Computational modeling of cholesterol metabolism

Paalvast, Thijs

Chapter 7

Developments in Intestinal Cholesterol Transport and Triglyceride Absorption

Yared Paalvast[1], Jan Freark de Boer[1] and Albert K. Groen[2,3]

Affiliations

[1]Department of Pediatrics, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands.

[2]Department of Laboratory Medicine, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands.

[3]Dept of Vascular Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands.

Current Opinion in Lipidology (2017), 28(3):248-254

Abstract

An important strategy in reducing atherosclerosis and risk of cardiovascular events is to increase the rate of reverse cholesterol transport, including its final step; cholesterol excretion from the body. The rate of removal is determined by a complex interplay between the factors involved in regulation of intestinal cholesterol absorption. One of these factors is a process known as trans-intestinal cholesterol excretion (TICE). This pathway comprises transport of cholesterol directly from the blood, through the enterocyte, into the intestinal lumen. In humans, this pathway accounts for 35% of cholesterol excretion in the feces. Mechanistic studies in mice revealed that, activation of the bile acid receptor FXR increases cholesterol removal via the TICE pathway as well as decreases plasma cholesterol and triglyceride providing an interesting target for treatment of dyslipidemia in humans. The physical chemical properties of bile acids are under control of FXR and determine intestinal cholesterol and triglyceride solubilization as well as absorption, providing a direct link between these two important factors in the pathogenesis of cardiovascular disease. Besides bile acids intestinal phospholipids are important for luminal lipid solubilization. Interestingly, phospholipid remodeling through LPCAT3 was shown to be pivotal for uptake of fatty acids by enterocytes, which may provide a mechanistic handle for therapeutic intervention.

Introduction

Plasma LDL-cholesterol and triglyceride levels are independent risk factors for atherosclerotic cardiovascular disease (CVD) [1]. The role of the intestine in control of both the plasma cholesterol and triglyceride concentration has long remained relatively unexplored. The last few years, the importance of the intestine in regulation of whole body lipid homeostasis is increasingly appreciated. Cholesterol homeostasis is determined by the balance between dietary cholesterol absorption, de novo cholesterol synthesis and fecal sterol excretion. Cholesterol absorption is typically around 50% in mice and humans, but can vary considerably (20% - 80%) [2]. The protein Nieman Pick C1-like 1 (NPC1L1) is crucial for cholesterol absorption in the intestine. Absence or inhibition of NPC1L1 in mice results in a >70% decreased cholesterol absorption [3]. In humans, treatment with the NPC1L1-inhibitor ezetimbe leads to a 54% decrease in cholesterol absorption [2]. The sterol transporter ATP-binding cassette G5/G8 (ABCG5/G8) antagonizes the activity of NPC1L1 by transporting sterols out of the enterocyte into the intestinal lumen [3]. Absence of ABCG5/G8 leads to sitosterolemia due to plant sterols, absorbed by NPC1L1, not being effluxed back into the intestinal lumen. Apart from the activity of transporters involved in uptake and export of sterols, solubilization of cholesterol in the intestinal lumen is an important factor impacting its

absorption. Bile acids are synthesized from cholesterol in the liver and play a central role in solubilization of lipids in the intestine by acting as biological detergents. In the absence of bile acids, only 20% of cholesterol is absorbed [4]. On the other hand, when the bile acid cholic acid is administered, cholesterol absorption is greatly enhanced [4,5]. Bile acids also affect the absorption of dietary fat, albeit with considerably less impact compared to the effect on the absorption of cholesterol. Without bile acids, absorption of fatty acids takes place more distally in the small intestine where the pH is higher [6]. It is still unclear which proteins are involved in intestinal uptake of fatty acids. CD36 could be involved, especially for long chain fatty acids. However, in absence of CD36 fatty acid uptake still takes place [7].

What is new in intestinal cholesterol absorption

Regulation of NPC1L1

The regulation of NPC1L1 is incompletely understood. The differential expression of NPC1L1 along the length of the intestinal tract appears to be regulated, at least in part, by epigenetic mechanisms involving methylation of the promoter region [8]. In addition, NPC1L1 was recently found to be negatively regulated by the transcription factor cAMP responsive element binding protein 3-like 3 (CREB3L3) [9,10], which was orignally identified as inducer of LPL co-activators [11]. In Caco-2 cells, CREB3L3 bound to the promoter region of NPC1L1 and reduced its activity in reporter assays. CREB3L3 overexpression reduced NPC1L1 expression in a dose-dependent manner in these cells [9]. Whereas chow-fed intestine-specific CREB3L3 knock out mice showed no differences compared with WT mice [10], mice expressing human CREB3L3 in the intestine showed reduced plasma cholesterol levels. Intestinal NPC1L1 expression was downregulated in these transgenic mice, while it increased in CREB3L3 knock out mice [9,10]. These data suggest that intestinal CREB3L3 is an important modulator of NPC1L1 expression.

