Liver Research 3 (2019) 25–30

Contents lists available at ScienceDirect

Liver Research

journal homepage: http://www.keaipublishing.com/en/journals/liver-research

Review Article

Role of gut microbiota in the development of non-alcoholic fatty liver disease☆

Xuemei Wang, Jialin Xia, Changtao Jiang*

Department of Physiology and Pathophysiology, School of Basic Medical Sciences, Peking University, The Key Laboratory of Molecular Cardiovascular Science, Ministry of Education, Beijing, China

ARTICLE INFO

Article history:
Received 29 June 2018
Received in revised form
1 November 2018
Accepted 25 January 2019

Keywords:
Non-alcoholic fatty liver disease (NAFLD)
Gut microbiota
Bile acid
Farnesoid X receptor (FXR)
Ceramide
Bile salt hydrolase (BSH)

ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is a chronic liver disease characterized by hepatic steatosis in the absence of other causes, such as chronic alcohol consumption, that cause secondary hepatic fat accumulation. NAFLD has become the most common liver disease worldwide over the past two decades, and the prevalence of NAFLD is 20–30% in Western countries. However, the mechanism of NAFLD remains unclear. The gut microbiota plays an important role in the metabolism of the host; in fact, it has been implicated in inflammatory diseases, metabolic syndrome and cardiovascular disease. Accumulating evidence has indicated that gut microbiota component changes are linked to human obesity, insulin resistance (IR), type 2 diabetes and NAFLD. Here, we provide insight into the role of gut microbiota, especially bile salt hydrolase (BSH) in modulating the bile acid pool and farnesoid X receptor (FXR), which promotes the synthesis of ceramide and contributes to the development of NAFLD.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) has become the most common liver disease worldwide over the past two decades. The mechanisms of NAFLD remain largely unknown. Previous studies have reported that alterations of the gut microbiota are related to obesity, insulin resistance (IR), type 2 diabetes and NAFLD.[1–5] The gut microbiota derives nutrients from fermenting indigestible host dietary components and generates metabolites, including short-chain fatty acids (SCFAs), trimethylamine (TMA) and bile acids, which play a vital role in host metabolism after they are reabsorbed into the blood.

Bile acids, synthesized mainly from cholesterol in hepatocytes, are a class of important endogenous metabolites that are metabolized by the gut microbiota. Bile acids regulate lipid and glucose metabolism and energy expenditure via endogenous receptors, which include farnesoid X receptor (FXR) and G-protein-coupled bile acid receptor 1 (GPBAR1, also known as TGR5). Studies have demonstrated that upon high-fat diet (HFD) feeding, intestinal-specific FXR inhibition substantially reduced hepatic triglyceride accumulation by suppressing ceramide biosynthesis.[6,7] In this review, we will discuss the relationship between the gut microbiota and NAFLD as well as potential therapeutic targets for NAFLD.

2. NAFLD

NAFLD is a chronic liver disease characterized by hepatic steatosis in the absence of other causes of secondary hepatic fat accumulation. NAFLD includes the stages of non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH).[8] NAFLD may lead to fibrosis, cirrhosis and hepatocellular carcinoma (HCC) and is a risk factor for cardiovascular disease and type 2 diabetes mellitus.[9]

Studies have reported that the prevalence of NAFLD is 20–30% in Western countries, and approximately 2–3% of NAFLD cases develop into NASH, which is associated with hepatic inflammation and is more severe than NAFL.[10] Research has suggested that the global prevalence of NAFLD has increased to 24%. The Middle East, South America, Asia, USA and Europe are the top five areas where NAFLD was most commonly reported, with prevalence rates of 32%, 31%, 27%, 24% and 23%, respectively.[11]

To date, the mechanism of NAFLD remains unclear. Previous studies have shown that NAFLD begins with lipid accumulation in the liver. Under normal conditions, the lipid accumulated in hepatocytes comes from fatty acids (FAs) that are released by

☆ Edited by Peiling Zhu and Genshu Wang.

* Corresponding author.
E-mail address: jiangchangtao@bjmu.edu.cn (C. Jiang).

https://doi.org/10.1016/j.livres.2019.01.006

peripheral adipose tissue, absorbed from the diet and synthesized via *de novo* lipogenesis in the liver. Lipids are eliminated by FA oxidation and triacylglycerol (TG) secretion.[12] When this balance is deregulated and accumulation exceeds elimination, NAFLD occurs.

It has been shown that patatin-like phospholipase domain containing 3 (PNPLA3) is related by circulating TGs.[13] PNPLA3 can increase the size of lipid droplets by co-expression with the TG lipase patatin-like phospholipase domain containing 2 (PNPLA2) activator abhydrolase domain containing 5 (ABHD5/CGI-58).[14] Members of the family with sequence similarity 3 (*FAM3*) gene family are also important regulators of glucose and lipid metabolism. *FAM3A* represses hepatic gluconeogenesis and lipogenesis by inhibiting the expression of gluconeogenic and lipogenic genes, including phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase (G6Pase) and fatty acid synthase (FAS), and increasing the expression of genes involved in lipid oxidation, such as adiponectin receptor 1 (AdipoR1), uncoupling protein 2 (UCP2) and peroxisome proliferator-activated receptor-α (PPAR-α). FAM3B overexpression reduces phosphorylated RAC-α serine/threonine-protein kinase (Akt), phosphorylated AMP-activated protein kinase (AMPK) and phosphorylated Forkhead box protein O1 (FOXO1) protein levels but increases FOXO1, PPAR-γ, sterol regulatory element-binding protein-1 (SREBP-1) and FAS protein levels. FAM3C induces the activation of the calmodulin (CaM)-phosphatidylinositol 3-kinase (PI3K)-Akt pathway. Imbalance among hepatic FAM3A, FAM3B and FAM3C expression levels and signaling networks contributes to the progression of steatosis.[15] The activation of transcription factors, such as SREBP-1, carbohydrate response element-binding protein (ChREBP) and PPAR-γ, can increase hepatic *de novo* lipogenesis.[16] An isoform of SREBP-1, SREBP-1c, significantly regulates the activation of *de novo* lipogenesis.[17] Moreover, SREBP-2 is involved in cellular cholesterol homeostasis. The dysregulation of these two genes is involved in hepatic fat accumulation.[18]

