Intestinal nuclear receptors in HDL cholesterol metabolism

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Abstract The intestine plays a pivotal role in cholesterol homeostasis by functioning as an absorptive and secretory organ in the reverse cholesterol transport pathway. Enterocytes control cholesterol absorption, apoAI synthesis, HDL biogenesis, and nonbiliary cholesterol fecal disposal. Thus, intestine-based therapeutic interventions may hold promise in the management of diseases driven by cholesterol overload. Lipid-sensing nuclear receptors (NRs) are highly expressed in the intestinal epithelium and regulate transcriptionally the handling of cholesterol by the enterocytes. Here, we discuss the NR regulation of cholesterol fluxes across the enterocytes with special emphasis on NR exploitation as a bona fide novel HDL-raising strategy.—Degirolamo, C., C. Sabbà, and A. Moschetta. Intestinal nuclear receptors in HDL cholesterol metabolism. J. Lipid Res. 2015, 56: 1262–1270.

Supplementary key words atherosclerosis • gene expression • lipoprotein • transport • transcription • high density lipoprotein

THE ENTEROCYTE, CHOLESTEROL HOMEOSTASIS, AND HDL

Cholesterol is essential in all mammalian cells as a structural component of cell membranes and as a precursor of a large variety of molecules critical in biological functions such as bile acids (BAs), steroid hormones, and vitamin D (1). Cellular cholesterol requirements are met through de novo synthesis; however, in the presence of plasma lipoproteins, hepatocytes and steroidogenic cells obtain cholesterol through internalization of exogenous cholesterol (2). At the cellular level, de novo cholesterol synthesis and uptake of lipoprotein cholesterol are modulated through a negative feedback loop responding to elevations in intracellular cholesterol and regulated by a family of membrane-bound transcription factors named sterol-regulatory element binding proteins (SREBPs) (3). Earlier studies by Dietschy, Spady, and colleagues (4–6) provided evidence that, although virtually every tissue can synthesize sterol from acetyl-CoA, cholesterol may also be absorbed into the body from dietary sources, thus supporting an intestinal route of sterol influx. The elucidation of the molecular mechanisms underlying cholesterol absorption and the identification of pharmacological compounds able to interfere with the absorptive process have greatly endorsed the intestinal apical and basolateral proteins as promising targets to modulate cholesterol metabolism (7, 8).

The handling of cholesterol by the enterocyte controls the metabolic fate of dietary and biliary cholesterol. In the lumen of the small intestine, free cholesterol (FC) from dietary intake and biliary secretion is solubilized in mixed micelles containing BAs and phospholipids. The apical protein Niemann-Pick C1-like 1 (NPC1L1) is both the crucial and major determinant of the amount of cholesterol absorbed by the enterocytes, as both NPC1L1 deficiency and treatment with the inhibitor ezetimibe result in a markedly reduced intestinal cholesterol absorption (9–13). Furthermore, BA presence in the intestinal lumen is an essential prerequisite for absorption to occur and, accordingly, cholesterol-7α-hydroxylase (CYP7A1)-deficient mice, which are unable to synthesize BAs in the liver, display virtually zero cholesterol absorption (14).

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This work was funded by NR-NET FP7 Marie Curie ITN, Italian Association for Cancer Research (AIRC, IG 14732), Italian Ministry of University and Education (Finanziamenti per la Ricerca di Base IDEAS RBID08C9N7; PRIN 2010FHF52M-002), Italian Ministry of Health (Young Researchers Grant GR-2008-1143546; GR-2010-2314703), and University of Bari (IDEA GRBA0802SJ-2008).

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Abbreviations: BA, bile acid; CE, cholesteryl ester; CYP7A1, cholesterol-7α-hydroxylase; FC, free cholesterol; FXR, farnesoid X receptor; LBD, ligand binding domain; LXR, liver X receptor; MTP, microsomal transfer protein; NPC1L1, Niemann-Pick C1-like 1; NR, nuclear receptor; RCT, reverse cholesterol transport; RXR, retinoid X receptor; SREBP, sterol-regulatory element binding protein; TICE, trans-intestinal cholesterol efflux; VDR, vitamin D receptor.