NPC1L1–mediated cholesterol absorption may not be dependent on endocytosis

The molecular mechanism of NPC1L1-mediated cholesterol absorption is not clear. It has been suggested that NPC1L1 shuttles cholesterol molecules into the cell via endocytosis of the protein with bound cholesterol, and that this process is blocked by ezetimibe [12]. The more recent finding that in the absence of Numb, a clathrin-adaptor, both NPC1L1-endocytosis and cholesterol absorption is impeded in CRL1601 cells, further supports this mechanism [13]. Notably, both these studies used cyclodextrin to deplete medium of cholesterol. Since cyclodextrin is known to interfere with clathrin-mediated endocytosis in general, Johnson et al. recently re-investigated

cholesterol absorption using the same cell line, however in cyclodextrin-free conditions [14**]. Under these conditions, it was found that ezetimibe does not affect endocytosis rates. Furthermore, a functional mutation in NPC1L1 that slows down endocytosis did not result in decreased cholesterol absorption, and blocking of endocytosis had no effect on ezetimibe-dependent or -independent cholesterol absorption [14**]. It should be noted however, that cholesterol absorption was measured using tritium-labeled cholesterol, and that therefore no distinction could be made between cholesterol entering the plasma membrane, and cholesterol being shuttled towards intracellular compartments [14**]. That, in combination with studies in an intestine-specific knock out mouse model of Numb showing decreased cholesterol absorption in vivo, still favors the view that endocytosis of NPC1L1 is important for cholesterol absorption [13].

The molecular structure of ABCG5/G8 has been elucidated, revealing a cholesterol binding site within the transmembrane domain

The X-ray structure of ABCG5/G8 was reported this year [15*]. The ATP-free structure revealed clues as to how the asymmetry in the transmembrane domains between G5 and G8, could contribute to the mechanism of cholesterol transport. Lee et al. found that G5 forms three stabilized alfa-helices within the transmembrane domains that are adjacent to the non-active nucleotide binding site. The corresponding helices in G8 miss a glutamate-arginine salt bridge and are adjacent to the active nucleotide binding site. They therefore propose that mechanistically, the flexible part in G8 may undergo a conformational change upon ATP-hydrolysis. Molecular dynamic simulations suggest that a movement of the respective nucleotide binding domains inward is coupled with a movement of the transmembrane domain upward. A mutation associated with sitosterolemia is predicted to abrogate this coupling by disruption of a transmembrane domain polar relay. Furthermore, a possible entry site for cholesterol into the transporter was identified. Occluding the presumed entry site by A540F substitution in G5 resulted in a 6-fold reduction of transport activity in vivo.

Trans-intestinal cholesterol excretion is active in both mice and humans

In addition to the long-known hepatobiliary cholesterol secretion route, it has become clear that an alternative pathway for cholesterol removal exists that mediates cholesterol transport directly from the blood, through the enterocyte, into the intestinal lumen. This pathway is known as trans-intestinal excretion (TICE) and has been shown to be a major pathway for fecal cholesterol excretion in mice. Recently, we demonstrated TICE to be active in healthy humans as well [16**]. Using an enterotest device we were able to sample bile non-invasively in humans. Then, by estimating the bile acid pool size and biliary secretion rate using stable isotopes, and by making use of

the cholesterol/cholate ratio, the biliary cholesterol secretion could be calculated. By carefully monitoring dietary intake and taking daily fecal samples, it could be established that, under basal conditions, TICE contributes to about 35% of fecal neutral sterol excretion, although the interindividual variation was substantial.

The molecular mechanism underlying TICE is still incompletely understood. Recently, Nakano et al. suggested that TICE is mostly a function of increased brush border membrane to lumen efflux of cholesterol [17]. By oral administration of tritium-labeled cholesterol followed by perfusion of a piece of intestine still connected to the circulation, they were able to determine changes in TICE. Interestingly, an inverse relation between cholesterol absorption and TICE was observed. Furthermore, and consistent with our study, they found that ABCG5/G8 potentiates the ezetimibe-mediated induction of TICE [18]. In vitro, using Caco-2 cells, they found that cholesterol is still able to enter the brush-border when ezetimibe is supplied. The authors proposed a model where the cholesterol content in the brush-border is the main driving force behind TICE, and that ezetimibe probably interferes with NPC1L1 transporting cholesterol from brush-border to intracellular locations. While this model explains why fractional cholesterol absorption and TICE correlate negatively with each other, it fails to explain some important observations made by others. One, ezetimibe-induced increase in fecal neutral sterol excretion has not been observed to correlate with fractional cholesterol absorption [16**]. Two, while the model predicts increased ABCG5/G8-efflux after ezetimibe-mediated inhibition of NPC1L1, which is in line with observations since TICE is then increased, it would also predict increased cholesterol absorption in the absence of ABCG5/G8. Cholesterol absorption is, however, not enhanced in ABCG5/G8 – knock out mice [19]. Furthermore, the theory does not address how cholesterol enters the enterocyte from the plasma compartment in the first place.