Decreased lipid elimination contributes to NAFLD. Lipids as energy are utilized by mitochondria. The inhibition of free fatty acid (FFA) β-oxidation further promotes hepatic lipid accumulation. Excess TGs are secreted into the circulation as very low-density lipoprotein (VLDL) particles with the help of apolipoproteins.[19] Liver-specific apolipoprotein A5 (ApoA5) might influence lipid accumulation mediated by the PPAR-γ pathway.[20] ApoA5 is also downregulated by liver X receptor (LXR)'s target gene SREBP-1c.[21]

In addition to liver lipid metabolism imbalance, adipose tissue dysfunction is also crucial for NAFLD. Adipose tissue depots increase FFA flux, leading to greater long-chain fatty acyl-coenzyme A (CoA) availability. Adipose tissue dysfunction results in an imbalance of adipokines that may profoundly affect not only the adipose tissue itself but also the liver.[22]

The gut has complex crosstalk with liver, especially in lipid metabolism. Intestine-specific FXR disruption reduces hepatic TG accumulation. The inhibition of intestinal FXR signaling suppresses ceramide synthesis, resulting in decreased serum ceramide levels. Decreased circulating ceramides downregulate hepatic SREBP-1c expression, resulting in decreased hepatic steatosis.[6] The gut microbiota plays an important role in lipid metabolism through mediating the gut-liver axis.

3. Effect of gut microbiota on metabolites

In the adult intestine, there are 10^{13}–10^{14} microorganisms that are collectively called the gut microbiota.[23] A healthy gut microbiota consists mainly of Phylum Bacteroidetes and Phylum Firmicutes.[24] Accumulating evidence has shown the role of the gut microbiota in inflammatory disease and metabolic syndrome, including obesity, IR, fatty liver, dyslipidemia, hypertension and diabetes.[4,25–29] Some studies have indicated that the gut microbiota plays an important role in the development of atherosclerotic cardiovascular disease and other related diseases.[30,31]

In intestinal microbiota studies, we have to consider the difference between human and mice. Because of the difference of dietary habits between the human and mice, it is no surprise that the intestinal microbiota varies.[32] It was reported that while some genera was found in both mouse and human, the abundance was quite different.

Furthermore, only 4% of the bacteria were the shared genera between mouse and human, about 80 genera.[33,34] For instance, the murine intestinal tract harbors a specific Firmicutes, called Candidatus arthromitus, which is not found in human and plays an important role in the innate immune system.[35–37]

It seems clear that changes in the quantity and variety of organisms within the gut microbiota are correlated to the host physiology, but the mechanism underlying how the gut microbiota influences the host remains largely unknown. The gut microbiota derives nutrients from fermenting indigestible host dietary components and generates metabolites, including SCFAs, TMA and bile acids, which play a vital role in host metabolism after they are absorbed into the blood. In addition, organisms from the gut microbiota enter the blood and affect the host metabolism directly.[38]

3.1. SCFAs

SCFAs are an essential energy source for the gut microbiota and host, and are composed of butyrate, propionate and acetate[39,40]; the gut microbiota uses indigestible carbohydrates to generate SCFAs mainly in the caecum and proximal colon, and these are absorbed into the bloodstream from the colon.[41,42] In the intestine, most bacteria participate in the production of SCFAs; these types of bacteria include *Bacteroides*, *Bifidobacterium*, *Eubacterium*, *Ruminococcus*, *Lactobacillus*, *Clostridium*, and *Streptococcus*.

SCFAs are produced by the gut microbiota and absorbed by the colon, where the concentration of SCFAs reaches 100 mM[43]; the SCFA concentration in the blood is 0.1–10 mM.[44] It is well established that G-protein-coupled receptors (GPCRs) are the receptors of SCFAs in the host, and they sense signals from the microbes.[45] The well-studied SCFA receptors include GPR43, GPR41, GPR109a and olfactory receptor 78 (OLFR78); through these receptors, SCFAs affect glucagon-like peptide-1 (GLP-1) and peptide tyrosine tyrosine (PYY) release, fat accumulation, energy homeostasis, insulin sensitivity and inflammatory responses.[46–48]

3.2. TMA

TMA is formed from dietary choline, L-carnitine and betaine; this process is mediated by the two-component Rieske-type oxygenase/reductase (CntAB) from human microbiotas,[49] which can be oxidized to trimethylamine N-oxide (TMAO) by hepatic flavin-containing monooxygenase 3 (FMO3).[50] It is reported that FMO3 is up-regulated by bile acids that activate FXR, but the basal levels of FMO3 are not controlled by FXR.[51] TMAO is well known as a risk factor for atherosclerosis because it reduces reverse cholesterol transport, changes bile acid metabolism and activates the inflammatory response to promote macrophage foam cell formation.[52] 3,3-dimethyl-1-butanol (DMB), an analogue of choline, has been reported to suppress TMAO formation by inhibiting microbial TMA lyases, thus alleviating the progression of atherosclerosis and related cardiovascular disease.[53] An epidemiological study has shown that circulating TMA levels are an independent risk factor for NAFLD in Chinese adults.[54]

3.3. Bile acids

Bile acids, synthesized from cholesterol in the liver, are an important class of endogenous metabolites that are metabolized by the gut microbiota. Primary bile acids include cholic acid (CA) and chenodeoxy-cholic acid (CDCA) in humans, while mice also produce muricholic acid (MCA); these bile acids are conjugated with glycine or taurine and stored in the gallbladder.

