in cell membrane function. Campesterol and sitosterol, the 24-methyl and the 24-ethyl analog of cholesterol, respectively, are the most abundant plant sterols, and they are found at low concentrations in human plasma. They are part of the diet and are exclusively taken up from the intestine. Over the past decade, plant sterols as ingredients in functional foods have been found to reduce plasma cholesterol levels (7, 8, 123). The effective doses are 1.5–3 g per day, which leads to a 8–16% reduction in plasma LDL cholesterol concentrations. The dietary intake of cholesterol and plant sterols is almost equal, but plant sterols are absorbed poorly or not at all. For example, the absorption efficiencies of sitosterol and campesterol are 5–8% and 9–18%, respectively (124). Most of the plant sterols that do enter the enterocyte most likely are pumped back rapidly into the intestinal lumen for excretion. In addition to poor net absorption, researchers have observed rapid biliary excretion of plant sterols. These mechanisms keep plasma plant sterol levels to <1 mg dl^{-1} in humans. Furthermore, plant sterols are poorly soluble in aqueous systems and require formulation for bioactivity. To increase their lipid solubility, it is imperative to esterify plant sterols and to dissolve them at high concentrations in the triglyceride phase of margarines (125). Cholesterol absorption from dietary and biliary sources is significantly reduced in the presence of plant sterols, and consequently the unabsorbed cholesterol excreted in the feces is increased markedly. The commonly accepted basic mechanisms of inhibitory action of these compounds are that plant sterols can become efficiently incorporated into micelles in the intestinal lumen, displace the cholesterol, and lead to its precipitation with other, nonsolubilized plant sterols (126–129). Moreover, competition between cholesterol and plant sterols for incorporation into micelles and for transfer into the brush border membrane, as well as competition within the enterocyte for ACAT, may partly explain the inhibitory effect of large amounts of plant sterols on cholesterol

absorption. This process reduces both hepatic cholesterol and triglyceride contents, which is compensated for by two different mechanisms: an increase in cholesterol synthesis and an increase in LDL receptor levels. In contrast, Δ^{22}-sterols (stigmasterol) markedly reduce cholesterol synthesis via competitive inhibition of sterol Δ^{24}-reductase, which is an interesting secondary mechanism for future research (130).

Ezetimibe

Ezetimibe (SCH 58235), 1-(4-fluorophenyl)-(3*R*)-[3-(4-fluorophenyl)-(3*S*)-hydroxypropyl]-(4*S*)-(4-hydroxyphenyl)-2-azetidinone, and an analog, SCH 48461, (3*R*)-(3-phenylpropyl)-1,(4*S*)-bis(4-methoxyphenyl)-2-azetidinone, are highly selective intestinal cholesterol absorption inhibitors that effectively and potently prevent the absorption of cholesterol by inhibiting the uptake of dietary and biliary cholesterol across the brush border membrane of the enterocyte. The high potency of these compounds is reflected by a 50% inhibition dose of 0.0005 mg kg^{-1} and 0.05 mg kg^{-1} in a series of different animal models (98, 131). Following oral administration, ezetimibe undergoes rapid glucuronidation in the enterocyte during its first pass (131–133). Both ezetimibe and its glucuronide are circulated enterohepatically and repeatedly delivered back to the site of action in the intestine, resulting in multiple peaks of the drug and accounting for an elimination half-life of approximately 22 hours (134). This may explain why ezetimibe displays a longer duration of action and the effort of treatment persists for several days after its cessation; thus, once-daily dosing should be sufficient for an adequate therapeutic effect. Furthermore, after oral administration of the glucuronide (SCH-60663), >95% of the compound remains in the intestine (131). That the glucuronide is more potent than ezetimibe in inhibiting cholesterol absorption confirms that ezetimibe acts directly in the intestine, as does glucuronide (131). Because ezetimibe and its analogs are relatively small molecular structures, they do not change the physical-chemical nature of the intraluminal environment, nor do they affect the enterohepatic flux of bile acids. Additionally, ezetimibe does not affect absorption of triglycerides, fatty acids, bile acids, or fat-soluble vitamins, including vitamins A, D, and E and α- and β-carotenes.