By activating the farnesoid X receptor (FXR) using a non-steroid agonist, we recently demonstrated that the TICE pathway has a tremendous capacity and that its rate is affected by the bile acid pool composition [20**]. Our studies in mice revealed that activation of intestinal FXR leads to a robust increase of TICE. The data strongly suggest that hydrophilic bile acids (i.e., muricholic acids) stimulate cholesterol removal via the TICE pathway. Importantly, the effects were independent of cholesterol absorption, as the magnitude of TICE induction by the FXR agonist was similar in animals in which cholesterol absorption had been blocked by ezetimibe. The combined treatment of the FXR agonist and ezetimibe stimulated daily cholesterol loss up to 60% of the entire estimated pool in the mice [20**]. Activation of FXR also strongly decreased plasma cholesterol and triglyceride, however, this occurred independent of the effect on TICE. The role of FXR in control of cholesterol and triglyceride

homeostasis is complex and subject to considerable controversy. For an in depth discussion we refer to a recent review [21**].
In vitro systems are of prime interest to facilitate elucidation of the physical and molecular mechanisms underlying transintestinal cholesterol trafficking. Dugardin et al. developed such a model using Caco-2/TC7 cells in a trans-well system [22]. Model systems like this allow for more detailed study of cholesterol transport than in vivo flux measurements and can therefore be expected to contribute to the further unravelling of the mechanisms involved in TICE.

Increased fecal neutral sterol secretion rather than inhibition of cholesterol synthesis may underlie the statin-induced reduction of plasma cholesterol
It is generally presumed that the LDLc lowering effect of statins is achieved through reduced cholesterol synthesis due to inhibition HMG-CoA reductase. However, conflicting results have been reported regarding the effect of statins in vivo. Studies using biomarkers as indirect means to estimate cholesterol synthesis suggested inhibited cholesterol synthesis upon statin treatment [23], whereas a study using stable isotopes showed increased cholesterol synthesis in pravastatin-treated individuals [24]. Now, Schonewille et al. demonstrated that statins paradoxically increase hepatic cholesterol synthesis in mice. Interestingly, statin-treatment caused augmented cholesterol disposal in these mice [25**]. Of particular note, this study also showed that commonly measured biomarkers of cholesterol synthesis, such as the plasma lathosterol/cholesterol ratio, did not correspond with the substantial increase in cholesterol synthesis. This may have been a reason underlying the conflicting results reported in previous studies

What is new in Triglyceride Absorption

CD36 redistribution from the brush-border membrane is inhibited in hyperinsulinemic states
Buttet et al. provided evidence for development of a kind of resistance against acute effects of HFD on CD36 expression showing that the gene regulatory response in mice on HFD is delayed in response to HFD. In response to an oral fat bolus, CD36 was no longer redistributed from the brush border membrane to intracellular compartments in mice on HFD, while this did occur in mice on LFD. Intriguingly, the study indicated that insulin may mediate the long-term effect of HFD because streptozotocin-induced diabetes reversed these effects [26]. Fat absorption and chylomicron production were, however, enhanced in mice on HFD, suggesting that CD36 disengagement from the brush border membrane is not required for chylomicron assembly.

SR-B1 inhibitors decrease intestinal uptake capacity of fat

Inhibitors of the HDL-receptor scavenger receptor- B1 (SR-B1) have been shown to alter triglyceride metabolism in hamsters and rats [27]. Inhibition as well as genetic deficiency of SR-B1 decreased the TG excursion in plasma during an oral fat tolerance test. Moreover, the reduction of the appearance of orally administered triglycerides in the blood circulation of mice treated with a liver X receptor (LXR) agonist, was reported to depend on SR-B1. Most conceivably, activation of LXR mediated both, a loss of SR-B1 stabilization in the brush border membrane by PDZK1 and interference with *Scarb1* transcription by miR-96-5p [28].