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DOI 10.1194/jlr.R052704

1262 Journal of Lipid Research Volume 56, 2015

This article is available online at http://www.jlr.org
The metabolic fate of the absorbed cholesterol within the enterocyte involves an integrated network consisting of apical and basolateral uptake proteins, microsomal proteins, and transcriptional programs. Newly absorbed FC can be transported to the endoplasmic reticulum where it can be converted in cholesteryl ester (CE) by the microsomal enzyme ACAT2 (15–18) or be effluxed back into the intestinal lumen by the heterodimer ABCG5/G8 (19–23). The role of ACAT2 in intestinal sterol absorption is of special interest because the physicochemical state of the cholesterol molecule determines its fate in the body. FC is soluble in membrane phospholipids and represents an important structural component of cellular membranes; by contrast, CE is mostly insoluble in membranes and must be packaged into lipid droplets or incorporated into the core of lipoprotein particles for export out of the cell. Studies in ACAT2 knockout mice have reported that the lack of CE formation results in reduced cholesterol absorption (24, 25), and that CE formation by ACAT2 selectively utilizes chylomicron particles for quantitative transport of newly absorbed cholesterol out of the enterocyte into the lymphatic system, and subsequently into the body (26, 27). Along the basolateral membrane of the enterocyte, CE gets to be assembled into the core of chylomicron particles and be secreted into the lymph in a microsomal transfer protein (MTP)-dependent manner. The assembly of CE into chylomicron particles is critically dependent on MTP and apoB48; accordingly, absence of either of these proteins completely abolishes cholesterol absorption (28). Along the apical membrane of enterocytes, the heterodimer ABCG5/G8 counteracts the cholesterol absorption inhibition (27). The nonbiliary RCT pathway involves the targeting of plasma cholesterol to the proximal part of the small intestine and the subsequent cellular cholesterol secretion into the lumen of this organ. Intestinal perfusion studies indicated that plasma cholesterol can transverse the small intestine in a basolateral to apical direction thanks to the luminal presence of acceptors such as BAs and phospholipids (60). Although TICE accounts for a small amount of total fecal sterol loss (61), it is a highly dynamic pathway that can be efficiently upregulated under conditions of biliary insufficiency via activation of nuclear receptor (NR)-mediated transcriptional programs (52, 53, 62, 63). Indeed, there is an emerging interest in the molecular elucidation of the lipoproteins delivering cholesterol to the small intestine, the potential receptors involved in intestinal lipoprotein cholesterol uptake, factors controlling the trafficking of cholesterol across enterocyte and into the intestinal lumen, and luminal acceptor molecules promoting TICE. So far, the molecular mechanisms underlying TICE are not fully explored, although some progress has been made with regard to the role of donor particles delivering cholesterol for TICE, as well as the potential role of the heterodimer ABCG5/G8 as a TICE contributor (57, 58, 64–69) (Fig. 1).

A complex network consisting of cellular components (including sterol transporters and cholesterol-metabolizing enzymes) and transcriptional sensors balances intestinal cholesterol absorption and synthesis with biliary excretion and conversion to BAs. Mounting evidence supports a crucial role of intestinal NRs in cholesterol whole-body homeostasis, as they orchestrate the cholesterol fluxes across the enterocytes and out of the body by transcriptionally regulating cholesterol absorption, HDL biogenesis, RCT, and TICE (70–76). Thus, intestinal lipid-sensing NRs could be exploited as a therapeutic means in the management of chronic disorders where deregulated sterol metabolism drives the pathogenesis, including atherosclerosis, inflammation, and cancer.
INTESTINAL MUCOSA EXPRESSION PATTERN OF NRs

The relationship between NR expression, function, and physiology has been explored by clustering NR tissue expression distribution profiles and has revealed the existence of a hierarchical network tying NR function to development, basal metabolic functions, dietary lipid metabolism, and energy homeostasis. The NR expression pattern was first investigated in normal mouse samples, and has mined initial information about the regulatory elements required to govern a specific network of receptors sharing common expression profiles (88). Lipid-sensing NRs, including LXR, FXR, PPAR, and vitamin D receptors (VDRs), appeared expressed at moderate to high levels along the murine gastro-enteric system, thus supporting the concept of the intestine as gatekeeper of lipid homeostasis being the intestinal mucosa responsible for sensing luminal contents. Thus, an “enteric NR team” composed of LXR, FXR, PPARs, and other NRs sits at the crossroad of nutritional and hormonal signals modulating the intertwined interactions between dietary lipid fluxes across the enterocyte and the intestinal epithelium homeostasis. The intestine is the most rapidly self-renewing tissue, and the homeostasis of its epithelium relies on the integrated control of proliferation, differentiation, and apoptosis, as well as on the functional architecture of the enterocytes. The permanent renewal takes place in the crypt-to-villus axis and is accomplished by the stem cells located at the bottom of the crypts that generate four differentiated cell types:

