Dietary cholesterol induces trafficking of intestinal Niemann-Pick Type C1 Like 1 from the brush border to endosomes

Marianne Skov, Carina K. Tønnesen, Gert H. Hansen, and E. Michael Danielsen

Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen, Denmark

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Skov M, Tønnesen CK, Hansen GH, Danielsen EM. Dietary cholesterol induces trafficking of intestinal Niemann-Pick Type C1 Like 1 from the brush border to endosomes. Am J Physiol Gastrointest Liver Physiol 300: G33–G40, 2011. First published November 4, 2010; doi:10.1152/ajpgi.00344.2010.—The transmembrane protein Niemann-Pick C1 Like 1 (NPC1L1) belongs to the Niemann-Pick C1 (NPC1) family of cholesterol transporters and is mainly expressed in the liver and the small intestine. NPC1L1 is believed to be the main transporter responsible for the absorption of dietary cholesterol. Like NPC1, NPC1L1 contains a sterol sensing domain, suggesting that it might be sensitive to dietary cholesterol. To test this hypothesis, mucosal explants were cultured in the presence or absence of cholesterol. In the absence of cholesterol NPC1L1 was localized mainly in the brush border of the enterocyte, colocalizing with the brush border enzyme aminopeptidase N (APN), and only a minor part was present in intracellular compartments. In contrast, following culture in the presence of cholesterol a major part of NPC1L1 was found in intracellular compartments positive for the early endosomal marker early endosome antigen 1, whereas only a minor fraction was left in the brush border. Neither APN, lactase, nor sucrase-isomaltase was endocytosed in parallel, demonstrating that this is a selective cholesterol-induced endocytosis of NPC1L1. Conceivably either the induced internalization could be due to NPC1L1 acting as an endocytic cholesterol receptor or it could be a mechanism to reduce the cholesterol uptake. The fluorescent cholesterol analog NBD-cholesterol readily labeled the cytoplasm also under conditions nonpermissible for endocytosis, arguing against a receptor-mediated uptake. We therefore propose that cholesterol is absorbed by NPC1L1 acting as a membrane transporter and that NPC1L1 is internalized to an endosomal compartment to reduce the absorption of cholesterol.

enterocyte; lipid raft; NBD-cholesterol; transporter

ABSORPTION OF DIETARY CHOLESTEROL in the proximal part of the small intestine plays a major role in the overall body cholesterol homeostasis. Previously it was believed that dietary cholesterol diffused passively across the brush border membrane (22). However, since the uptake of cholesterol is more efficient than that of the structurally similar phytosterols (34), and since genetic variations are thought to underlie the individual differences in cholesterol absorption, the process is now believed to be protein mediated (47). During the last decade several proteins have been proposed to be responsible for the cholesterol uptake. One of the candidates, scavenger receptor B1 (SR-B1), was proposed because less cholesterol was taken into brush border membrane vesicles derived from SR-B1 knockout mice (46). Another member of the class B scavenger receptors, cluster determinant 36 (CD36), has been implied because less cholesterol is taken up by enterocytes in CD36 knockout mice (32). However, recently it was proposed that SR-B1 and CD36-mediated uptake of cholesterol only becomes limiting during high-cholesterol diets (33). To further identify proteins that could be involved in cholesterol uptake a jejunal cDNA library was screened for features expected of a cholesterol transporter and Niemann-Pick C1 Like 1 (NPC1L1) emerged as the only credible candidate. Moreover, NPC1L1 knockout mice exhibited a significant reduction of cholesterol absorption (1). The subsequent identification of NPC1L1 as the target of the cholesterol absorption inhibitor ezetimibe (13, 15, 50) has confirmed its role in uptake of cholesterol, and today it is considered the main player in intestinal cholesterol absorption.

NPC1L1 belongs to the NPC1 family of lipid transport facilitators and shares 51% amino acid similarity with NPC1 (11). Both are membrane proteins containing 13 putative transmembrane domains, 5 of which are similar to the sterol sensing domain (SSD) found in, for example, 3-hydroxy-3 methylglutaryl coenzyme A reductase (HMG CoA reductase), the rate-controlling enzyme in the cholesterol synthesis (9–11, 48). NPC1 is a ubiquitously expressed protein involved in egress of cholesterol from the lysosomes to the endoplasmic reticulum and the plasma membrane (6). NPC1L1 on the other hand is mainly expressed in the liver and small intestine (1). NPC1 and NPC1L1 have similar membrane topology (48), and the binding site for cholesterol in NPC1 has been mapped to the NH2-terminal luminal loop 1 (21, 26). The binding site for cholesterol in NPC1L1 has yet to be mapped, but, because of the high degree of homology to NPC1, it is expected to reside in the luminal oriented NH2-terminal. Ezetimibe has been found to bind to a region between the transmembrane helix 2 and 3 at the luminal surface of the enterocyte (50).