Intestinal fat absorption requires phospholipid remodeling through the Land's pathway

Fat absorption is considered to be the result of both active and passive transport of free fatty acids across the brush border membrane. A series of recent studies on phospholipid remodeling sheds more light on how these processes interact [29–33]. Phospholipids with unsaturated chains are mostly produced through a process called phospholipid remodeling, the Land's cycle, where one of the acyl –chains of a phospholipid is exchanged for a poly-unsaturated acyl chain. Intriguingly, mice with whole body knockout of LPCAT3, the protein responsible for phospholipid remodeling in the enterocyte, show an almost complete lack of fat absorption in the proximal intestine, resulting in increased death rates during the suckling period, and the inability to survive on a high-fat diet. Paradoxically, the animals show decreased food intake when put on a high-fat diet compared to their wild-type littermates [29**]. Since exendin-9 can partly reverse the decreased food intake, GLP-1 is thought to be at least in part responsible for this phenomenon [29**]. It was found that deleting LPCAT3 in mice changed the composition of the phospholipids in the brush border membrane, and that NPC1L1, CD36 and ABCG8 were decreased at the protein level, while MTP was unchanged and FATP4 was increased [32*]. Importantly, the villi were distorted and the abundance of NPC1L1 was decreased at the brush border membrane, especially at the top of the villi [32*]. Similar findings were reported by Li et al. in the total body LPCAT3 knockout, though these authors found decreased FATP4 [34]. Since both the change in phospholipid composition and the decrease in brush border membrane – resident proteins may be expected to contribute to decreased absorption of fatty acids, it remains to be established whether the change in phospholipid composition mainly interferes with active or passive transport. The fact that intestinal fatty acid transport is almost normal in CD36 – null mice suggests however that interference with the passive component of fatty acid uptake may be more important [7].

Perturbing repackaging into triglycerides affects intestinal triglyceride absorption

After absorption of free fatty acids into the enterocytes, they need to be repackaged into triglyceride to allow transport out of the enterocyte within chylomicrons. Interfering with this process, through knocking out monoacylglycerol-acyltransferase (MGAT), decreases triglyceride absorption. More recently, it was established that interfering with more minor routes of triglyceride synthesis via glycerol-3-phosphate acyltransferase 3 (GPAT3), diacylglycerol-acyltransferase (DGAT) and acyl-CoA synthase 5 (ACSL5) respectively, also results in decreased uptake [35–37]. It should be noted that this effect only becomes evident once the animals are properly challenged, e.g. by a high-fat diet. Interestingly, DGAT knock out has been reported to increase fecal neutral sterol excretion in addition to blocking fatty acid absorption [35], suggesting interdependency of the cholesterol and triglyceride absorption pathways.

Genes newly implicated in fat absorption

The incretin GLP-2 has been shown to be involved in chylomicron production. Hsieh et al. show that GLP-2 stimulation of chylomicron production is dependent on NO-signaling [38]. However, the contribution of GLP-2 to postprandial hyperlipidemia appears to be limited, at least in apparently healthy obese men [39]. Sar1b is a GTPase involved in intracellular vesicle transport and mutations in the corresponding gene result in chylomicron retention disease. Recently, it has been shown that enhanced expression of Sar1b leads to increased fat absorption [40]. Similarly, Tm6sf2 has been implicated in lipid absorption through reduced incorporation of TG into apoB-containing lipoproteins which results in both decreased VLDL-TG secretion and reduced intestinal lipid absorption [41]. Another new gene connected to lipid absorption is Park2, which is involved in mitophagy. Park2 knock out mice present with reduced intestinal lipid absorption [42]. Future studies will have to demonstrate the significance of the impact of these genes on fat absorption.

Bile acids, regulation by hydrophobic / hydrophilic balance and effect of ASBT

No effect of the bile acid sequestrant colesevelam on GLP-1 secretion

Colesevelam, a bile acid sequestrant, inhibits bile acid reuptake, in turn leading to increased production of bile acids (from cholesterol) resulting in lower LDL-c. Colesevelam has further been shown to enhance glucose tolerance in type 2 diabetes patients. It has been suggested that this effect is mediated through *basolateral* activation of TGR5, a bile acid receptor, in L-cells, in turn stimulating GLP-1 secretion [43]. In contrast, it has also been suggested that bile acids may stimulate TGR5 directly from within the intestinal lumen, explaining why colesevelam, at least in mice, has

been observed to paradoxically increase GLP-1 secretion as well [44]. The latter mechanism however, would imply that colesevelam and exogenous supply of bile acids would have an additive effect on GLP-1 secretion. Hansen et al. have now observed in humans, that under fasting conditions, in contrast to exogenous supply of chenodeoxycholic acid, colesevelam does not increase GLP-1 secretion [43]. Moreover, no additive effect was found of co-administration of colesevelam with chenodeoxycholic acid. These findings argue against direct activation of TGR5 from within the intestinal lumen. It may be speculated, that previous findings of enhanced GLP-1 secretion by colesevelam are due to differences in nutrient sensing in the L-cells.