THE NR SUPERFAMILY: FOCUS ON THE RETINOID X RECEPTOR HETERODIMERS

NRs are gaining increasing importance in the lexicon of functional biology because they represent a well-characterized, powerful, and fast-track bridge between pharmacology and physiology. NRs compose a large superfamily of evolutionarily related DNA transcription factors that transduce different metabolic signals into modulation of gene transcription. Thus, NRs control a broad range of biological processes and genetic programs, including development, cell growth and differentiation, metabolism, and immune response. An increasing understanding of the role of NRs in the physiology and pathophysiology of common and chronic diseases, including diabetes, obesity, and cancer, fostered the transitions of NRs through the tipping point as therapeutic targets in the treatment of human diseases.

As elegantly discussed by Evans, Mangelsdorf, and colleagues, the discovery of the retinoid X receptor (RXR) and its endogenous ligand (9-cis-retinoic acid, a vitamin A metabolite) triggered a “Big Bang” of molecular endocrinology and led to the discovery of RXR heterodimerization with other NRs as a mechanism to control gene-specific transcription (77–82). On its own, RXR functions as a self-sufficient homodimer, binding to a direct repeat of half-sites separated by one nucleotide (DR1), and displays a ligand binding domain (LBD) that allows the adoption of multiple conformations and the dimerization with different NRs. Interestingly, the RXR heterodimer partners, with the exception of the NR-related 1 protein (NURR1), are all ligand dependent (77, 83–85).

As transcription factors, NRs rely on DNA sequence-specific binding to transactivate their target genes. NRs possess a DNA binding domain composed of two zinc finger motifs which bind a specific response element that can differ in terms of extension, duplication, and orientation of the repeat; accordingly response elements can be selective for a given NR or a class of receptors (86). Moreover NRs exhibit a LBD containing a ligand-dependent activation function-2 (AF-2) motif that mediates coactivator recruitment. In the absence of ligand, the LBD is bound to transcriptional corepressor complexes, while ligand binding to the NR triggers changes in the NR three-dimensional conformation which results in the dissociation of corepressor and in a subsequent recruitment of tissue-specific coregulators (87). Of note, the conserved modular structure of NRs is completed by an N-terminal ligand-independent activation function domain (AF-1) and a carboxyl-terminal domain. Among the RXR heterodimers, lipid sensing NRs, including liver X receptor (LXR), farnesoid X receptor (FXR), and PPAR, are abundantly expressed along the gastrointestinal system (88), thus suggesting a crucial role of these transcription factors in the intestine with relevance to regulation of HDL-cholesterol metabolism, RCT, and TICE. Indeed, it is tempting to hypothesize that the intestinal epithelium may take advantage of these molecular sensors of nutrients and lipids to monitor cholesterol fluxes across enterocytes, as they sit at the crossroad of sterol disposal.
Paneth cells, goblet cells, enteroendocrine cells, and the absorptive enterocytes. The last three cell types differentiate during their migration upward from crypts toward the tip of the villi (89–92). The mechanisms controlling cell transition from crypt-to-villus tip involve transcription factors that switch on and off compartment- and cell-specific genes (93). By using in situ hybridization and systematic real-time quantitative polymerase chain reaction, the enteric NR family has been clustered into different quantitative expression and qualitative cell-type-specific networks (93). The fatty acid sensor, PPARβ/δ, is mostly localized between the proliferative progenitor cells and the lower third of the crypt, while a group of NRs including LXRβ, PPARα, (and PPARγ only in the small intestine) is more ubiquitously expressed in the intestinal mucosal epithelium. Finally, the BA and vitamin D sensors, namely FXR and VDR, as well as the oxysterol sensor, LXRα, are mostly expressed in the fully differentiated cells lining the intestinal epithelium (Fig. 2). Importantly, the NR expression pattern was found to predict the modulation of its expression in tumors (93). The degree of NR enteric expression is suggestive of their great potential as targets with regard to the transcriptional modulation of the multifaceted functions of the small intestine as both a cholesterol-absorbing and an excretory organ.