Although NPC1L1 has been reported to reside primarily in the apical membrane of rate (1, 23) and human enterocytes (38), intracellular localization of NPC1L1 has been observed in a hepatoma cell line (12). Furthermore, by use of enhanced green fluorescent protein-tagged NPC1L1, the transporter has been shown to traffic between the plasma membrane and intracellular compartments in rat hepatoma cells following cholesterol depletion by methyl-β-cyclodextrin (36, 52). In hepatoma cells NPC1L1 was proposed to be internalized via clathrin-coated pits, and binding of ezetimibe blocks both the endocytosis of NPC1L1 as well as the uptake of cholesterol, suggesting that cholesterol is internalized by NPC1L1 via a receptor-mediated mechanism (16).

In the present work we studied the cholesterol dependency of the localization of NPC1L1 in the enterocyte as well as the uptake of 22-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-23, 24-bisnor-5-cholen-3β-ol (NBD-cholesterol), a fluorescent cholesterol analog, using a mucosal explant system (8). We found that NPC1L1 traffics from the brush border membrane to an endosomal compartment after

Address for reprint requests and other correspondence: M. Skov, Dept. of Cellular and Molecular Medicine, the Panum Institute, Bldg. 6.4, Blegdamsvej 3, DK-2200 Copenhagen N, Denmark (e-mail: mskov@sund.ku.dk).

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exposure to cholesterol. In contrast, NBD-cholesterol rapidly appeared throughout the cytosol, also under conditions nonpermissible for endocytosis, indicating an uptake by a membrane transporter rather than via an endocytic receptor. We therefore propose that cholesterol is transported through the brush border membrane by NPC1L1 acting as a membrane transporter. In addition we propose that NPC1L1 senses the luminal cholesterol concentration and traffics to an endosomal compartment to downregulate the absorption of cholesterol.

MATERIALS AND METHODS

Materials. Rabbit anti-NPC1L1 and rabbit anti-NPC1 were purchased from Novus Biological (http://www.novusbio.com/), goat anti-early endosome antigen 1 (EEA1) was from Santa Cruz (http://www.scbt.com/), rabbit anti-alkaline phosphatase was from Biogenesis (http://www.biogenesis.co.uk/); the following antibodies were previously described; mouse anti-aminopeptidase N (APN) (14), rabbit anti-lactase (42), rabbit anti-human intestinal brush border (41), and rabbit anti-sucrase-isomaltase (40). Secondary Alexa 488/594 conjugated antibodies, Prolong Gold antifade reagent with 4,6-diamidino-2-phenylindole (DAPI). Image-iT FX signal enhancer and NBD-cholesterol were from Invitrogen (www.probes.invitrogen.com). Fluorescent mounting media, secondary horseradish peroxidase (HRP)-conjugated antibodies, and secondary anti-rabbit antibodies for conjugation to gold particles were purchased from DAKO (http://www.dako.dk/). The secondary anti-rabbit antibodies were conjugated to gold particles of ~13 nm in diameter prepared according to Ref. 43. Ox gall powder, pancreatin, cholesterol, and chlorpromazine were from Sigma (http://www.sigmaaldrich.com). ECL Western blot detection kit was from GE Healthcare, Little Chalfont, UK.

Organ culture of small intestinal explants. Porcine jejunum was obtained from fasted pigs by licensed staff from Department of Experimental Medicine, the Panum Institute, Copenhagen, Denmark. Mucosal explants from porcine jejunum were cultured in MEM media or MEM media enriched with 1% ox gall powder and 1% cholesterol for 0.5 or 1 h at 37°C. In some experiments the tissue was not cultured but fixed immediately after excision from the pig.