Perturbing bile acid production results in reduced lipid absorption

Hydrophobic bile acids are better able to stimulate cholesterol absorption than more hydrophilic bile acids [45]. Therefore, shifting the composition towards hydrophilic bile acids decreases intestinal cholesterol absorption. A number of research groups have recently explored this effect further by changing the amount and composition of bile acids by interfering in the major bile acid synthetic pathways. Ferrell et al. show that Cyp7a1-KO mice were found to be protected from high-fat/high-cholesterol diet-induced metabolic disorders [46]. Cyp7a1-KO mice had a decreased bile acid pool size, but the alternative bile acid synthesis pathway (including the expression Cyp7b1) was upregulated. This resulted in a shift of the bile acid pool composition towards more hydrophilic species (i.e. muricholic acids). Bonde et al., generated mice lacking the expression of sterol 12□-hydroxylase (Cyp8b1). Due to the absence of Cyp8b1 activity, the mice were forced to produce more chenodeoxycholic acid, the precursor of muricholic acid, instead of cholic acid. Cyp8b1-deficient mice displayed reduced weight gain, increased fecal cholesterol and FFA excretion compared to controls when fed a HFD [47]. In line with these and other findings, Xu et al. used obeticholic acid-mediated downregulation of both Cyp7a1 and Cyp8b1, inducing a muricholic acid-enriched bile acid pool, and observed increased fecal cholesterol excretion [48].

Cyp2c70 may be responsible for production of muricholic acids in mice

Bile acid metabolism in the mouse differs considerably from man. Where mice synthesize ample amounts of muricholic acids, these bile acid species are not found in humans. It is known that chenodeoxycholic acid is the precursor of muricholic acids, but the responsible enzymes have never been identified. Takahashi et al., now report that mice without the Cyp2c gene cluster are not able to produce muricholic acids. They further found that, of the genes in the Cyp2c gene cluster, only transfection with Cyp2c70 resulted in the production of muricholic acids by human HepG2 hepatoma cells. Furthermore, knock-down of Cyp2c70 mRNA in mouse primary hepatocytes

significantly reduced the production of muricholic acids [49**]. This pivotal finding requires confirmation. Unpublished studies in our laboratory show no correlation between expression of Cyp2c70 and muricholic acid synthesis. A proteomics study of Huang et al. indicated the enzyme to be more abundant in males whereas there is no clear gender difference in muricholate synthesis [50].

Circadian control of bile acids is partly mediated by KLF15

Klf15 was previously identified as an important circadian regulator, and total body knock-out mice were shown to have reduced expression of *Cyp7a1*, and as a result cholesterol and triglyceride absorption is impeded. Han et al. now show that this effect is not mediated by the liver, but by the ileum. Increasing Klf15 expression in mouse primary epithelial cells from ileal villi resulted in decreased FGF15 expression, while knock-down of Klf15 resulted in increased FGF15 expression [51*]. Of particular interest, increased Fgf15 expression and reduced bile acid syntheses were maintained in bile duct diverted Klf15 knock-out mice. This demonstrates that the effects on bile acid synthesis in these mice are independent of the presence of bile acids in the intestine and suggest that Klf15 may regulate Fgf15 directly and independent of FXR [51*].

Concluding Remarks

How are the new findings going to change the perspective on new drugs for treating atherosclerosis.

TICE has now been shown to be present in man and to be inducible by ezetimibe. While trials like IMPROVE-IT have already shown how this may be of benefit [52], the observation that TICE can be stimulated in humans validates targeting this pathway as a strategy for development of new drugs. This therapeutic potential may spur efforts to identify the basolateral players in TICE, and thereby generate new drug targets.

Shifting bile acids to a more hydrophilic profile in mice leads to effects in fat and cholesterol homeostasis that could prove beneficial to humans as well. However, considering the differences in bile acid production between mice and man, it is too early to say whether those observations may eventually be translated to the human population.

When findings in LPCAT3 knock out mice are translatable to humans, its inhibition would result in both decreased fat absorption and reduced food intake. In the current obesity epidemic, and in light of the propensity of human beings to negate an intended decrease in food intake, a drug with those combined effects would be invaluable.

Acknowledgements

This work was supported in part by European Union grant FP7- HEALTH n°305707 and Netherlands Heart Foundation Grant Genius.

