Development and Physiological Regulation of Intestinal Lipid Absorption.

III. Intestinal transporters and cholesterol absorption

Hui DY, Labonte ED, Howles PN. Development and Physiological Regulation of Intestinal Lipid Absorption. III. Intestinal transporters and cholesterol absorption. Am J Physiol Gastrointest Liver Physiol 294: G839–G843, 2008. First published February 14, 2008; doi:10.1152/ajpgi.00061.2008.—Intestinal cholesterol absorption is modulated by transport proteins in enterocytes. Cholesterol uptake from intestinal lumen requires several proteins on apical brush-border membranes, including Niemann-Pick C1-like 1 (NPC1L1), scavenger receptor B-I, and CD36, whereas two ATP-binding cassette half transporters, ABCG5 and ABCG8, on apical membranes work together for cholesterol efflux back to the intestinal lumen to limit cholesterol absorption. NPC1L1 is essential for cholesterol absorption, but its function as a cell surface transporter or an intracellular cholesterol absorption protein needs clarification. Another ATP transporter, ABCA1, is present in the basolateral membrane to mediate HDL secretion from enterocytes.

Elevated plasma cholesterol level is a well-established risk factor of atherosclerosis. Major contributors to the amount of cholesterol in plasma include exogenous cholesterol absorbed through the gastrointestinal tract and endogenously synthesized cholesterol. As much as 60% of the cholesterol entering the body each day is derived from the diet. In addition, the amount of dietary cholesterol absorbed also influences endogenous cholesterol biosynthesis in the liver. Dietary influence on plasma cholesterol and LDL levels is extremely variable in the general population, suggesting that genetic and environmental factors are involved. One difference in individual response to a specific dietary challenge is the amount of dietary cholesterol absorbed through the gastrointestinal tract. These individual differences may be due to polymorphisms in genes that participate in the cholesterol absorption pathway.

Protein-Mediated Cholesterol Absorption in the Intestine

There are two major hypotheses on the mechanism by which cholesterol in the intestinal lumen is taken up through the brush-border membranes of the intestinal mucosa. One long-standing hypothesis suggests that cholesterol absorption is an energy-independent passive diffusion process in which micellar cholesterol in the lumen is in equilibrium with monomolecular cholesterol in solution, and the monomeric cholesterol is absorbed to the brush-border membrane down a concentration gradient. More recent data suggest that cholesterol absorption is a protein-mediated process. In support of the latter hypothesis is the observation that cholesterol uptake by brush-border membranes in vitro follows a second-order reaction kinetics and that the reaction reverts to a low-affinity first-order kinetics mechanism upon proteolytic digestion of proteins on the surface of the brush-border membranes (20). The recent discovery of inhibitors selectively blocking cholesterol absorption at very low dosage and their binding to intestinal mucosa in a specific and saturable manner is supportive of the protein-mediated cholesterol absorption hypothesis (12).

Ezetimibe Inhibition of Cholesterol Absorption

One of the most significant advances in lipid-lowering therapy in recent years is the development of the cholesterol absorption inhibitor ezetimibe (trade name Zetia). Ezetimibe is absorbed and modified by uridine 5-diphosphate-glucuronosyl transferases in the intestine and liver. The glucuronidated form of ezetimibe is excreted in the bile and delivered to the intestinal lumen where it can inhibit cholesterol absorption. The glucuronidated form of ezetimibe is more potent than the native unmodified drug in inhibiting cholesterol absorption by binding more avidly to enterocyte brush-border membranes. Ezetimibe does not affect fat-soluble vitamin absorption but specifically inhibits the absorption of cholesterol and other sterols into enterocytes.

It is interesting to note that several clinical trials in humans showed a wide range of interindividual variation in cholesterol-lowering response to ezetimibe. Patients who are hyperresponders to statin therapy are typically hyperresponders to ezetimibe treatment. Since hyperresponders to statin therapy are thought to be hyperresponders to dietary cholesterol, this observation suggests that intestinal proteins and genetic factors that are responsible for mediating cholesterol absorption by intestinal cells may be the ezetimibe target(s).