In addition, during ezetimibe treatment, there is a marked compensatory increase in cholesterol synthesis in the liver, but not in the peripheral organs, and an accelerated loss of cholesterol in the feces, with little or no change in the rate of conversion of cholesterol to bile acids. Thus, the combination of ezetimibe with HMG-CoA reductase inhibitors such as atorvastatin or simvastatin is a powerful new therapeutic approach with which to control plasma LDL cholesterol levels in the general population as well as to provide a complementary treatment strategy for high-risk patients, e.g., patients with primary hypercholesterolemia (15–18, 135, 136), homozygous familial hypercholesterolemia (137), or sitosterolemia (138).

Among the most frequently reported adverse events of combination therapy of ezetimibe and statins are elevated plasma aminotransferase and γ-glutamyltransferase activities (131). The Federal Drug Agency in Germany has recently released a cautious note on ezetimibe with respect to the number of myopathies and increases of liver enzymes reported (at least 15 patients, most of whom were on combination therapy with statins) (139). Because systemic concentrations of ezetimibe are maintained continually during treatment, the side effects of ezetimibe need further investigation in patients undergoing long-term therapy before this regimen is widely recommended. In addition, on the basis of its mechanism of action, ezetimibe may provide additional reduction in plasma total and LDL cholesterol concentrations when used in combination with fibrate, niacin, or bile acid–binding resins, but clinical trial data are not yet available. Because

intestinal cholesterol absorption shows a wide variation in the general population, it may be reasonable to distinguish prospective responders from nonresponders to therapy with ezetimibe so as to optimize cholesterol-lowering therapy in the future.

CONCLUSIONS

A strong genetic factor in the regulation of intestinal cholesterol absorption has been established, indicating that intestinal cholesterol absorption is a complex and a multistep process that is regulated by multiple genes at the enterocyte level. Also, this process provides multiple therapeutic targets for preventing the absorption of cholesterol from dietary and biliary sources by suppressing uptake and transport of cholesterol through the enterocytes. A better understanding of the molecular genetics of intestinal cholesterol absorption will lead to novel approaches for the prevention and treatment of hypercholesterolemia.

SUMMARY POINTS

1. There are interindividual differences and interstrain variations in intestinal cholesterol absorption efficiency in humans and other animals. Genetic factors at the enterocyte level are crucial in determining the variations of intestinal cholesterol absorption efficiency, and high cholesterol absorption is a dominant trait.
2. The ABCG5 and ABCG8 transporters represent apical sterol export pumps that promote active efflux of cholesterol and plant sterols from enterocytes back into the intestinal lumen for excretion. This explains why cholesterol absorption is a selective process; plant sterols and other noncholesterol sterols are absorbed poorly or not at all.
3. The complete insensitivity of *Npc1l1*-deficient mice to ezetimibe indicates that NPC1L1 plays a critical role in the ezetimibe-sensitive cholesterol absorption pathway.
4. Cholesterol absorption is a multistep process that is regulated by multiple genes at the enterocyte level (**Figure 1**). Any factors that change the transportation of cholesterol from the intestinal lumen to the lymph can influence the efficiency of intestinal cholesterol absorption (**Table 1** and **Figure 2**).
5. The absorption efficiency of cholesterol is most likely determined by the net effect between influx and efflux of intraluminal cholesterol molecules across the brush border of the enterocyte.
6. Combination therapy using a novel, specific, and potent cholesterol absorption (NPC1L1) inhibitor (ezetimibe) and HMG-CoA reductase inhibitors (statins) offers an efficacious new approach to the prevention and treatment of hypercholesterolemia.
7. The detergency of bile acids through micellar solubilization of intraluminal sterols is obligatory for intestinal cholesterol absorption.
8. Plant sterols reduce cholesterol absorption by displacing cholesterol from micelles, primarily because they have higher affinity to micelles than does cholesterol.

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