Food intake stimulates the release of bile acids into the duodenum to facilitate the absorption of dietary lipids. In the gastrointestinal tract, primary bile acids are modified into secondary bile acids by the gut microbiota, are reabsorbed through the ileum and travel back to the liver via the portal blood; this pathway is called the enterohepatic circulation. In addition, unconjugated bile acids are excreted in the feces; these excreted bile acids account for 5% of the bile acid pool.[55]

In the liver, there are 17 enzymes that participate in bile acid biosynthesis via two different pathways. The classic pathway produces CA and CDCA and accounts for 75% of bile acid generation. Cholesterol 7α-hydroxylase (CYP7A1) is the rate-limiting enzyme of this classic pathway.[56] The alternative pathway generates primarily CDCA, and CDCA is converted to MCA in mice. There are some differences in the composition of bile acids between humans and mice. Ursodeoxycholic acid (UDCA) is the secondary bile acid in human but the primary bile acid in mice. In addition, CA and CDCA are the most abundant bile acids in human, but in mice, CA and MCA are the primary bile acids. What's more, glycine-conjugated bile acids are the major form and the ratio of glycine and taurine is about 3:1 in human, but in mice, taurine-conjugated bile acids are the most abundant. What's more, UDCA is the secondary bile acid in human and the primary bile acid in mice.

In bile acid metabolism, the gut microbiota takes part in deconjugating primary bile acids via bile salt hydrolase (BSH) activity. BSH is produced by *Lactobacilli*, *Bifidobacterium*, *Clostridium* and *Bacteroides* and is associated with bile toxicity resistance.[57]

Bile acids regulate lipid and glucose metabolism and energy expenditure via endogenous receptors, mainly FXR and TGR5. Many studies have demonstrated that FXR plays an important role in bile acid synthesis by inhibiting the transcription of the *CYP7A1* and sterol 12α-hydroxylase (*CYP8B1*) genes via inducing small heterodimer partner (SHP), which represses the trans-activation of hepatic nuclear factor 4α (HNF4α) and liver receptor homologue 1 (LRH1).[58] In the intestine, bile acids activate FXR to induce fibroblast growth factor 15 (FGF15, FGF19 in humans), thus activating hepatic FGF receptor 4 (FGFR4) and downstream signaling molecules to inhibit the transcription of *CYP7A1* and *CYP8B1* via the c-Jun N-terminal kinases (JNK)/extracellular signal-regulated kinase (ERK) pathway.[59]

4. Interaction of the gut microbiota and NAFLD

The composition of the gut microbiota is affected by many factors, including diet.[60] Undoubtedly, diet is an important regulator of the gut microbiota that impacts gut microbiota metabolites, which modulate host metabolism. Accumulating evidence has indicated that alterations to gut microbiota components are linked to human obesity, IR, type 2 diabetes and NAFLD.[1–5] Metagenomic studies showed that the gut microbiota in obese mice had a high capacity for harvesting energy, and this ability was reversed when the gut microbiota from obese mice was transplanted into germ-free mice, which then accumulated more fat.[61] The amount of abundant *Bacteroides* species was decreased in obese people, particularly *B. thetaiotaomicron*, which is positively associated with resistance to adiposity.[62] When mice were exposed to 12 °C for 4 weeks, the gut microbiota composition was characterized by increased *Adlercreutzia*, *Mogibacteriaceae*, *Ruminococcaceae*, and *Desulfovibrio* and reduced *Bacilli* and *Erysipelotrichaceae*; these changes contributed to reduced diet-induced obesity.[63] A study of obese humans administered antibiotics (amoxicillin or vancomycin) for 7 days indicated that vancomycin decreased the gut microbiota diversity and the abundance of Firmicutes, which is related to SCFA and bile acid generation.[64] Calorie-restriction (CR) is the most common strategy for weight loss. Intermittent fasting, one form of CR, shifts the gut microbiota composition and results in higher SCFA production, which promotes white adipose browning and alleviates obesity.[65] The antidiabetic medication acarbose increased *Lactobacillus* and *Bifidobacterium* and the ratio of primary bile acids to secondary bile acids, which contributed to improving the metabolic states of type 2 diabetes patients.[66]

Evidence has shown that mice treated with antibiotics or tempol are resistant to HFD-induced NAFLD due to alterations to the bile acid pool; tauro-β-muricholic acid (T-β-MCA), which inhibits intestinal FXR signaling, was increased. The same phenomenon was also observed with reductions to the genus *Lactobacillus*.[1] Increased levels of T-β-MCA resulted from reduced BSH with decreases to the genus *Lactobacillus*.[6] Treating mice with a BSH inhibitor, caffeic acid phenethyl ester (CAPE), increased the T-β-MCA levels in the intestine and inhibited intestinal FXR signaling.[7] A high-affinity FXR antagonist, glycine-β-muricholic acid (Gly-MCA), which could not be hydrolyzed by BSH was also designed. Gly-MCA reversed diet-induced obesity and IR by inhibiting intestinal FXR signaling.[67] The suppression of intestinal FXR reduced FGF15 levels, consequently increasing *CYP7A1* transcription and bile acid biosynthesis in the liver. Furthermore, HFD feeding and intestinal FXR disruption specifically reduced hepatic triglyceride levels by suppressing ceramide biosynthesis.[6,7] Moreover, colesevelam, a bile acid binding resin, could improve cholestatic liver injury in multidrug resistance-2 $(Mdr2)^{-/-}$ mice via inhibiting intestinal FXR.[68,69]