NPC1L1 in Intestinal Cholesterol Absorption

Considerable effort has been expended over the past several years on identifying the cholesterol transporter(s) and ezetimibe target(s) in enterocyte brush-border membranes. A leading candidate was identified with the use of a genomic/bioinformatics approach with the assumption that the cholesterol transporter should be a membrane-bound protein expressed in jejunal enterocytes and should contain a sterol-binding motif (2). This protein, called Niemann-Pick C1-like 1 protein (NPC1L1), has the typical features of a membrane-bound protein with a signal peptide sequence, 13 predicted transmembrane domains, and extensive N-linked glycosylation sites in the extracellular loop. It also contains a sterol-sensing domain, and its expression in intestine parallels the efficiency...
of cholesterol absorption along the gastrocolic axis, with the highest level of NPC1L1 expression and cholesterol absorption observed in the proximal intestine and minimal NPC1L1 expression and cholesterol absorption in the ileum. Furthermore, in situ hybridization and immunohistochemistry analysis of the jejunum for NPC1L1 mRNA and protein expression reveal discrete NPC1L1 localization to the epithelial layer bordering the luminal space along the crypt-villus axis (2). These observations are consistent with the possibility that NPC1L1 may participate in cholesterol absorption at the brush-border membrane.

Direct evidence documenting the importance of NPC1L1 in cholesterol absorption was obtained in experiments showing that ablating this gene in mice reduces cholesterol absorption by $>70\%$ (2). Ezetimibe treatment of $Npc1l1^{-/-}$ mice did not result in further reduction of cholesterol absorption (2). In humans, multiple sequence variations in NPC1L1 have been found and correlated to plasma cholesterol response to ezetimibe therapy (6, 11, 19). Taken together, both mouse and human data established that NPC1L1 participates in the ezetimibe-sensitive cholesterol absorption pathway.

The mechanism by which NPC1L1 participates in cholesterol absorption is controversial. One study used immunohistochemical techniques to localize NPC1L1 to the brush-border membranes of the enterocytes to suggest its role as the cholesterol transporter on the cell surface responsible for mediating cellular cholesterol uptake (2). Subsequently, the same group of investigators transfected Chinese hamster ovary (CHO) cells with FLAG epitope-tagged rat NPC1L1 cDNA and demonstrated the cell surface location of the FLAG epitope-tagged protein (13). However, the latter study did not reveal whether CHO cells expressing the FLAG-tagged NPC1L1 on the cell surface displayed increased cholesterol transport characteristics compared with nontransfected cells. Previous studies from the same group have failed to demonstrate increased cholesterol transport activities in cells transfected with nonepitope-tagged NPC1L1 (2). An independent study by Ioannou and colleagues (7) found NPC1L1 residing in a subcellular vesicular compartment but not in the plasma membrane. These investigators presented two independent studies to confirm the mostly intracellular instead of cell surface location of NPC1L1. First, Yu et al. (23) have localized NPC1L1 to intracellular vesicular compartments. Second, Ioannou and colleagues (7) showed that NPC1L1 is associated with vesicular compartments rich in the small GTPase Rab5 protein. The well-documented role of Rab GTPases in regulation of intracellular vesicle fusion and transport clearly suggests that NPC1L1 is a protein important for shuffling cholesterol newly acquired from extracellular milieu to the cell interior where it can be processed and packaged into lipoproteins for secretion into the circulation. Third, cholesterol uptake by the enterocyte-like Caco-2 cells was only moderately reduced (by $\sim 20\%$) by NPC1L1 small interfering (si)RNA (18). Finally, in a more definitive experiment, Field et al. (9) showed that incubating Caco-2 cells with ezetimibe caused only a modest decrease in uptake of micellar cholesterol from the apical side but markedly suppressed its esterification. Their data also revealed that cholesterol trafficking from the plasma membrane to the endoplasmic reticulum was dramatically reduced and plasma cholesterol concentration in the apical membrane was concomitantly elevated (9). In view of previous studies showing that cholesterol absorption is a two-step process, initially involving the partitioning of cholesterol from micelles in the intestinal lumen to the apical membrane of enterocytes, followed by intracellular transfer of cholesterol from the plasma membrane to an intracellular compartment for esterification and packaging into chylomicrons, the Field study clearly indicates that NPC1L1 participates in the second step of the cholesterol absorption process, namely the transfer of cholesterol from the apical plasma membrane to the endoplasmic reticulum. This model implies that ezetimibe inhibition of NPC1L1 function decreases esterification of plasma membrane cholesterol, thereby decreasing the amount of cholesterol available for packaging into chylomicrons for secretion into the lymph circulation.

