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Interactions between gut microbiota and non-alcoholic liver disease: the role of microbiota-derived metabolites

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Abstract

There is increasing evidence that the intestinal microbiota plays a mechanistic role in the etiology of non-alcoholic fatty liver disease (NAFLD). Animal and human studies have linked small molecule metabolites produced by commensal bacteria in the gut contribute to not only intestinal inflammation, but also to hepatic inflammation. These immunomodulatory metabolites are capable of engaging host cellular receptors, and may mediate the observed association between gut dysbiosis and NAFLD. This review focuses on the effects and potential mechanisms of three specific classes of metabolites that synthesized or modified by gut bacteria: short chain fatty acids, amino acid catabolites, and bile acids. In particular, we discuss their role as ligands for cell surface and nuclear receptors regulating metabolic and inflammatory pathways in the intestine and liver. Studies reveal that the metabolites can both agonize and antagonize their cognate receptors to reduce or exacerbate liver steatosis and inflammation, and that the effects are metabolite- and context-specific. Further studies are warranted to more comprehensively understand bacterial metabolite-mediated gut-liver in NAFLD. This understanding could help identify novel therapeutics and therapeutic targets to intervene in the disease through the gut microbiota.

Graphical Abstract

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Keywords

NAFLD; inflammation; microbiota metabolites; indole; short chain fatty acids; bile acids

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most prevalent chronic liver disease in Western countries [1]. In the United States, the number of NAFLD patients is about 25% of the population [2]. NAFLD has multiple stages, with each stage having distinct characteristics [3]. The first stage is steatosis, or the deposition of fat in the liver. Steatosis by itself is thought to be reversible and a benign condition in most individuals. However, for some individuals, steatosis progresses to nonalcoholic steatohepatitis (NASH). This second stage is characterized by inflammation in the liver (infiltration of immune cells, production of inflammatory cytokines and reactive oxygen species) and damage to hepatocytes [3]. A subset of NASH patients develops cirrhosis, and eventually hepatocellular carcinoma (HCC) [4].

A “two-hit” model has been widely used to explain the pathogenesis of NAFLD. In this model, the first hit is lipid accumulation in the liver. The second hit is an additional, inflammation-inducing insult such as an increase in the levels of pro-inflammatory cytokines and reactive oxygen species (ROS) [5]. However, the idea that NASH always progresses from steatosis is debatable. Studies in mice have found that inflammatory stress can exacerbate hepatic lipid accumulation [6], suggesting that inflammation may precede steatosis. It is possible that the factors driving NAFLD pathogenesis vary between different individuals or even different animal models. Recently, a multiple hit model has been proposed as a more comprehensive alternative to the two-hit model [7]. In this updated model, NAFLD pathogenesis is explained by a combination of nutritional and environmental factors that together induce disease onset and progression in genetically predisposed individuals. One such factor is dysbiosis of the intestinal microbiota, which has been implicated in various GI conditions with an inflammatory component as well as chronic metabolic diseases closely associated with NAFLD.

Seminal work by the Gordon laboratory [8, 9] demonstrated that dysbiosis could be a contributing mechanism for obesity and related diseases, including NAFLD. Fecal transplantation experiments in mice showed that both obesity and NAFLD are transmissible by microbial components in the feces [9, 10]. Le Roy et al. reported that HFD-fed germ-free

mice inoculated with bacterial isolates from feces of HFD-fed hyperglycemic mice developed steatohepatitis, whereas germ-free mice fed a high-fat diet inoculated with bacteria from HFD-fed, but normoglycemic mice only showed mild steatosis [11]. These findings suggest that the gut microbiota plays a direct, possibly causal, role in the progression of NAFLD.

Several mechanisms have been hypothesized to explain the role of the intestinal microbiota in NAFLD [12, 13]. One possibility is that dysbiosis of the microbiota could promote hepatic fat deposition by enhancing energy harvest. Alternatively, changes in the microbiota composition could lead to increased generation of toxic metabolic byproducts (e.g., ethanol), resulting in an elevated chemical burden on the liver. Another possibility is that dysbiosis establishes a pro-inflammatory state in the intestine, leading to weakened intestinal barrier function, translocation of bacterial endotoxins, and systemic immune response [14, 15]. This third explanation is supported by animal as well as human studies linking microbiota dysbiosis and intestinal inflammation [12, 16].

An intriguing molecular-level explanation for the link between dysbiosis and inflammation is that the metabolites produced by the microbiota have immunomodulatory properties. Well-known products of microbial metabolism in the intestine include short chain fatty acids (e.g., butyrate, propionate), tryptophan metabolites (e.g., indole, tryptamine), and bile acids [17], which have been reported to attenuate intestinal inflammation [17]. These metabolites are also present in circulation [18, 19], suggesting that they could exert immunomodulatory effects in and outside of the intestine. In this light, dysbiosis of the microbiota could alter the balance of immunomodulatory metabolites, which in turn could elevate inflammation both in the intestine and the liver. However, the molecular mechanisms underlying the immunomodulatory effect of microbiota metabolites in distal tissues such as the liver are still poorly understood.