For the uptake experiments with NBD-cholesterol, the following solution was prepared: NBD-cholesterol (0.5 mg) was dissolved in 0.1 ml ethanol and mixed with 0.5 ml of corn oil. 10 mg of bile and 10 mg of pancreatin were dissolved in 4.5 ml of RPMI medium. The two solutions were mixed and incubated at 37°C overnight (ON) to generate a medium containing mixed micelles with NBD-cholesterol. Explants were cultured for 15 min at 37°C or for 1 h at 4°C. For the uptake experiments using chlorpromazine the explants were preincubated in RPMI media in the presence or absence of 10 μg/ml chlorpromazine for 1 h at 37°C and then in NBD-cholesterol media formulated as above in the presence or absence of the inhibitor for 15 min at 37°C.

Immuno fluorescence microscopy. Explants were fixed in 4% paraformaldehyde in 0.1 M sodium phosphate, pH 7.2 (PB) for 2 h at 4°C, briefly rinsed in PB, and stored in 1% paraformaldehyde in PB. The explants were either embedded in paraffin or snap frozen in precooled 2-methylbutane. In some experiments the explants were cryoprotected by infusion ON with 25% sucrose in PB before being snap frozen in 2-methylbutane. Paraffin-embedded tissue was cut into sections of 4–5 μm in a Leica RM 2165 rotary microtome, and frozen explants were cut into sections of 6- to 7-μm thickness in a Leica CM 1850 cryostat. The sections were rinsed with 50 mM Tris, 150 mM NaCl, containing 20 mM glycine, pH 7.4 (buffer A), incubated with Image-iT FX signal enhancer for 30 min, rinsed with 50 mM Tris, 150 mM NaCl, containing 0.5% ovalbumin, 0.1% gelatin, 0.05% Tween 20, and 0.2% Teleostan gelatin, pH 7.4 (buffer B). The sections were then incubated with rabbit anti-NPC1L1, rabbit anti-NPC1, mouse anti-APN and goat anti-EEA1, in buffer B, rinsed with buffer B and incubated with secondary Alexa 594/488 conjugated antibodies in buffer B. The sections were subsequently rinsed with buffer B, with 50 mM Tris, 150 mM NaCl, pH 7.4 (buffer C), and with water before being mounted with fluorescent mounting media or antifade reagent with DAPI. The sections were finally examined in a Leica DM 4000 B microscope equipped with a Leica DC 300 FX digital camera. Control experiments were included in parallel in which the primary antibodies were omitted.

Immunoelectron microscopy. Explants were fixed in 4% paraformaldehyde for 2 h at 4°C and infused ON with PB containing 2.3 M sucrose and 1% paraformaldehyde before being snap frozen in liquid N2. Ultrathin sections of ~50 nm were cut in a RMC MT 6000-XL ultramicrotome and collected at Formvar-coated grids. The ultrathin sections were then treated with buffer A, followed by buffer B and antibodies (anti-NPC1L1 or anti-NPC1) diluted in buffer B. Following rinsing in buffer B the sections were incubated with secondary gold-conjugated antibody diluted in buffer B. Subsequently the sections were rinsed in buffer B, in buffer C, and briefly in water. The sections were finally stained by using 0.4% uranyl acetate in Methocell and examined in a Zeiss EM 900 electron microscope equipped with a Mega View II digital camera. A morphometric analysis of the labeling intensity of NPC1L1 in the brush border membrane and in the apical part of enterocytes from mucosal explants cultured in the presence or absence of cholesterol was performed as follows: From each culture condition the number of gold particles was counted in the brush border membrane (from tight junction to tight junction) in 20 randomly selected enterocytes and in the apical...
part of the enterocyte (−2 µm into the enterocyte). The ratio between the number of gold particles in the brush border membrane and the apical part of the enterocyte was estimated in the two different culture conditions. A Student’s t-test was performed using the Microsoft Office Excel 2007 to determine whether the obtained ratios from enterocytes cultured in the presence or absence of cholesterol were statistically significantly different.