NRs IN THE ENTEROCYTE

LXR

The NRs LXRα and LXRβ are oxysterol-activated transcription factors and function as chief regulators of cholesterol homeostasis by controlling cholesterol influx, transport, and efflux (94, 95). Studies in LXR-deficient mice (96, 97) and use of LXR agonists have provided compelling evidence that LXRs are critical for cholesterol homeostasis and that they exert atheroprotective effects including promotion of RCT, elevation of HDL-cholesterol levels, inhibition of cholesterol absorption, and stimulation of TICE (8, 31, 61, 98, 99). However, the use of LXR agonists in therapy has been hampered by the observation that LXR-driven atheroprotection is accompanied by hypertriglyceridemia, which has been attributed to the LXRα isoform, mostly expressed in the liver (100). As LXRs are expressed in multiple tissues involved in RCT, including macrophages, liver, and intestine, there has been an increasing interest in the development of tissue-selective LXR modulators. An earlier study in LDL receptor-deficient mice showed the ability of a LXR agonist, named ATI-829, to selectively activate LXR target gene expression in mouse intestine and macrophages, but not in the liver, thus providing initial evidence of the feasibility of a tissue-specific LXR activation (100). A few years later, Dan Rader’s group reported the identification of an intestine-specific LXR agonist, named GW6340, which was able to promote macrophage RCT in vivo, thus suggesting that macrophage LXR itself plays a nondominant role in promoting RCT in response to a LXR agonist (101). Finally, constitutive intestinal LXRα activation has been found to reduce cholesterol absorption, increase preβ HDL particles, and protect from diet-induced atherosclerosis without any side effects such as liver steatosis and increased fatty acid synthesis (71).

In the intestine, LXRs control both cholesterol excretion and luminal reabsorption by transcriptionally regulating critical uptake and delivery proteins involved in RCT and TICE (33, 38, 61, 103). Studies with LXR agonists clarified that regulation of sterol loss by LXR depends on the function of the ABCG5/G8 heterodimer (31) while...
being independent from ABCA1 (102). In contrast, studies in intestine-specific ABCA1-deficient mice implicated ABCA1 in the LXR-dependent increase in plasma HDL (99). These studies highlighted the importance of intestine-specific cholesterol absorption and efflux pathways in regulating HDL biogenesis and cholesterol homeostasis. Whether cholesterol absorption per se is required for the HDL-raising properties of the LXR agonist has been studied in NPC1L1-deficient mice (103) and was recently evaluated in intestine-specific MTP-deficient mice (73). Xie et al. (73) demonstrated that LXR agonist administration led to an increase in HDL biogenesis in the absence of chylomicron secretion, thus illustrating the complex and interrelated pathways that modulate cholesterol efflux pathways in vivo. Because two isoforms exist, the significance of LXRα and LXRβ on cholesterol absorption was investigated (72). Hu et al. (72) reported that selective LXRβ activation increased cholesterol absorption and apoB-containing lipoprotein secretion, which seem to be counteracted by LXRα isoform; thus, these data underscored the relevance of an isoform-specific LXR modulation.

Collectively, strategies targeting LXR in an isoform-specific or tissue-selective manner may hold promise in our understanding of how we can exploit LXR activation as a powerful tool in the management of cholesterol overload-induced chronic disorders.

**PPARs**

PPARs are ligand-activated transcription factors that act as fatty acid sensors to control metabolic programs and to regulate energy homeostasis (104). PPAR ligands are represented by native and modified (oxidized and nitrated) fatty acids, eicosanoids, derivatives of polyunsaturated fatty acids, fibrate, and thiazolidinedione. Three isoforms exist in mammals (α, β/δ, and γ), characterized by a very specific tissue distribution and physiological functions with the PPARα isoform mediating fibrate action on HDL-cholesterol levels via transcriptional induction of synthesis of the major HDL apolipoproteins, apoAI and apoAII (105–110). With regard to intestinal expression, PPARα and PPARβ/δ are localized to the small intestine while PPARγ is mostly confined in the large intestine (colon) (86). Thus, PPARα and PPARβ activation has been found to stimulate both RCT and TICE (62, 70, 74, 75). PPARα’s ability to increase HDL cholesterol levels has been typically attributed to activation of PPARα in the liver; however, an earlier study by Colin et al. (74) highlighted the role of PPARα in the intestine in increasing HDL and showed that upon PPARα activation, apoAI and ABCA1 mRNA were increased, thus leading to augmented HDL lipidaemia and number of HDL particles secreted by the enterocytes. More recently, studies in mice receiving PPARα/δ modulator (named GFT505) suggested that intestinal PPARα upregulation regulates HDL production by inducing ABCA1 expression and apoAI secretion, and reducing cholesterol esterification (74).