The goal of this review is to discuss recent findings regarding the potential role of microbiota metabolites in the development and severity of NAFLD. We focus on three classes of microbiota metabolites - short chain fatty acids (SCFAs), aromatic amino acid-derived metabolites, and bile acids – for which the literature has identified specific host cellular receptors that could anchor immunomodulatory pathways. The associations between microbiota composition changes and NAFLD is only briefly discussed as this topic has been extensively reviewed previously [16, 20].

MICROBIOTA DYSBIOSIS AND NAFLD

The human intestinal microbiota is dominated by four phyla - *Bacteroidetes*, *Firmicutes*, *Proteobacteria* (including *E. coli* species) and *Actinobacteria* - which account for nearly 90% of the intestinal microbial community [21]. Of these, *Bacteroidetes* and *Firmicutes* are the most abundant and cover nearly 70% of the bacteria in the intestinal tract [22]. Alterations in microbiome composition and the abundance of specific taxa have been correlated with NAFLD in both human and experimental models [23-25]. NAFLD patients harbor more Gram-negative and fewer Gram-positive bacteria compared to healthy subjects [26], and disease progression correlates with phylum-level changes, such as increase in *Bacteroidetes*

and decrease in *Firmicutes* [26]. At the genus level, a significant increase in the abundance of *Bacteroides* and decrease in *Prevotella* in fecal samples has been observed in NASH patients compared to NAFLD patients without NASH [13]. Since plant material-rich diet favors *Prevotella* and HFD favors *Bacteriodes*, it has been proposed that changes in the diet can cause imbalance between the two genera [27]. Similar observations on increased abundance of *Bacteroides* in rats with NASH [28] and *Ruminococcus* in patients with fibrosis [13] have also been reported.

A consequence of microbiota dysbiosis is the increase in the translocation of bacterial products from the gut lumen into circulation, which plays a significant role in NASH. Tight junctions formed by intestinal epithelial cells regulate the permeability of solutes and limits bacterial translocation across the epithelial layer [29]. The expression of proteins involved in formation of tight junctions is reduced in NAFLD patients and HFD fed mice [30, 31], which compromises barrier integrity and increases intestinal permeability, resulting in enhanced translocation of bacterial components such as endotoxins [31, 32]. The translocation of bacteria and their products across the epithelial barrier leads to activation of intestinal immune cells [33], which elevates inflammatory cytokine production in the intestinal mucosa. This in turn further impairs epithelial barrier integrity, and exacerbates intestinal inflammation [34, 35].

Translocation of bacterial products also increases inflammation in distal sites such as the liver [36]. Endotoxin is a potent toll-like receptor 4 (TLR4) ligand, and its translocation from gut lumen to circulation activates the immune cells in the liver to induce inflammation [12]. Homozygous TLR4 null mice are protected from diet-induced steatosis and liver inflammation [37], suggesting that activation of TLR4-dependent signaling leads to exacerbation of inflammation. Treatment of HFD-fed mice with an anti-inflammatory molecule (5-aminosalicylic acid) targeting the gut reduced not only intestinal inflammation, but also liver steatosis and insulin resistance [38], indicating that intestinal inflammation is a contributing factor in NAFLD.

ROLE OF MICROBIOTA METABOLITES IN NAFLD

There is increasing evidence that alterations in the levels of molecules produced by the microbiota play an important role in NAFLD. For example, an elevation in bacterially-synthesized deoxycholic acid (DCA) in the liver has been observed in HFD-mediated NASH [39], and has been correlated to the increased abundance of *Bacteroides*. The increase in *Bacteriodes* has also been linked to decreased levels of SCFAs and amino acids [40].

Short chain fatty acids

The microbiota in the colon produce SCFAs through fermentation of plant-derived polysaccharides that are only partially digested by host enzymes [41]. Supplementation of SCFAs to the diet has been shown to have a protective effect against NAFLD. MA et al. [42] reported that mice treated with a butyrate pro-drug, tributyrin, were protected from diet-induced obesity, insulin resistance, and hepatic steatosis. Weitkunat et al. [43] found that acetate or propionate supplementation reduced hepatic lipogenesis and fatty acid uptake, and protected mice from HFD induced weight gain, steatosis and insulin resistance.

The primary mechanism through which SCFAs impact NAFLD is through modulation of inflammation. Butyrate and propionate have well established anti-inflammatory roles in the intestine [44]. Increased SCFA production through dietary supplementation has been shown to ameliorate intestinal inflammation [45]. Conversely, colonic inflammation lowers the abundance of SCFA-producing bacteria (*Bacteroidetes*, *Firmicutes*) [45-48].