Scavenger Receptor B-I in Intestinal Cholesterol Absorption

The observation that NPC1L1-transfected cells displayed high-affinity binding to ezetimibe suggests that NPC1L1 expression may induce the expression and/or translocation of another ezetimibe-binding cholesterol transport protein to the plasma membrane. A potential candidate is scavenger receptor B-I (SR-BI). This protein is highly expressed in the apical side of proximal small intestine villus, and neutralizing antibodies against SR-BI abolished high-affinity binding of cholesterol to brush-border membrane vesicles from both wild-type and $Npc1l1^{-/-}$ mice (15). High-affinity cholesterol binding to these brush-border membrane vesicles is inhibited by ezetimibe treatment in vivo and in vitro (15). The ability of both SR-BI antibodies and ezetimibe to inhibit high-affinity binding to brush-border membrane vesicles prepared from the intestine of $Npc1l1^{-/-}$ mice displayed similar high-affinity cholesterol binding (15) and transport properties (14) as brush-border membrane vesicles from intestine of wild-type mice. These latter observations suggest that, although NPC1L1 is a necessary participant in the cholesterol absorption pathway and may be present in brush-border membrane under cholesterol-deprived conditions, it does not function as a typical cell surface transporter for cholesterol uptake.
cholesterol binding and transport to brush-border membrane vesicles suggests that ezetimibe interacts directly with SR-BI. This conclusion was supported by an earlier study using photoaffinity-labeled ezetimibe to identify potential targets, which revealed a direct interaction between ezetimibe and SR-BI (1). Ezetimibe treatment or siRNA knockdown of NPC1L1 expression in Caco-2 cells resulted in decreased expression of SR-BI (8, 18), suggesting that SR-BI may be involved in the ezetimibe-sensitive NPC1L1-mediated cholesterol absorption pathway. The participation of SR-BI in cholesterol absorption was suggested by experiments showing that SR-BI cDNA-transfected cells displayed increased cholesterol uptake from micellar substrates compared with mock-transfected cells with low SR-BI expression (1, 22). Moreover, the increase in cholesterol uptake by SR-BI-transfected cells was sensitive to ezetimibe inhibition in both of these studies (1, 22).

Despite the strong evidence suggesting that SR-BI may participate in cholesterol absorption as an ezetimibe-sensitive cholesterol transporter on the surface of brush-border membranes, its role in cholesterol absorption in a physiological setting remains controversial. The controversy stems from studies showing that the Scarb1−/− mice, with targeted ablation of the SR-BI gene, absorbed cholesterol efficiently and in an ezetimibe-sensitive manner similar to wild-type mice (2). Interestingly, cholesterol absorption is increased in transgenic mice with SR-BI-specific overexpression in the intestine (5). These disparate results may be interpreted collectively to indicate that SR-BI may participate in the initial step of cholesterol absorption as a high-affinity cholesterol-binding protein in the brush-border membrane and that alternative cholesterol transporter(s) may compensate for the absence of SR-BI in mediating cholesterol absorption. This interpretation is consistent with the recent report demonstrating the presence of multiple high-affinity and ezetimibe-sensitive cholesterol receptors in intestinal brush-border membranes (14). These receptors are distinct from NPC1L1, thus implying that SR-BI, the compensatory transport protein(s), and NPC1L1 are all participants in the ezetimibe-sensitive cholesterol absorption pathway. Moreover, the effectiveness of ezetimibe in inhibiting cholesterol absorption in the Scarb1−/− mice, together with the observation of direct interaction and inhibition of SR-BI, suggests the presence of multiple ezetimibe targets in the cholesterol absorption pathway. A schematic diagram depicting this hypothesis is illustrated in Fig. 1.

Role of CD36 in Intestinal Cholesterol Absorption

A second protein that has been implicated to function as the cholesterol transporter in brush-border membranes is the fatty acid translocase CD36. Like SR-BI, CD36 is expressed in numerous tissues and organs where it facilitates cellular uptake of long-chain fatty acids. In humans and mice, CD36 is also expressed in the epithelial cells of the small intestine along the gastrocolic and crypt-to-villus axes, similar to the expression of genes important for fat and cholesterol absorption and transport. The importance of CD36 in fatty acid absorption is well established, with results showing that CD36 deficiency leads to abnormal lipid processing in enterocytes. The potential role of CD36 in cholesterol absorption was implicated in studies showing enhanced cholesterol uptake from micellar substrates by CD36-transfected COS-7 cells compared with that observed in mock-transfected cells (22). The CD36-mediated cholesterol uptake properties in the transfected cells are similar to that observed with SR-BI-transfected cells as well as those observed with brush-border membrane vesicles prepared from wild-type mice (22). The importance of CD36 in mediating cholesterol absorption was demonstrated recently in experiments showing significant reduction of cholesterol trans-
port from the intestinal lumen to the lymph in CD36-null mice (17). Interestingly, the CD36-facilitated cholesterol uptake process is similar to that observed for SR-BI-mediated cholesterol uptake in their sensitivity to ezetimibe inhibition (22). Taken together, these results implied that both SR-BI and CD36 are potential ezetimibe-sensitive cholesterol transporters in the gastrointestinal tract.