Abundant studies have reported that gut microbiota dysbiosis might contribute to the development of NAFLD due to impaired intestinal barrier function, which meanwhile leads to the excess production of gut-derived endotoxin, such as lipopolysaccharide (LPS).[70] The portal circulation delivers the blood from gut directly to the liver, which makes the liver become the first to be affected by the gut-derived endotoxin.[71] LPS is responsible for several liver diseases. The activation of toll-like receptor (TLR) by LPS mediated innate immunity, which is considered one factor of NAFLD. In particular the activation of TLR4 in Kupffer cells and stellate cells stimulates pro-inflammatory and accelerates the progression of NASH.[72] Moreover, the TLR4 and TLR9 can activate the nucleotide-binding oligomerization domain-, leucine-rich repeat- and pyrin domain-containing 3 (NLRP3) inflammasomes, contributing to the fibrosis process.[73]

5. FXR as new therapeutic target for NAFLD

Currently, there are few effective treatments for NAFLD. Some studies have demonstrated that calorie restriction and exercise have beneficial effects on weight loss. It is recognized that lifestyle modifications combined with target therapy are more effective.

Due to its distinct glucose-lowering effects, metformin has been the most widely prescribed oral antidiabetic drug in Europe since 1957 and in the USA since 1995.[74] Accumulating evidence indicates that metformin can positively ameliorate NAFLD in obese mice.[75–77] Furthermore, other studies suggest that metformin treatment improves glucose homeostasis in diet-induced obese mice by modulating the gut microbiota.[78–81] Metformin altered the composition and significantly reduced the diversity of the gut microbiota, but the SCFA-producing bacteria were increased.[79] This finding indicated that SCFAs may play a beneficial role in host

metabolism. Evidence indicated that *Akkermansia muciniphila* and *Clostridium cocleatum* abundance was increased when obese mice were treated with metformin,[78] and the administration of *Akkermansia muciniphila* prevented diet-induced obesity in mice.[82,83] Amuc_1100, a protein of *Akkermansia muciniphila*, partly caused the beneficial effect of reducing fat mass accumulation and IR.[84]

It was reported that the serum concentration of the FXR agonist CDCA was reduced and that FXR signaling was suppressed in patients with NAFLD.[85] Obeticholic acid (OCA), a semisynthetic CDCA derivative and FXR agonist, have been approved to treat primary biliary cirrhosis (PBC) by the USA food and drug administration (FDA). Moreover, OCA improved the histological features of NASH in clinical trials.[86] Treatment with 25 or 50 mg OCA for 6 weeks improved insulin sensitivity and reduced liver inflammation in NAFLD patients.[87]

Since the activation of intestinal FXR signaling mediates HFD-induced hepatic lipid accumulation, it is considered a potential drug target for the treatment of NAFLD (Fig. 1). Results indicate that inhibition of intestinal FXR signaling is a protective strategy for the development of NAFLD. In the intestine, T-β-MCA and Gly-MCA inhibited FXR to reduce plasma ceramide levels, thus alleviating NAFLD. T-β-MCA is rapidly hydrolyzed by BSH, which is expressed by the gut microbiota. Gly-MCA is more stable in the intestine where it is resistant to BSH. The beneficial effects of T-β-MCA and Gly-MCA were reversed by the administration of the FXR agonist GW4064.[7,67]

A study has reported that the intestinal FXR agonist fexeramine promoted adipose tissue browning to improve IR and hepatic steatosis.[88] The reasons for the different results are as follows. In that study, the fexeramine dose (100 mg/kg) was ten-fold higher than that of Gly-MCA (10 mg/kg). Furthermore, fexeramine increased the proportion of lithocholic acid (LCA), an endogenous ligand of TGR5, which may be different from the effect of FXR. A recent mechanistic study showed that fexeramine metabolic improvements resulted from the higher abundance of the genus *Acetatifactor*, and *Bacteroides* increased taurolithocholic acid (TLCA) levels, which produced CDCA and UDCA, to activate TGR5/GLP-1 signaling.[89]

In addition, the administration of UDCA was found to inhibit intestinal FXR activation and decrease circulating FGF19 levels, thus resulting in TG accumulation in the liver.[90] Gly-MCA appears to be the best intestinal FXR antagonist for treating NAFLD, but its safety should be evaluated. More studies should be carried out to identify the side effects of Gly-MCA.

Ceramides are important lipids for maintaining skin and cell functions. However, in HFD-induced obese mice, serum ceramide levels are increased and are associated with hepatic inflammation, lipotoxicity, IR, ER stress and mitochondrial dysfunction. It has reported that ceramide induces IR by inhibiting mediators of the insulin signaling pathway, including insulin receptor substrate 1 (IRS1), PI3K and Akt/protein kinase B (PKB).[91] Ceramide treatment enhanced the expression of mRNA involved in ER stress.[7] Ceramide has three synthesis pathways: *de novo* pathway from palmitate; sphingomyelin pathway, and salvage pathway.[92] Sphingomyelinase (SMase) is one synthesis pathway of ceramide and the activation of SMase response to stress and pro-inflammatory by JNK signaling induces apoptosis in liver and aggravates the process of NASH.[93]

Study has revealed that decreased ceramide levels decreased hepatic mitochondrial acetyl-CoA levels and pyruvate carboxylase (PC) activity, thus reducing hepatic gluconeogenesis.[7] Fibroblast growth factor 21 (FGF21) improves the IR by reducing cellular