Of the several PPAR isoforms, PPARβ/δ is the least understood, but the recent discovery of PPARβ agonists and their ability to increase HDL cholesterol levels and RCT (111, 112) shed light on the therapeutic potential of PPARβ modulators in cholesterol overload-induced clinical conditions. As PPARβ agonists increase HDL-cholesterol concentrations, they therefore have the potential to stimulate macrophage-to-faeces RCT. In vivo studies have shown that PPARβ agonists promote RCT by reducing NPC1L1 expression rather than by stimulating macrophage cholesterol efflux, and increase HDL cholesterol without any ABCA1 upregulation either in the liver or in the small intestine (70). Similarly, van der Veen et al. (69) reported that PPARβ activation is associated with elevated plasma HDL and reduced cholesterol absorption efficiency that may be related to downregulation of NPC1L1 expression.

The role of PPARγ in the intestine with regard to cholesterol homeostasis has been poorly investigated. Recent studies indicate close intriguing links between microbial communities and regulation of intestinal PPARγ expression by colonocytes, and suggests that lipopolysaccharide from Gram-negative bacteria is critical in colonic steady state PPARγ expression through Toll-like receptor 4 (112). Studies in vitro have shown that PPARγ agonist (troglitazone) treatment was able to lower cholesterol synthesis via reduced concentration of nuclear SREBP2 (113), while studies in vivo reported that combined deficiency of MTP and ABCA1 was associated with increased PPARγ expression and accompanied with a significant reduction of plasma cholesterol levels (114). Further studies are ongoing to assess commensal bacteria ability to induce PPARγ expression and activation and to evaluate whether this may have relevance with regard to cholesterol homeostasis. In mammals, it has long been known that luminal cholesterol of both exogenous and endogenous origin is metabolized by the intestinal microbial community and the end-product is mostly the poorly absorbable coprostanol, whose formation facilitates cholesterol removal from the body. Whether PPARγ may participate in cholesterol homeostasis via involvement in bacterial conversion to coprostanol may be the focus of future studies. Moreover, the ability of the PPARγ agonist, pioglitazone, to inhibit cholesterol absorption in rats (115) and to increase HDL-cholesterol levels in diabetic patients (116, 117) provides the impetus to further investigate the hypocholesterolemic potential of PPARγ activation.

**FXR**

FXR is the master regulator of BA homeostasis by controlling BA synthesis, efflux, influx, and detoxification in the gut/liver axis (118–121). In the liver, FXR stimulates BA and phospholipid secretion into bile and BA conjugation and detoxification, and represses the BA-synthesizing enzyme CYP7A1 (122–124). In the intestine FXR regulates fibroblast growth factor 15 (FGF15) expression (124) as well as genes involved in apical BA uptake [apical sodium BA cotransporter (ASBT)] (125), intra-enterocyte BA transport [intestinal BA binding protein (IBABP)] (126–129), and basolateral BA secretion [organic solute transporter α and β (OSTαβ)] (129). Although FXR activation has been reported to decrease serum cholesterol and apoAI concentrations while increasing phospholipid transfer protein (PLTP)-mediated HDL remodeling (130, 131), the role of
intestinal-specific FXR activation has not yet been studied with regard to changes in cholesterol metabolism. Indeed, constitutive intestinal FXR activation has been reported to protect mice from cholestasis and to lower both biliary BA and cholesterol (132). These data seem to implicate a link between upregulation of intestinal FXR transcriptome and modulation of cell and tissue sterol content. In line with this, a recent study highlighted a relationship among cholesterol levels, SREBP2, and intestinal FXR transcriptional machinery, thus suggesting that SREBP2 negatively regulates FXR-mediated induction of the FGF19 gene in human intestinal cells (133). Future studies aiming at investigating how intestinal FXR-mediated CYP7A1 repression along with modulation of luminal BA content may impact cholesterol homeostasis are urgently awaited.