Subcellular fractionation of tissue explants. The mucosa of the explants was fractionated into microvillus membranes (Mic) and intracellular and basolateral membranes (Mg2+) and according to the divalent cation precipitation method (2). Procedures were carried out at 4°C unless otherwise stated. The mucosa was homogenized in mannitol buffer (50 mM mannitol, 2 mM Tris, and 10 µg/ml aprotinin-leupeptin, pH 7.1) in a Potter-Elvehjem tissue grinder. The homogenate was cleared by centrifugation at 120 g for 5 min. The supernatant was added MgCl2 to a final concentration of 10 mM and incubated for 10 min before being centrifuged at 1,100 g to pellet intracellular and basolateral membranes (Mg2+-pellet). To pellet the microvillus membranes (Mic-pellet) the supernatant was centrifuged at 48,000 g for 30 min. For detergent-resistant membrane (DRM) analysis Mic-pellets were resuspended in 25 mM HEPES and 150 mM NaCl, pH 7.1 (HB) containing 10 µg/ml aprotinin-leupeptin and solubilized with ice-cold 1% Triton X-100 at ice before being mixed with 80% sucrose to obtain a final sucrose concentration of 40%. A 35 to 15% sucrose gradient was layered above the extracted pellet according to (4). The gradient was centrifuged for 20–22 h at 217,000 g in a Beckman SW40 Ti rotor before fractions of 1 ml were collected by use of a peristaltic pump.

SDS-PAGE. Protein samples were separated in 10% polyacrylamide gels according to Laemmli (27) and electrotransferred to polyvinylidene difluoride membranes. Following transfer, membranes were treated with blocking reagent (0.5% ovalbumin, 0.1% gelatin, 0.2% Teleostan gelatin, and 2% Tween 20 in HB) after which the membranes were incubated with anti-NPC1L1 and anti-alkaline phosphatase, a glycosylphosphatidylinositol (GPI) anchoring intracellular and basolateral membranes (Fig. 1). The observed distribution was similar to that of the intestinal alkaline phosphatase, a glycosylphosphatidylinositol (GPI) anchored brush border membrane protein demonstrating that NPC1L1 mainly resides in the brush border membrane of the porcine enterocytes.

Organ culture of explants in the presence and absence of bile and cholesterol was used in the present work to mimic the in vivo postprandial and interprandial/fasting state, respectively. In the absence of cholesterol NPC1L1 resided mainly in the apical membrane of the jejunal enterocyte, but a small fraction was also found in small punctate intracellular compartments (Fig. 2A). The basolateral membrane (Fig. 2A) and goblet cells (Fig. 3) were free of labeling, and in general less intense labeling was seen in the crypt region compared with the villus region (not shown). Following culture with cholesterol a substantial amount of the NPC1L1 was found in intracellular compartments but some labeling was still present in the brush border (Fig. 2B). In contrast to NPC1L1, NPC1 resided in intracellular compartments irrespective of the presence or ab-

![Fig. 2. Subcellular localization of NPC1L1 and NPC1. Immunofluorescence microscopy of sections of paraffin-embedded mucosal explants cultured in the absence (Control) or presence (+ chol) of cholesterol for 1 h. In the control NPC1L1 resided mainly in the brush border (A), but a minor fraction was found in punctate intracellular compartments (arrow in high-magnification inset). In the presence of cholesterol NPC1L1 was mainly seen in intracellular compartments (B). NPC1 resided almost exclusively in the intracellular compartments regardless of the presence or absence of cholesterol (C and D). Bar: 20 µm.](http://ajpgi.physiology.org/)
presence of cholesterol (Fig. 2, C and D). This localization is in agreement with its role in egress of cholesterol from the endosomal and lysosomal compartments.

For further characterization of the trafficking of NPC1L1, double immunolabeling of NPC1L1 and APN, a major brush border enzyme, was carried out. Figure 3 shows that, whereas NPC1L1 colocalized extensively with amino peptidase (APN; A), but in explants cultured in the presence of cholesterol (B) NPC1L1 was found in intracellular compartments in the enterocytes. Both the goblet cells (arrow) and the nuclei (arrowhead) were free of immunoreactivity. In contrast, APN (B), the crude fraction of brush border enzymes (C and D), sucrase-isomaltase (E and F), and lactase (G and H) all remained in the brush border following culture in the presence of cholesterol. Bar: 20 μm.

Fig. 3. Selective trafficking of NPC1L1. Immunofluorescence microscopy of cryosections (A and B) and sections of paraffin-embedded explants (C–H) cultured in the presence or absence of cholesterol. In the control NPC1L1 colocalized extensively with amino peptidase (APN; A), but in explants cultured in the presence of cholesterol (B) NPC1L1 was found in intracellular compartments in the enterocytes. Both the goblet cells (arrow) and the nuclei (arrowhead) were free of immunoreactivity. In contrast, APN (B), the crude fraction of brush border enzymes (C and D), sucrase-isomaltase (E and F), and lactase (G and H) all remained in the brush border following culture in the presence of cholesterol. Bar: 20 μm.