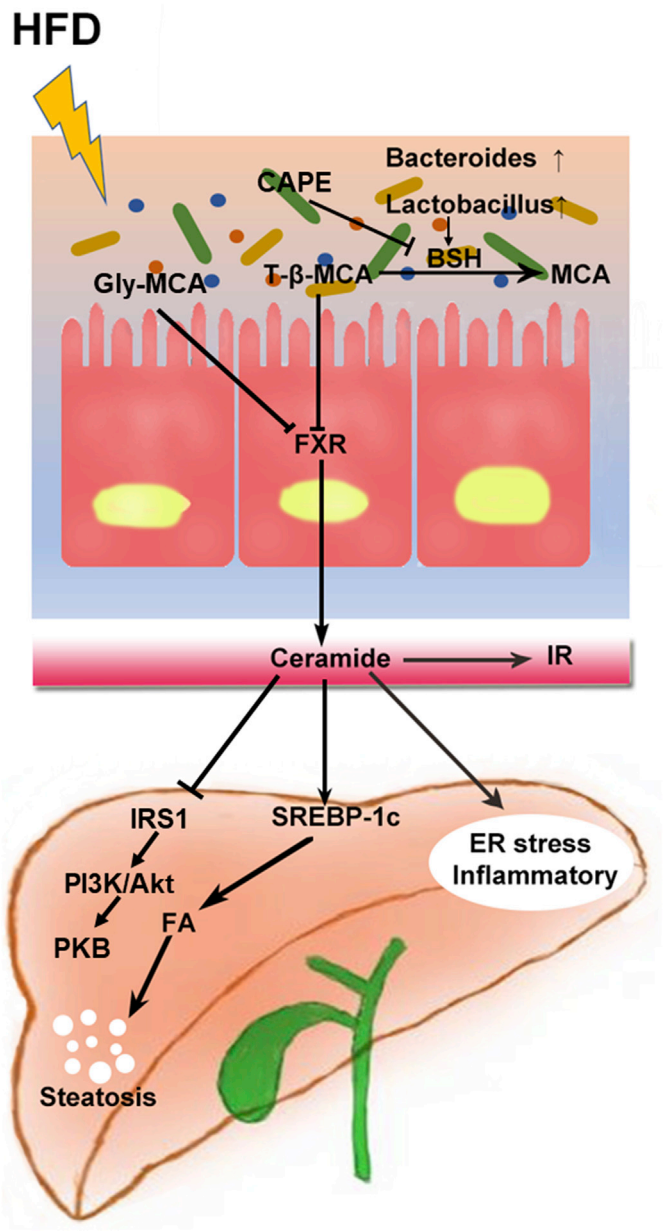

Fig. 1. Role of intestinal FXR in the development of NAFLD. On chow diet, intestinal FXR signaling is inhibited by T-β-MCA, which is generated in the liver and secreted into intestine. Gly-MCA, the analogue of T-β-MCA has the same effect. Inhibited FXR downregulated ceramide level by suppressing expression of genes involved in ceramide synthesis. Decreased circulated ceramide level repressed hepatic SREBP-1c pathway, reduced the FA level and alleviated hepatic steatosis. HFD remodeled the gut microbiota, characterized by increased *Bacteroides* and *Lactobacillus* genus, which can generate BSH. BSH is a bacterial enzyme that hydrolyzes T-β-MCA to MCA. The reducing of T-β-MCA released suppression of intestinal FXR signaling, increased serum ceramide level and aggravated hepatic steatosis, IR, ER stress and inflammatory. Ceramide induces IR by inhibiting mediators of the insulin signaling pathway, including IRS1, PI3K and Akt/PKB. Inhibiting the BSH by CAPE can increase the level of T-β-MCA and improve metabolic disorder. Abbreviations: Akt, threonine-protein kinase; BSH, bile salt hydrolase; CAPE, caffeic acid phenethyl ester; ER, endoplasmic reticulum; FA, fatty acid; FXR, farnesoid X receptor; Gly-MCA, glycine-β-muricholic acid; HFD, high-fat diet; IR, insulin resistance; IRS1, insulin receptor substrate 1; MCA, muricholic acid; PI3K, phosphatidylinositol 3-kinase; PKB, protein kinase B; SCFAs, short-chain fatty acids; SREBP-1c, sterol regulatory element-binding protein-1c; TMA, trimethylamine; T-β-MCA, tauro-β-muricholic acid.

ceramide.[94] Some studies have reported that inhibition of intestinal FXR signaling by CAPE could downregulate ceramide levels and ceramide synthesis-related genes were reduced.[7]

6. Conclusions

Several lines of evidence have indicated that the gut microbiota participates in diverse metabolic diseases, including IR, obesity and metabolism-related cardiovascular disease, of which NAFLD has attracted considerable attention in recent years because of its increasing prevalence. A consensus has been reached that food restriction and exercise have good effects on NAFLD. However, the lack of efficacious drugs has increased the risk of complications in patients. Here, we propose that inhibiting intestinal FXR signaling alleviates NAFLD by decreasing the intestinal production of ceramide. The FXR agonists T-β-MCA and Gly-MCA caused significant improvements in NAFLD.

Authors' contributions

X. Wang and J. Xia contributed equally to this study. X. Wang, J. Xia, and C. Jiang wrote the manuscript. All the authors edited the manuscript and approved the final manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

This work was supported by the National Key Research and Development Program of China (2016YFC0903100, 2016YFC0903102), the National Natural Science Foundation of China (81522007, 81470554, 31401011), and the Fundamental Research Funds for the Central Universities: Clinical Medicine Plus X - Young Scholars Project of Peking University (PKU2018LCXQ013).