PERSPECTIVES FROM PHYSIOLOGY TO PHARMACOLOGY

The role of the intestine in the regulation of whole-body cholesterol homeostasis has been underestimated for a long time, but during the last 10 years a compelling body of evidence has greatly contributed to the appreciation of the gatekeeper role of the intestine in cholesterol homeostasis maintenance. The intestine has thus emerged as a dynamic organ with tremendous therapeutic potential, as it houses a large variety of proteins that sense (NRs), modify (microsomal enzymes), and transport (apical and basolateral transporters) cholesterol across the intestinal epithelium, thus priming its metabolic fate. The intestine functions as an absorptive and excretory organ regulating cholesterol fluxes across the enterocytes and out of the body. While the intestinal cholesterol absorption mechanisms have been largely explored, thus providing the knowledge necessary for the development of pharmacological tools able to interfere with the numerous cellular factors involved, only recently, the intestinal role as a cholesterol excretory organ in RCT and partly via TICE has received attention. The process of RCT involves principally HDL-mediated delivery of peripheral cholesterol to the liver for biliary secretion. However, the observation that a small percentage of RCT persists in genetic or surgical models of biliary insufficiency (33, 56, 134) supports the existence of a nonbiliary fecal cholesterol disposal pathway (60). However, HDL lipoproteins do not appear to be involved in this nonbiliary RCT (58). Nevertheless, the biliary and nonbiliary RCT pathway appeared to be highly dynamic and amenable to NR-based pharmacologic interventions (52, 53, 56, 62, 63). Pharmacological activation of both LXR and PPARβ has been reported to independently stimulate each of these intestinal pathways for cholesterol removal and promote the identification of the lipoproteins delivering cholesterol to the small intestine, the potential receptors involved in intestinal lipoprotein cholesterol uptake, and the factors controlling the trafficking of cholesterol across enterocyte and into the intestinal lumen. Unfortunately, most of these vexing questions are still unanswered and recent studies linking microbiota, RCT, and atherosclerosis (135) have opened new possibilities of targeting the intestine as a source of molecules displaying a pro/anti-atherogenic potential. To this end, a deeper knowledge of PPARγ function in the colon and its potential involvement in the regulation of the bacterial communities, particularly those able to convert cholesterol to coprostanol, may hold promise and deserve further investigation. Moreover, the intestinal NR network includes members whose functions in the intestine with regard to RCT have never been investigated, including FXR and VDR. Finally, targeting the multifaceted functions of the intestine as a solid gatekeeper of plasma cholesterol homeostasis may be a burgeoning area for pharmaceutical efforts with the goal of managing the clinical conditions arising from or subsequent to deregulated sterol metabolism.\footnote{The authors apologize to their distinguished colleagues whose work has not been cited owing to space limitations. The authors are indebted to Dr. Roberta Le Donne for the artwork.}