The NPC1L1-positive intracellular compartment was further probed with specific antibodies to sucrase-isomaltase and lactase as well as antibodies recognizing a mixture of the major brush border enzymes. None of these brush border markers were internalized in the presence of cholesterol, indicating that the endocytosis of NPC1L1 is a selective process.

The NPC1L1-positive intracellular compartment was characterized by use of the early endosomal marker EEA1. In explants cultured in the presence of cholesterol, a substantial
colocalization between NPC1L1 and EEA1 was seen (Fig. 4B). However, a minor degree of colocalization was also observed in explants cultured without cholesterol (Fig. 4A). These findings indicate that NPC1L1 traffics from the brush border to early endosomes following culture in the presence of cholesterol.

The above findings were further substantiated by immunoelectron microscopy. In the absence of cholesterol, labeling of NPC1L1 was mainly seen in the brush border whereas a less dense labeling was observed in intracellular compartments (Fig. 5A). On the other hand, exposure to cholesterol resulted in reduced labeling of the brush border and increased labeling of the terminal web region just below the microvilli (Fig. 5B) and in endosomal-like structures (Fig. 5C). By morphometric analysis the relative labeling of the brush border in explants cultured with cholesterol was found to be reduced to approximately one-third compared with control explants (Fig. 5D). Regardless of the presence or absence of cholesterol NPC1 was primarily found in endosomal and lysosomal structures and in mitochondria (not shown).

Together the immunofluorescence and the immunogold labeling show that cholesterol, within a time frame of 0.5–1 h, induces a selective internalization of NPC1L1 to an endosomal compartment.

NBD-cholesterol rapidly enters the cytoplasm. Fluorescent NBD-cholesterol has previously been used to track intestinal cholesterol absorption in vivo (44). Most NBD-cholesterol was detected in the enterocytes 2 h after oral gavage, with the fluoros-terol found in cytoplasmic droplets as well as in the cytoplasm. However, the analog was also found in lipoprotein particles in the lamina propria and detected in the blood already 30 min after oral gavage; after 4 h only a minor fraction was still present in the enterocytes. To address the mechanism of cholesterol absorption mucosal explants were cultured in the presence of NBD-cholesterol. Following 15 min of culture at 37°C the cholesterol analog was readily absorbed into the enterocytes (Fig. 6A). It localized diffusively over the entire cytoplasm, with the most intense fluorescence seen at the apical side of the nucleus. Preincubation and culture in the presence of chlorpromazine, which is known to inhibit clathrin-mediated endocytosis (49), caused a similar cytoplasmic distribution of the cholesterol analog (Fig. 6B), indicating that cholesterol is not taken up by clathrin-mediated endocytosis. Likewise, culture at 4°C, which generally blocks all membrane traffic, also did not prevent uptake of NBD-cholesterol in explants cultured for 1 h at 4°C (Fig. 6C). Unlike the NBD-cholesterol, the NPC1L1 remained in the brush border following culture at 4°C for 1 h (Fig. 6D). Together these results thus show that NBD-cholesterol is not taken up by endocytosis but enters the cytoplasm directly after translocation across the brush border membrane. In addition they suggest that NPC1L1 acts as a membrane transporter rather than as an endocytic receptor, since if cholesterol were to be absorbed by an endocytic receptor a labeling pattern of the analog similar to that seen for NPC1L1 cultured in the presence of cholesterol would have been expected.

NPC1L1 is largely excluded from lipid rafts. The cellular plasma membrane is known to be organized into transient cholesterol- and sphingolipid-enriched microdomains, also known as lipid rafts (28). However, the brush border membrane of the enterocyte contains lipid rafts that are independent of cholesterol and more stable compared with rafts in other cell types (20). To determine whether NPC1L1 resides in lipid rafts, a DRM analysis by extraction with ice-cold 1% Triton X-100 followed by sucrose density centrifugation was carried out. Figure 7 shows that NPC1L1 was detected in all the gradient fractions; however, most was found in the heavy fractions representing the detergent-soluble fraction and only a smaller part resided in the lighter fractions representing DRMs. Unlike NPC1L1, the GPI-anchored

**Fig. 4. Colocalization of NPC1L1 with early endosome antigen 1 (EEA1). In the absence of cholesterol only a minor fraction of NPC1L1 resided in intracellular compartments positive for EEA1 (A, arrowhead). However, following culture in the presence of cholesterol extensive colocalization of NPC1L1 and EEA1 was seen (B). Arrow indicates a goblet cell devoid of labeling. Bar: 3 μm.**
intestinal alkaline phosphatase resides predominantly in the DRM fractions. Since lipid rafts in the enterocyte are considered to be stable, this finding suggests that NPC1L1 might reside in a relative mobile part of the brush border membrane.