References

1. Li F, Jiang C, Krausz KW, et al. Microbiome remodelling leads to inhibition of intestinal farnesoid X receptor signalling and decreased obesity. *Nat Commun*. 2013;4:2384.
2. Tilg H, Moschen AR. Microbiota and diabetes: an evolving relationship. *Gut*. 2014;63:1513–1521.
3. Qin J, Li Y, Cai Z, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature*. 2012;490:55–60.
4. Aqel B, DiBaise JK. Role of the gut microbiome in nonalcoholic fatty liver disease. *Nutr Clin Pract*. 2015;30:780–786.
5. Le Roy T, Llopis M, Lepage P, et al. Intestinal microbiota determines development of non-alcoholic fatty liver disease in mice. *Gut*. 2013;62:1787–1794.
6. Jiang C, Xie C, Li F, et al. Intestinal farnesoid X receptor signaling promotes nonalcoholic fatty liver disease. *J Clin Invest*. 2015;125:386–402.
7. Xie C, Jiang C, Shi J, et al. An intestinal farnesoid X receptor-ceramide signaling axis modulates hepatic gluconeogenesis in mice. *Diabetes*. 2017;66:613–626.
8. Sattar N, Forrest E, Preiss D. Non-alcoholic fatty liver disease. *BMJ*. 2014;349:g4596.
9. Bellentani S. The epidemiology of non-alcoholic fatty liver disease. *Liver Int*. 2017;37:81–84.
10. Bellentani S, Scaglioni F, Marino M, Bedogni G. Epidemiology of non-alcoholic fatty liver disease. *Dig Dis*. 2010;28:155–161.
11. Younossi Z, Anstee QM, Marietti M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol*. 2018;15:11–20.
12. Mardinoglu A, Wu H, Bjornson E, et al. An integrated understanding of the rapid metabolic benefits of a carbohydrate-restricted diet on hepatic steatosis in humans. *Cell Metab*. 2018;27:559–571 (e5).
13. Hyysalo J, Gopalacharyulu P, Bian H, et al. Circulating triacylglycerol signatures in nonalcoholic fatty liver disease associated with the I148M variant in PNPLA3 and with obesity. *Diabetes*. 2014;63:312–322.
14. Chamoun Z, Vacca F, Parton RG, Gruenberg J. PNPLA3/adiponutrin functions in lipid droplet formation. *Biol Cell*. 2013;105:219–233.
15. Zhang X, Yang W, Wang J, Meng Y, Guan Y, Yang J. FAM3 gene family: a promising therapeutical target for NAFLD and type 2 diabetes. *Metabolism*. 2018;81:71–82.
16. George J, Liddle C. Nonalcoholic fatty liver disease: pathogenesis and potential for nuclear receptors as therapeutic targets. *Mol Pharm*. 2008;5:49–59.
17. Schultz JR, Tu H, Luk A, et al. Role of LXRs in control of lipogenesis. *Genes Dev*. 2000;14:2831–2838.
18. Musso G, Gambino R, Cassader M. Cholesterol metabolism and the pathogenesis of non-alcoholic steatohepatitis. *Prog Lipid Res*. 2013;52:175–191.
19. Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *J Clin Invest*. 2005;115:1343–1351.
20. Ress C, Moschen AR, Sausgruber N, et al. The role of apolipoprotein A5 in non-alcoholic fatty liver disease. *Gut*. 2011;60:985–991.
21. Jakel H, Nowak M, Moitrot E, et al. The liver X receptor ligand T0901317 down-regulates APOA5 gene expression through activation of SREBP-1c. *J Biol Chem*. 2004;279:45462–45469.
22. Gregor MF, Hotamisligil GS. Thematic review series: adipocyte Biology. Adipocyte stress: the endoplasmic reticulum and metabolic disease. *J Lipid Res*. 2007;48:1905–1914.
23. Ramakrishnan SK, Shah YM. Role of intestinal HIF-2alpha in health and disease. *Annu Rev Physiol*. 2016;78:301–325.
24. Jandhyala SM, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Nageshwar Reddy D. Role of the normal gut microbiota. *World J Gastroenterol*. 2015;21:8787–8803.
25. Hakansson A, Molin G. Gut microbiota and inflammation. *Nutrients*. 2011;3:637–682.
26. Schachter J, Martel J, Lin CS, et al. Effects of obesity on depression: a role for inflammation and the gut microbiota. *Brain Behav Immun*. 2018;69:1–8.
27. Boursier J, Diehl AM. Implication of gut microbiota in nonalcoholic fatty liver disease. *PLoS Pathog*. 2015;11:e1004559.
28. Delzenne NM, Cani PD. Interaction between obesity and the gut microbiota: relevance in nutrition. *Annu Rev Nutr*. 2011;31:15–31.
29. Lin CJ, Wang MC. Microbial metabolites regulate host lipid metabolism through NR5A-Hedgehog signalling. *Nat Cell Biol*. 2017;19:550–557.
30. Jie Z, Xia H, Zhong SL, et al. The gut microbiome in atherosclerotic cardiovascular disease. *Nat Commun*. 2017;8:845.
31. Tang WH, Kitai T, Hazen SL. Gut Microbiota in cardiovascular health and disease. *Circ Res*. 2017;120:1183–1196.
32. Ley RE, Hamady M, Lozupone C, et al. Evolution of mammals and their gut microbes. *Science*. 2008;320:1647–1651.
33. Hugenholtz F, de Vos WM. Mouse models for human intestinal microbiota research: a critical evaluation. *Cell Mol Life Sci*. 2018;75:149–160.
34. Nguyen TL, Vieira-Silva S, Liston A, Raes J. How informative is the mouse for human gut microbiota research? *Dis Model Mech*. 2015;8:1–16.
35. Snel J, Heinen PP, Blok HJ, et al. Comparison of 16S rRNA sequences of segmented filamentous bacteria isolated from mice, rats, and chickens and proposal of "Candidatus Arthromitus". *Int J Syst Bacteriol*. 1995;45:780–782.
36. Gaboriau-Routhiau V, Rakotobe S, Lécuyer E, et al. The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. *Immunity*. 2009;31:677–689.
37. Ivanov II , Atarashi K, Manel N, et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell*. 2009;139:485–498.
38. Tao X, Wang N, Qin W. Gut microbiota and hepatocellular carcinoma. *Gastrointest Tumors*. 2015;2:33–40.
39. Macfarlane S, Macfarlane GT. Regulation of short-chain fatty acid production. *Proc Nutr Soc*. 2003;62:67–72.
40. Sartor RB. Microbial influences in inflammatory bowel diseases. *Gastroenterology*. 2008;134:577–594.
41. Wong JM, de Souza R, Kendall CW, Emam A, Jenkins DJ. Colonic health: fermentation and short chain fatty acids. *J Clin Gastroenterol*. 2006;40:235–243.
42. Scott KP, Gratz SW, Sheridan PO, Flint HJ, Duncan SH. The influence of diet on the gut microbiota. *Pharmacol Res*. 2013;69:52–60.
43. Natarajan N, Pluznick JL. From microbe to man: the role of microbial short chain fatty acid metabolites in host cell biology. *Am J Physiol Cell Physiol*. 2014;307:C979–C985.
44. Le Poul E, Loison C, Struyf S, et al. Functional characterization of human receptors for short chain fatty acids and their role in polymorphonuclear cell activation. *J Biol Chem*. 2003;278:25481–25489.
45. Blad CC, Tang C, Offermanns S. G protein-coupled receptors for energy metabolites as new therapeutic targets. *Nat Rev Drug Discov*. 2012;11:603–619.
46. Kasubuchi M, Hasegawa S, Hiramatsu T, Ichimura A, Kimura I. Dietary gut microbial metabolites, short-chain fatty acids, and host metabolic regulation. *Nutrients*. 2015;7:2839–2849.
47. Canfora EE, Jocken JW, Blaak EE. Short-chain fatty acids in control of body weight and insulin sensitivity. *Nat Rev Endocrinol*. 2015;11:577–591.
48. Kaji I, Karaki S, Kuwahara A. Short-chain fatty acid receptor and its contribution to glucagon-like peptide-1 release. *Digestion*. 2014;89:31–36.
49. Zhu Y, Jameson E, Crosatti M, et al. Carnitine metabolism to trimethylamine by an unusual Rieske-type oxygenase from human microbiota. *Proc Natl Acad Sci U S A*. 2014;111:4268–4273.
50. Fennema D, Phillips IR, Shephard EA. Trimethylamine and trimethylamine N-oxide, a flavin-containing monooxygenase 3 (FMO3)-mediated host-