REFERENCES

1. Ikonen, E. 2008. Cellular cholesterol trafficking and compartmentalization. Nat. Rev. Mol. Cell Biol. 9: 125–138.
2. Liscum, L., and N. K. Dahl. 1992. Intracellular cholesterol transport. J. Lipid Res. 33: 1239–1254.
3. Horton, J. D., J. L. Goldstein, and M. S. Brown. 2002. SREBP: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J. Clin. Invest. 109: 1125–1131.
4. Dietzchy, J. M., and M. D. Siperstein. 1967. Effect of cholesterol feeding and fasting on sterol synthesis in seventeen tissues of the rat. J. Lipid Res. 8: 97–104.
5. Spady, D. K., and J. M. Dietzchy. 1983. Sterol synthesis in vivo in 18 tissues of the squirrel monkey, guinea pig, rabbit, hamster, and rat. J. Lipid Res. 24: 303–315.
6. Spady, D. K., L. A. Woollett, and J. M. Dietzchy. 1993. Regulation of plasma LDL-cholesterol levels by dietary cholesterol and fatty acids. Annu. Rev. Nutr. 13: 355–381.
7. Kruit, J. K., A. K. Groen, T. J. van Berkel, and F. Kuipers. 2006. Emerging roles of the intestine in control of cholesterol metabolism. World J. Gastroenterol. 12: 6429–6439.
8. Bonamassa, B., and A. Moschetta. 2013. Atherosclerosis: lessons from LXR and the intestine. Trends Endocrinol. Metab. 24: 120–128.
9. Altmann, S. W., H. R. Davis, Jr., L. J. Zhu, X. Yao, L. M. Hoos, G. Tetzloff, S. P. Iyer, M. Maquire, A. Golovko, M. Zeng, et al. 2004. Niemann-Pick C1 like 1 protein is critical for intestinal cholesterol absorption. Science. 303: 1201–1204.
10. Davies, J. P., C. Scott, K. Oishi, A. Liapis, and Y. A. Ioannou. 2005. Inactivation of NPC1L1 causes multiple lipid transport defects and protects against diet-induced hypercholesterolemia. J. Biol. Chem. 280: 12710–12720.
11. Davis, H. R., Jr., L. J. Zhu, L. M. Hoos, G. Tetzloff, M. Maquire, J. Liu, X. Yao, S. P. Iyer, M. H. Lam, E. G. Lund, et al. 2004. Niemann-Pick C1 like 1 (NPC1L1) is the intestinal phytosterol and cholesterol transporter and a key modulator of whole-body cholesterol homeostasis. J. Biol. Chem. 279: 33586–33592.
12. Clader, J. W. 2004. The discovery of ezetimibe: a view from outside the receptor. J. Med. Chem. 47: 1–9.
13. Garcia-Calvo, M., J. Lisonick, H. G. Bull, B. E. Hawes, D. A. Burnett, M. P. Braun, J. H. Crona, H. R. Davis, Jr., D. C. Dean, P. A. Detmers, et al. 2005. The target of ezetimibe is Niemann-Pick C1-like 1 (NPC1L1). Proc. Natl. Acad. Sci. USA. 102: 8132–8137.
14. Schwarz, M., D. W. Russell, J. M. Dietzchy, and S. D. Turley. 1998. Marked reduction in bile acid synthesis in cholesterol 7alpha-hydroxylase-deficient mice does not lead to diminished tissue cholesterol turnover or to hypercholesterolemia. J. Lipid Res. 39: 1833–1843.
15. Anderson, R. A., C. Joyce, M. Davis, J. W. Reagan, M. Clark, G. S. Shelnos, and L. L. Rudel. 1998. Identification of a form of Intestinal regulation of HDL cholesterol 1267
acyl-CoA cholesterol acyltransferase specific to liver and intestine in nonhuman primates. J. Biol. Chem. 273: 26747–26754.

16. Rudel, L. L., R. G. Lee, and T. L. Cockman. 2001. Acyl coenzyme A: cholesterol acyltransferase types 1 and 2: structure and function in atherosclerosis. Curr. Opin. Lipidol. 12: 121–127.

17. Willner, E. D., J. Trow, K. J. Bardin, M. Wilson, D. A. Sanan, L. L. Rudel, and R. V. Farese, Jr. 2003. Deficiency of acyl CoA cholesterol acyltransferase 2 prevents atherosclerosis in apolipoprotein E-deficient mice. Proc. Natl. Acad. Sci. USA. 100: 1206–1207.

18. Lee, R. G., R. Shah, J. K. Sawyer, R. L. Hamilton, J. S. Parks, and L. L. Rudel. 2005. ACAT2 contributes cholesterol esters to newly secreted VLDL, whereas LCAT adds cholesterol ester to LDL in mice. J. Lipid Res. 46: 1209–1212.

19. Lee, M. H., K. Lu, S. Hazard, H. Yu, S. Shulenin, H. Hidaka, H. Kojima, R. Allikmets, N. Sakuma, R. Pegoraro, et al. 2001. Identification of a gene, ABCG5, important in the regulation of dietary cholesterol absorption. Nat. Genet. 27: 79–83.

20. Berge, K. E., H. Tian, G. A. Graf, L. Yu, N. V. Grishin, J. Schultz, P. Kvetonovich, B. Shan, R. Barnes, and H. H. Hobbs. 2000. Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. Science. 290: 1771–1775.