References

1. Tenenbaum A, Klempfner R, Fisman EZ. Hypertriglyceridemia: a too long unfairly neglected major cardiovascular risk factor. Cardiovasc Diabetol. 2014;13(1):159.
2. Sudhop T, Lütjohann D, Kodal A, Igel M, Tribble DL, Shah S, et al. Inhibition of intestinal cholesterol absorption by ezetimibe in humans. Circulation. 2002;106(15):1943–8.
3. van der Wulp MYMM, Verkade HJ, Groen AK. Regulation of cholesterol homeostasis. Mol Cell Endocrinol. 2013 Apr 10;368(1–2):1–16.
4. Woollett L a, Wang Y, Buckley DD, Yao L, Chin S, Granholm N, et al. Micellar solubilisation of cholesterol is essential for absorption in humans. Gut. 2006;55(2):197–204.
5. Woollett LA, Buckley DD, Yao L, Jones PJH, Granholm NA, Tolley EA, et al. Cholic Acid Supplementation Enhances Cholesterol Absorption in Humans. Gastroenterology. 2004;126(3):724–31.
6. Morgan RGH, Borgström B. the Mechanism of Fat Absorption in the Bile Fistula Rat. Q J Exp Physiol Cogn Med Sci. 1969;54(2):228–43.
7. Drover VA, Nguyen D V., Bastie CC, Darlington YF, Abumrad NA, Pessin JE, et al. CD36 mediates both cellular uptake of very long chain fatty acids and their intestinal absorption in mice. J Biol Chem. 2008;283(19):13108–15.
8. Malhotra P, Soni V, Kumar A, Anbazhagan AN, Dudeja A, Saksena S, et al. Epigenetic modulation of intestinal cholesterol transporter Niemann-Pick C1-like 1 (NPC1L1) gene expression by DNA methylation. J Biol Chem. 2014;289(33):23132–40.
9. Kikuchi T, Orihara K, Oikawa F, Han S iee, Kuba M, Okuda K, et al. Intestinal CREBH overexpression prevents high-cholesterol diet-induced hypercholesterolemia by reducing Npc1l1 expression. Mol Metab. 2016;5(11):1092–102.
10. Nakagawa Y, Oi F, Mizuno S, Ohno H, Yagishita Y. Hyperlipidemia and hepatitis in liver-specific CREB3L3 knockout mice generated using a one-step CRISPR / Cas9 system. Sci Rep. 2016;(November 2015):1–11.
11. Lee JH, Giannikopoulos P, Duncan S a, Wang J, Johansen CT, Brown JD, et al. The transcription factor cyclic AMP-responsive element-binding protein H regulates triglyceride metabolism. Nat Med. 2011;17(7):812–5.
12. Ge L, Wang J, Qi W, Miao HH, Cao J, Qu YX, et al. The Cholesterol

Absorption Inhibitor Ezetimibe Acts by Blocking the Sterol-Induced Internalization of NPC1L1. Cell Metab. 2008;7(6):508–19.

13. Li P-S, Fu Z-Y, Zhang Y-Y, Zhang J-H, Xu C-Q, Ma Y-T, et al. The clathrin adaptor Numb regulates intestinal cholesterol absorption through dynamic interaction with NPC1L1. Nat Med. 2014 Dec 15;20(1):80–6.

14. **Johnson TA, Pfeffer SR. Ezetimibe-sensitive cholesterol uptake by NPC1L1 protein does not require endocytosis. Mol Biol Cell. 2016;27. This study contradicts prevous claims that NPC1L1-dependent cholesterol absorption acts through endocytosis.

15. *Lee J-Y, Kinch LN, Borek DM, Wang JJ, Wang JJ, Urbatsch IL, et al. Crystal structure of the human sterol transporter ABCG5/ABCG8. Nature. 2016;533(7604):1–17. The first report of a crystal structure of ABCG5/G8, a key step in better understanding the mechanism behind cholesterol absorption and efflux.

16. **Jakulj L, van Dijk TH, Freark de Boer J, Kootte RS, Schonewille M, Paalvast Y, et al. Transintestinal Cholesterol Transport Is Active in Mice and Humans and Controls Ezetimibe-Induced Fecal Neutral Sterol Excretion. Cell Metab. 2016;24(6):1–12. This study is the first to unquivocally show the presence and relevance of TICE for cholesterol homeostasis in humans.

17. Nakano T, Inoue I, Takenaka Y, Ono H, Katayama S, Awata T, et al. Ezetimibe promotes brush border membraneto-lumen cholesterol efflux in the small intestine. PLoS One. 2016;11(3):1–27.

18. van der Veen JN, van Dijk TH, Vrins CLJ, van Meer H, Havinga R, Bijsterveld K, et al. Activation of the liver X receptor stimulates trans-intestinal excretion of plasma cholesterol. J Biol Chem. 2009 Jul 17;284(29):19211–9.

19. Yu L, Hammer RE, Li-Hawkins J, Von Bergmann K, Lutjohann D, Cohen JC, et al. Disruption of Abcg5 and Abcg8 in mice reveals their crucial role in biliary cholesterol secretion. Proc Natl Acad Sci U S A. 2002;99(25):16237–42.

20. **de Boer JF, Schonewille M, Boesjes M, Wolters H, Bloks VW, Bos T, et al. Intestinal Farnesoid X Receptor Controls Transintestinal Cholesterol Excretion in Mice. Gastroenterology. 2017; FXR agonist strongly activates TICE. After dual treatment with ezetimibe mice excrete up to 60% of their total cholesterol pool.