In an effort to differentiate the significance of both SR-BI and CD36 and CD36 expression in the cholesterol absorption process, van Bennekum et al. (22) examined cholesterol uptake properties of brush-border membrane vesicles prepared from proximal and distal intestines of wild-type and Scarb1−/− mice. Their interesting results showed that cholesterol uptake by proximal intestinal membrane vesicles (duodenum and proximal jejunum) are highly dependent on SR-BI expression, whereas cholesterol uptake by distal membrane vesicles is similar between wild-type and Scarb1−/− mice. Importantly, cholesterol uptake by the distal brush-border membrane vesicles from both wild-type and Scarb1−/− mice is sensitive to inhibition by CD36-neutralizing antibodies (22). These results suggest that, at least in rodents, SR-BI is important for cholesterol absorption in the duodenum and proximal jejunum, whereas CD36 is the protein that mediates cholesterol absorption in distal jejunum and ileum. Moreover, these results, along with observations of normal cholesterol absorption efficiency observed in the Scarb1−/− mice, suggest the possibility that the presence of CD36 in the distal intestine is sufficient to compensate for the lack of SR-BI in mediating cholesterol absorption in Scarb1−/− mice. This possibility can be examined by determining cholesterol absorption in SR-BI and CD36 double knockout mice.

Role of ABC Transporters-Mediated Efflux in Cholesterol Absorption

In addition to the cholesterol transport proteins on the brush-border membranes that facilitate cholesterol uptake into enterocytes, cholesterol absorption is also regulated by a group of ATP-binding cassette (ABC) proteins located on membranes of enterocytes where they function to export cholesterol out of the cells. The ABCA1 protein is located on the basolateral surface of intestinal cells, indicating that this protein does not have a direct role in cholesterol absorption from the intestinal lumen. Current literature indicates that ABCA1 expression in the basolateral membrane is crucial for intestinal secretion of HDL, which accounts for ~30% of HDL production in the body (3). It is important to note, however, that ABCA1 is present in other tissues and promotes reverse cholesterol transport to the liver for biliary excretion (21). Therefore, ABCA1 may indirectly impact cholesterol absorption through modulation of lipid composition in bile and in the intestinal lumen, as evident by the moderately lower cholesterol absorption in abca1−/− mice.

Two ABC half transporters, ABCG5 and ABCG8, are present in apical membranes of enterocytes and have been identified to participate in regulation of cholesterol absorption on the basis of studies of mutations affecting sitosterolemia and cholesterol absorption efficiency. Expression of ABCG5/ABCG8 is highest in the duodenum and jejunum compared with the ileum and limits the amount of cholesterol absorbed by excreting excess cholesterol back to the lumen (16). The importance of ABCG5/ABCG8 in regulation of cholesterol absorption is evident by experiments showing elevated cholesterol absorption in abcg5/abcg8-null mice and reduced cholesterol absorption in transgenic mice overexpressing the human ABCG5/ABCG8 gene (24, 25). Mutations that affect the normal function of ABCG5 and ABCG8 in humans enhance cholesterol absorption, and these patients are predisposed to atherosclerosis (4, 16).

Summary

Cholesterol absorption from intestinal lumen into enterocytes and secretion to plasma circulation is a complex process involving transporters on the apical surface of brush-border membranes that regulate the amount of cholesterol uptake, intracellular trafficking proteins to transport cholesterol from the plasma membrane to intracellular compartments where it can be packaged into lipoproteins, and transporters on the basolateral membrane for lipoprotein secretion. An essential role for NPC1L1 in cholesterol absorption is well established, although its function as a transporter or an intracellular cholesterol-trafficking protein needs to be clarified. Although the identity of the apical transporter(s) responsible for cholesterol uptake is still highly debated in the community, the current data suggest that multiple transporters are involved. The apical transporter responsible for cholesterol efflux back to the intestinal lumen in limiting excess cholesterol uptake is better defined and involves the coordinated activity of the two ABC half transporters, ABCG5 and ABCG8. The definitive identification of cholesterol transporters on apical brush-border membranes and defining the role of NPC1L1 in the cholesterol absorption process will be fruitful for the improvement on treatment strategies and to identify additional targets to suppress cholesterol absorption in reducing hypercholesterolemia and lowering the risk of cardiovascular disease.

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