21. Repa, J. J., K. E. Berge, C. Pomajzl, J. A. Mangelsdorf, D. J. Repa, and H. H. Hobbs. 2000. Regulation of ATP-binding cassette mediating cellular cholesterol efflux gene, ABCA1. Curr. Opin. Lipidol. 11: 859–860.

22. Graf, G. A., W. P. Li, R. D. Gerard, I. Gelissen, A. White, J. C. Cohen, and H. H. Hobbs. 2002. Coexpression of ATP-binding cassette proteins ABCG5 and ABCG8 permits their transport to the apical surface. J. Clin. Invest. 110: 659–669.

23. Graf, G. A., L. Yu, W. P. Li, R. Gerard, P. L. Tuma, J. C. Cohen, and H. H. Hobbs. 2003. ABCG5 and ABCG8 are obligate heterodimers for protein trafficking and biliary cholesterol excretion. J. Biol. Chem. 278: 48275–48282.

24. Buhman, K. K., M. Accad, S. Novak, R. S. Choi, J. S. Wong, R. L. Hamilton, S. Turley, and R. V. Farese, Jr. 2000. Resistance to diet-induced hypercholesterolemia and gallstone formation in ACAT2-deficient mice. Nat. Med. 6: 1341–1347.

25. Repa, J. J., K. K. Buhman, R. V. Farese, Jr., J. M. Dietschy, and S. D. Turley. 2004. ACAT2 deficiency limits cholesterol absorption in the cholesterol-fed mouse: impact on hepatic cholesterol homeostasis. Hepatology. 40: 1088–1097.

26. Nguyen, T. M., J. K. Sawyer, K. L. Kelley, M. A. Davis, and L. L. Rudel. 2012. Cholesterol esterification by ACAT2 is essential for efficient intestinal cholesterol absorption: evidence from thoracic lymph duct cannulation. J. Lipid Res. 53: 95–104.

27. Nguyen, T. M., J. K. Sawyer, K. L. Kelley, M. A. Davis, C. R. Kent, and L. L. Rudel. 2012. ACAT2 and ABCG5/G8 are both required for efficient cholesterol absorption in mice: evidence from thoracic lymph duct cannulation. J. Lipid Res. 53: 1598–1609.

28. Hussain, M. M., T. M. Leung, L. Zhou, and S. Abu-Merhi. 2013. Regulating intestinal function to reduce atherogenic lipoproteins. Curr. Opin. Lipidol. 24: 481–490.

29. Yu, L., J. Li-Hawkins, R. E. Hammer, K. E. Berge, J. D. Horton, J. C. Cohen, and H. H. Hobbs. 2002. Overexpression of ABCG5 and ABCG8 promotes biliary cholesterol secretion and reduces fractional absorption of dietary cholesterol. J. Clin. Invest. 110: 671–680.

30. Yu, L., J. York, K. von Bergmann, D. Lutjohann, J. C. Cohen, and H. H. Hobbs. 2003. Stimulation of cholesterol excretion by the liver X receptor agonist requires ATP-binding cassette transporters G5 and G8. J. Biol. Chem. 278: 15565–15570.

31. Yu, L., S. Gupta, F. Xu, A. D. Liverman, A. Moschetta, D. J. Mangelsdorf, J. J. Repa, H. H. Hobbs, and J. C. Cohen. 2005. Expression of ABCG5 and ABCG8 is required for regulation of biliary cholesterol secretion. J. Biol. Chem. 280: 8742–8747.

32. Temel, R. E., J. K. Sawyer, L. Yu, C. Lord, C. Degirolamo, A. McDaniel, S. M. Marshall, N. Wang, R. Shah, L. L. Rudel, et al. 2010. Biliary cholesterol secretion is ATP-dependent for macromolecule reverse cholesterol transport. Cell Metab. 12: 96–102.

33. Glomset, J. A. 1968. The plasma lecithins: cholesterol acyltransferase reaction. J. Lipid Res. 9: 155–167.

34. Glomset, J. A., and K. R. Norum. 1973. The metabolic role of lecithin:cholesterol acyltransferase: perspectives form pathology. Adv. Lipid Res. 10: 95–135.

35. Glickman, R. M., and P. H. Green. 1977. The intestine as a source of apolipoprotein A1. Proc. Natl. Acad. Sci. USA. 74: 2569–2573.
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