21. **Gonzalez FJ, Jiang C, Patterson AD. An Intestinal Microbiota-Farnesoid X Receptor Axis Modulates Metabolic Disease. Gastroenterology. 2016;151(5):845–59. Review discussing existing conflicting data on the effect of FXR activation/inactivation on energy metabolism.

22. Dugardin C, Briand O, Touche V, Schonewille M, Moreau F, Le May C, et al. Retrograde cholesterol transport in the human Caco-2/TC7 cell line: a model to study trans-intestinal cholesterol excretion in atherogenic and diabetic dyslipidemia. Acta Diabetol. 2016;1–9.

23. Miettinen TA, Gylling H. Synthesis and absorption markers of cholesterol in

serum and lipoproteins during a large dose of statin treatment. Eur J Clin Invest. 2003;33(11):976–82.

24. Kallien G, Lange K, Stange EF, Scheibner J. The pravastatin-induced decrease of biliary cholesterol secretion is not directly related to an inhibition of cholesterol synthesis in humans. Hepatology. 1999;30(1):14–20.

25. **Schonewille M, de Boer JF, Mele L, Wolters H, Bloks VW, Wolters JC, et al. Statins increase hepatic cholesterol synthesis and stimulate fecal cholesterol elimination in mice. J Lipid Res. 2016;57:jlr.M067488. First demonstration that statin treatment increases whole body cholesterol synthesis in mice.

26. Buttet M, Poirier H, Traynard V, Gaire K, Tran TTT, Sundaresan S, et al. Deregulated lipid sensing by intestinal CD36 in diet-induced hyperinsulinemic obese mouse model. PLoS One. 2016;11(1):1–19.

27. Lino M, Farr S, Baker C, Fuller M, Trigatti B, Adeli K. Intestinal scavenger receptor class B type I as a novel regulator of chylomicron production in healthy and diet-induced obese states. Am J Physiol - Gastrointest Liver Physiol. 2015 Sep 1;309(5):G350–9.

28. Briand O, Touche V, Colin S, Brufau G, Davalos A, Schonewille M, et al. Liver X Receptor Regulates Triglyceride Absorption Through Intestinal Down-regulation of Scavenger Receptor Class B, Type 1. Gastroenterology. 2016;150(3):650–8.

29. **Wang B, Rong X, Duerr MA, Hermanson DJ, Hedde PN, Wong JS, et al. Intestinal phospholipid remodeling is required for dietary-lipid uptake and survival on a high-fat diet. Cell Metab. 2016;23(3):492–504. Demonstrates the importance of LPCAT3 in passive intestinal free fatty acid absorption.

30. Hashidate-Yoshida T, Harayama T, Hishikawa D, Morimoto R, Hamano F, Tokuoka SM, et al. Fatty acyl-chain remodeling by LPCAT3 enriches arachidonate in phospholipid membranes and regulates triglyceride transport. Elife. 2015;2015(4):1–75.

31. Rong X, Wang B, Dunham MM, Hedde PN, Wong JS, Gratton E, et al. Lpcat3-dependent production of arachidonoyl phospholipids is a key determinant of triglyceride secretion. Elife. 2015 Mar 25;4.

32. *Kabir I, Li Z, Bui HH, Kuo MS, Gao G, Jiang XC. Small intestine but not liver lysophosphatidylcholine acyltransferase 3 (lpcat3) deficiency has a dominant effect on plasma lipid metabolism. J Biol Chem. 2016;291(14):7651–60. This study shows that LPCAT3 - effects are mainly mediated through the intestine and that the absence of intestinal LPCAT3 results in distorted villi and a decrease of NPC1L1 at the brush border membrane.

33. Cash JG, Hui DY. Liver-specific overexpression of LPCAT3 reduces postprandial hyperglycemia and improves lipoprotein metabolic profile in mice. Nutr Diabetes. 2016;6(4):e206.

34. Li Z, Jiang H, Ding T, Lou C, Bui HH, Kuo MS, et al. Deficiency in Lysophosphatidylcholine Acyltransferase 3 Reduces Plasma Levels of Lipids

by Reducing Lipid Absorption in Mice. Gastroenterology. 2015;149(6):1519–29.

35. Sachdev V, Leopold C, Bauer R, Patankar J V., Iqbal J, Obrowsky S, et al. Novel role of a triglyceride-synthesizing enzyme: DGAT1 at the crossroad between triglyceride and cholesterol metabolism. Biochim Biophys Acta - Mol Cell Biol Lipids. 2016;1861(9):1132–41.