microbiome metabolic axis implicated in health and disease. *Drug Metab Dispos*. 2016;44:1839–1850.
51. Bennett BJ, de Aguiar Vallim TQ, Wang Z, et al. Trimethylamine-N-oxide, a metabolite associated with atherosclerosis, exhibits complex genetic and dietary regulation. *Cell Metab*. 2013;17:49–60.
52. Koeth RA, Wang Z, Levison BS, et al. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nat Med*. 2013;19:576–585.
53. Wang Z, Roberts AB, Buffa JA, et al. Non-lethal inhibition of gut microbial trimethylamine production for the treatment of atherosclerosis. *Cell*. 2015;163:1585–1595.
54. Chen YM, Liu Y, Zhou RF, et al. Associations of gut-flora-dependent metabolite trimethylamine-N-oxide, betaine and choline with non-alcoholic fatty liver disease in adults. *Sci Rep*. 2016;6:19076.
55. de Aguiar Vallim TQ, Tarling EJ, Edwards PA. Pleiotropic roles of bile acids in metabolism. *Cell Metab*. 2013;17:657–669.
56. Thomas C, Pellicciari R, Pruzanski M, Auwerx J, Schoonjans K. Targeting bile-acid signalling for metabolic diseases. *Nat Rev Drug Discov*. 2008;7:678–693.
57. Jones BV, Begley M, Hill C, Gahan CG, Marchesi JR. Functional and comparative metagenomic analysis of bile salt hydrolase activity in the human gut microbiome. *Proc Natl Acad Sci U S A*. 2008;105:13580–13585.
58. Qi Y, Jiang C, Cheng J, et al. Bile acid signaling in lipid metabolism: metabolomic and lipidomic analysis of lipid and bile acid markers linked to anti-obesity and anti-diabetes in mice. *Biochim Biophys Acta*. 2015;1851:19–29.
59. Chiang JY. Recent advances in understanding bile acid homeostasis. *F1000Res*. 2017;6:2029.
60. Sonnenburg JL, Bäckhed F. Diet-microbiota interactions as moderators of human metabolism. *Nature*. 2016;535:56–64.
61. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*. 2006;444:1027–1031.
62. Liu R, Hong J, Xu X, et al. Gut microbiome and serum metabolome alterations in obesity and after weight-loss intervention. *Nat Med*. 2017;23:859–868.
63. Zietak M, Kovatcheva-Datchary P, Markiewicz LH, Ståhlman M, Kozak LP, Bäckhed F. Altered microbiota contributes to reduced diet-induced obesity upon cold exposure. *Cell Metab*. 2016;23:1216–1223.
64. Reijnders D, Goossens GH, Hermes GD, et al. Effects of gut microbiota manipulation by antibiotics on host metabolism in obese humans: a randomized double-blind placebo-controlled trial. *Cell Metab*. 2016;24:63–74.
65. Li G, Xie C, Lu S, et al. Intermittent fasting promotes white adipose browning and decreases obesity by shaping the gut microbiota. *Cell Metab*. 2017;26:672–685 (e4).
66. Gu Y, Wang X, Li J, et al. Analyses of gut microbiota and plasma bile acids enable stratification of patients for antidiabetic treatment. *Nat Commun*. 2017;8:1785.
67. Jiang C, Xie C, Lv Y, et al. Intestine-selective farnesoid X receptor inhibition improves obesity-related metabolic dysfunction. *Nat Commun*. 2015;6:10166.
68. Fuchs CD, Paumgartner G, Mlitz V, et al. Colesevelam attenuates cholestatic liver and bile duct injury in Mdr2(-/-) mice by modulating composition, signalling and excretion of faecal bile acids. *Gut*. 2018;67:1683–1691.
69. Trabelsi MS, Daoudi M, Prawitt J, et al. Farnesoid X receptor inhibits glucagon-like peptide-1 production by enteroendocrine L cells. *Nat Commun*. 2015;6:7629.
70. Henao-Mejia J, Elinav E, Jin C, et al. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. *Nature*. 2012;482:179–185.
71. Son G, Kremer M, Hines IN. Contribution of gut bacteria to liver pathobiology. *Gastroenterol Res Pract*. 2010;2010.
72. Mencin A, Kluwe J, Schwabe RF. Toll-like receptors as targets in chronic liver diseases. *Gut*. 2009;58:704–720.
73. Wree A, McGeough MD, Peña CA, et al. NLRP3 inflammasome activation is required for fibrosis development in NAFLD. *J Mol Med (Berl)*. 2014;92:1069–1082.