**DISCUSSION**

Although NPC1L1 has previously been shown to localize in the brush border of enterocytes (1, 23, 38), the cholesterol dependency of the localization of NPC1L1 was not addressed.

Fig. 5. Ultrastructural localization of NPC1L1 in the enterocyte. Electron micrographs of mucosal explants cultured in the absence (A) and in the presence of cholesterol and bile for 1 h (B) and 0.5 h (C). In the absence of cholesterol, NPC1L1 resided in the brush border scattered over the microvilli (A). In explants cultured in the presence of cholesterol the brush border harbored less NPC1L1, which was instead detected in the terminal web region below the microvilli (B; see arrow) and in endosomal structures (C). Bars: 0.5 μm (A and B), 0.2 μm (C). D: morphometric analysis showing the ratio of gold particles in the brush border to gold particles intracellular for explants cultured without and with cholesterol. *P = 10^{-6}. Error bars indicate SD.

Fig. 6. 22-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-23, 24-bisnor-5-cholen-3β-ol (NBD)-cholesterol uptake into the enterocytes. Fluorescence images of frozen (A and B) and paraffin-embedded (C and D) sections. Explants were cultured in the absence (A) or presence (B) of chlorpromazine as described in MATERIALS AND METHODS. At 37°C the NBD-cholesterol was readily absorbed and distributed diffusively in the cytoplasm of the enterocyte both in explants cultured in the presence and absence of chlorpromazine. NBD-cholesterol was found neither in the nuclei (A, arrowhead) nor in the goblet cells (B, arrow). Following culture at 4°C for 1 h (C) the distribution pattern of the NBD-cholesterol was similar to that seen at 37°C, with the NPC1L1 protein residing in the brush border in the explants cultured at 4°C (D). Bar: 20 μm.
in these studies. In the present study we have shown that in the absence of luminal cholesterol the major part of the NPC1L1 protein resides in the brush border of the enterocyte. However, following culture in the presence of cholesterol, mimicking a meal rich in cholesterol, NPC1L1 selectively relocates to EEA1-positive intracellular compartments. The purpose of this trafficking either could be that NPC1L1 acts as an endocytic cholesterol receptor equivalent to for instance the LDL-receptor (19) or it could be a mechanism to minimize the absorption of cholesterol by downregulating the presence of the transporter in the brush border. In favor of NPC1L1 acting as an endocytic receptor is the fact that, in a hepatoma cell line, it was shown that cholesterol was absorbed by clathrin-mediated endocytosis either by use of RNA interference to silence the expression of clathrin heavy chain (16) or by potassium depletion (5). Furthermore, ezetimibe blocked absorption by impeding endocytosis of cholesterol (16), leading to the hypothesis that NPC1L1 acts as a recycling cholesterol receptor. In support of NPC1L1 acting as a transporter stands the fact that NPC1 has been shown to act as a transmembrane pump using a proton motive force (9); with a similar membrane topology NPC1L1 can be expected to work by an equivalent mechanism. The observed uptake of NBD-cholesterol into the cytoplasm of enterocytes in explants where endocytosis was inhibited either by low temperature or by chlorpromazine implies that cholesterol is absorbed by a membrane transporter-mediated mechanism rather than by endocytosis. NBD-cholesterol has previously been shown to distribute like cholesterol in the enterocyte under iron-depleted conditions but found intracellularly following iron replenishing (39). From a whole body perspective this sensing mechanism makes sense as excess of both cholesterol and iron are considered to be toxic (18, 35).

It is a well-established fact that the cholesterol homeostasis is regulated by feedback inhibition of both HMG CoA reductase and the LDL receptor (19), and the observed trafficking of NPC1L1 might add another level to regulation of the body’s cholesterol homeostasis, but clearly more work is needed to fully elucidate the mechanisms of cholesterol uptake.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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