36. Bowman TA, O'Keeffe KR, D'Aquila T, Yan QW, Griffin JD, Killion EA, et al. Acyl CoA synthetase 5 (ACSL5) ablation in mice increases energy expenditure and insulin sensitivity and delays fat absorption. Mol Metab. 2016;5(3):210–20.

37. Khatun I, Clark RW, Vera NB, Kou K, Erion DM, Coskran T, et al. Characterization of a novel intestinal glycerol-3-phosphate acyltransferase pathway and its role in lipid homeostasis. J Biol Chem. 2016;291(6):2602–15.

38. Hsieh J, Trajcevski KE, Farr SL, Baker CL, Lake EJ, Taher J, et al. Glucagon-like peptide 2 (GLP-2) stimulates postprandial chylomicron production and postabsorptive release of intestinal triglyceride storage pools via induction of nitric oxide signaling in male hamsters and mice. Endocrinology. 2015 Oct;156(10):3538–47.

39. Matikainen N, Bjornson E, Soderlund S, Boren C, Eliasson B, Pietilainen KH, et al. Minor contribution of endogenous GLP-1 and GLP-2 to postprandial lipemia in obese men. PLoS One. 2016;11(1):1–15.

40. Levy E, Spahis S, Garofalo C, Marcil V, Montoudis A, Sinnet D, et al. Sar1b transgenic male mice are more susceptible to high-fat diet-induced obesity, insulin insensitivity and intestinal chylomicron overproduction. J Nutr Biochem. 2014 May;25(5):540–8.

41. Smagris E, Gilyard S, BasuRay S, Cohen JC, Hobbs HH. Inactivation of Tm6sf2, a gene defective in fatty liver disease, impairs lipidation but not secretion of very low density lipoproteins. J Biol Chem. 2016;291(20):10659–76.

42. Frank DN, Bales ES, Monks J, Jackman MJ, MacLean PS, Ir D, et al. Perilipin-2 modulates lipid absorption and microbiome responses in the mouse intestine. PLoS One. 2015;10(7):1–19.

43. Hansen M, Scheltema MJ, Sonne DP, Hansen JS, Sperling M, Rehfeld JF, et al. Effect of chenodeoxycholic acid and the bile acid sequestrant colesevelam on glucagon-like peptide-1 secretion. Diabetes, Obes Metab. 2016;571–80.

44. Potthoff MJ, Potts A, He T, Duarte JA, Taussig R, Mangelsdorf DJ, et al. Colesevelam suppresses hepatic glycogenolysis by TGR5-mediated induction of GLP-1 action in DIO mice. Am J Physiol Gastrointest Liver Physiol. 2013;304(4):G371-80.

45. Heuman DM. Quantitative estimation of the hydrophilic-hydrophobic balance of mixed bile salt solutions. J Lipid Res. 1989;30(5):719–30.

46. Ferrell JM, Boehme S, Li F, Chiang JYL. Cholesterol 7α-hydroxylase-deficient mice are protected from high-fat/high-cholesterol diet-induced metabolic

disorders. J Lipid Res. 2016;57(7):1144–54.

47. Bonde Y, Eggertsen G, Rudling M. Mice abundant in muricholic bile acids show resistance to dietary induced steatosis, weight gain, and to impaired glucose metabolism. PLoS One. 2016;11(1).

48. Xu Y, Li F, Zalzala M, Xu J, Gonzalez FJ, Adorini L, et al. Farnesoid X receptor activation increases reverse cholesterol transport by modulating bile acid composition and cholesterol absorption in mice. Hepatology. 2016;64(4):1072–85.

49. **Takahashi S, Fukami T, Masuo Y, Brocker CN, Xie C, Kristopher W, et al. Cyp2c70 is responsible for the species difference in bile acid metabolism between mice and humans. J Lipid Res. 2016;57. This study identifies the enzyme responsible for the synthesis of muricholic acids.

50. Huang HJ, Tsai ML, Chen YW, Chen SH. Quantitative shot-gun proteomics and MS-based activity assay for revealing gender differences in enzyme contents for rat liver microsome. J Proteomics. 2011;74(12):2734–44.

51. *Han SS, Zhang R, Jain R, Shi H, Zhang L, Zhou G, et al. Circadian control of bile acid synthesis by a KLF15-Fgf15 axis. Nat Commun. 2015;6:7231. Here it is shown that KLF15 has an important role in the FGF15-mediated control of circadian rhythm, independent of bile acid reabsorption, thereby providing an alternative pathway to perturb hepatic FXR-activity.

52. Murphy SA, Cannon CP, Blazing MA, Giugliano RP, White JA, Lokhnygina Y, et al. Reduction in Total Cardiovascular Events with Ezetimibe/Simvastatin Post-Acute Coronary Syndrome the IMPROVE-IT Trial. J Am Coll Cardiol. 2016;67(4):353–61.