74. Pernicova I, Korbonits M. Metformin-mode of action and clinical implications for diabetes and cancer. *Nat Rev Endocrinol*. 2014;10:143–156.
75. Lin HZ, Yang SQ, Chuckaree C, Kuhajda F, Ronnet G, Diehl AM. Metformin reverses fatty liver disease in obese, leptin-deficient mice. *Nat Med*. 2000;6:998–1003.
76. Kim EK, Lee SH, Jhun JY, et al. Metformin prevents fatty liver and improves balance of white/brown adipose in an obesity mouse model by inducing FGF21. *Mediators Inflamm*. 2016;2016:5813030.
77. Nobili V, Manco M, Ciampalini P, et al. Metformin use in children with nonalcoholic fatty liver disease: an open-label, 24-month, observational pilot study. *Clin Ther*. 2008;30:1168–1176.
78. Lee H, Ko G. Effect of metformin on metabolic improvement and gut microbiota. *Appl Environ Microbiol*. 2014;80:5935–5943.
79. Zhang X, Zhao Y, Xu J, et al. Modulation of gut microbiota by berberine and metformin during the treatment of high-fat diet-induced obesity in rats. *Sci Rep*. 2015;5:14405.
80. Duca FA, Côté CD, Rasmussen BA, et al. Metformin activates a duodenal Ampk-dependent pathway to lower hepatic glucose production in rats. *Nat Med*. 2015;21:506–511.
81. Wu H, Esteve E, Tremaroli V, et al. Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug. *Nat Med*. 2017;23:850–858.
82. Shin NR, Lee JC, Lee HY, et al. An increase in the Akkermansia spp. population induced by metformin treatment improves glucose homeostasis in diet-induced obese mice. *Gut*. 2014;63:727–735.
83. de la Cuesta-Zuluaga J, Mueller NT, Corrales-Agudelo V, et al. Metformin is associated with higher relative abundance of mucin-degrading Akkermansia muciniphila and several short-chain fatty acid-producing microbiota in the gut. *Diabetes Care*. 2017;40:54–62.
84. Plovier H, Everard A, Druart C, et al. A purified membrane protein from Akkermansia muciniphila or the pasteurized bacterium improves metabolism in obese and diabetic mice. *Nat Med*. 2017;23:107–113.
85. Jiao N, Baker SS, Chapa-Rodriguez A, et al. Suppressed hepatic bile acid signalling despite elevated production of primary and secondary bile acids in NAFLD. *Gut*. 2018;67:1881–1891.
86. Neuschwander-Tetri BA, Loomba R, Sanyal AJ, et al. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. *Lancet*. 2015;385:956–965.
87. Mudaliar S, Henry RR, Sanyal AJ, et al. Efficacy and safety of the farnesoid X receptor agonist obeticholic acid in patients with type 2 diabetes and nonalcoholic fatty liver disease. *Gastroenterology*. 2013;145:574–582 (e1).
88. Fang S, Suh JM, Reilly SM, et al. Intestinal FXR agonism promotes adipose tissue browning and reduces obesity and insulin resistance. *Nat Med*. 2015;21:159–165.
89. Pathak P, Xie C, Nichols RG, et al. Intestine farnesoid X receptor agonist and the gut microbiota activate G-protein bile acid receptor-1 signaling to improve metabolism. *Hepatology*. 2018;68:1574–1588.
90. Mueller M, Thorell A, Claudel T, et al. Ursodeoxycholic acid exerts farnesoid X receptor-antagonistic effects on bile acid and lipid metabolism in morbid obesity. *J Hepatol*. 2015;62:1398–1404.
91. Summers SA. Sphingolipids and insulin resistance: the five Ws. *Curr Opin Lipidol*. 2010;21:128–135.
92. Chavez JA, Summers SA. A ceramide-centric view of insulin resistance. *Cell Metab*. 2012;15:585–594.
93. Yabu T, Shiba H, Shibasaki Y, et al. Stress-induced ceramide generation and apoptosis via the phosphorylation and activation of nSMase1 by JNK signaling. *Cell Death Differ*. 2015;22:258–273.
94. Holland WL, Adams AC, Brozinick JT, et al. An FGF21-adiponectin-ceramide axis controls energy expenditure and insulin action in mice. *Cell Metab*. 2013;17:790–797.