The anti-inflammatory effects of SCFAs are observed in different cells in the gastrointestinal (GI) tract. Butyrate, acetate and propionate activate NLRP3 (NOD-, LRR- and pyrin domain-containing protein 3) inflammasome in intestinal epithelial cells to induce the secretion of IL-18 to improve epithelial barrier integrity [49, 50]. Mono-association of germ-free rats with the acetate producer *B. thetaiotaomicron* promotes the production of mucin by goblet cells and helps to maintain epithelial barrier function. However, when germ-free rats were colonized with *B. thetaiotaomicron* and an acetate-consuming bacterium (*F. prausnitzii*), the production of mucin and improvement of barrier integrity was reduced [51]. Butyrate and propionate block the development of dendritic cells from bone marrow stem cells [52]. Butyrate, acetate and propionate reduce the production of pro-inflammatory cytokines from LPS-activated neutrophils and macrophages through its inhibition of histone deacetylases [53, 54], and promotes the production of antibodies by modulating the energy metabolism of B cells [55]. Lastly, SCFAs also promote the expansion and *de novo* differentiation of regulatory T cells [56-59]. Smith et al. [59] found that butyrate, acetate and propionate increased the expansion of colonic regulatory T cells pool through G-protein coupled receptor 43 (GPCR43), and mice fed with these SCFAs were protected from colitis in a GPCR43 expressing regulatory T cells-dependent manner. Furusawa et al. [57] reported similar findings where butyrate induced differentiation of regulatory T cells *in vivo* protected mice from colitis through increased histone (H3) acetylation in the promoter and conserved non-coding sequence regions of the forkhead box P3 gene (*Foxp3*) locus. Singh et al. [58] also found that butyrate promoted the anti-inflammatory phenotype of colonic macrophages and dendritic cells and increased their ability to induce regulatory T cells differentiation through a hydroxycarboxylic acid receptor (Gpr109a). Mice deficient in *Niacr1* (gene that encodes Gpr109a) are susceptible to colonic inflammation, and activation of Gpr109a by a pharmacological agonist suppresses colonic inflammation in a Gpr109a-dependent manner.

In addition to their anti-inflammatory effects in the intestine, SCFAs can also impact NAFLD through their effects on lipid and glucose metabolism. SCFAs have been shown to regulate hepatic lipid metabolism through glucagon-like peptide-1 (GLP-1) signaling. Svegliati-Baroni et al. [60] reported that the expression of GLP-1 receptor (GLP-1r) is reduced in the hepatocytes of HFD-fed rats and NASH patients, and that the activation of GLP-1r in hepatocytes increased fatty acid β -oxidation and improved insulin sensitivity. Tolhurst et al. [61] found that acetate and propionate increased the expression of GPCR41 and GPCR43, and secretion of glucagon-like peptide-1 (GLP-1) from L cells, which leads to activation of GLP-1r in the liver. GPCR41 and GPCR43 knockout mice secrete less GLP-1 when treated with SCFAs and have impaired glucose tolerance. These studies demonstrate the protective role of SCFAs in NAFLD. On the other hand, SCFAs can enter the liver through the portal vein and directly impact hepatic lipid and glucose metabolism [62]. Acetate is a substrate for fatty acid synthesis and promotes hepatic triglyceride accumulation

[63], while propionate can be metabolized for hepatic gluconeogenesis [62, 64]. Both triglyceride accumulation and hepatic gluconeogenesis increase with NAFLD, suggesting that the direct effects of SCFAs in the liver are to promote NAFLD. These indirect and direct effects of SCFAs on NAFLD progression is summarized in Figure 1.

Amino acid derived metabolites

Metabolites derived from catabolism of amino acids have been shown to have protective and therapeutic effect on liver function. Sellmann et al. [65] found that oral supplementation of glutamine protected mice against Western diet-induced elevation of portal endotoxin levels, liver damage and hepatic TNF α levels. In a different study, Sellmann et al. [66] found that, oral supplementation of glutamine alleviated steatosis and hepatic inflammation in mice with diet-induced NASH. Lin et al. [67] also observed similar protective effects (decreased steatosis, hepatic TNF α , and NF- κ B activation) with glutamine supplementation in HFD-fed rats. The protective role of other amino acids such as arginine [68] and citrulline [28, 69] have also been observed in diet-induced animal models of NASH. Recent studies by our lab and other groups have shown that metabolites derived from aromatic amino acids (AAA) can have anti-inflammatory effects in host cells [70, 71]. Ritze et al. [72] reported that supplemental of tryptophan significantly reduced hepatic lipid accumulation while increased the expression of occludin, a tight-junction protein in a mice model of NAFLD.

A potential mechanistic link between amino acid derived metabolites and NAFLD is through the metabolites' effect on intestinal epithelial barrier function (Figure 2). Bansal et al. showed that indole, produced from dietary tryptophan by the bacterial enzyme tryptophanase A, attenuated TNF α -mediated NF- κ B activation and expression of IL-8, while promoting anti-inflammatory IL-10 production and increasing tight junction resistance in HCT-8 intestinal epithelial cells (human ileocecal colorectal adenocarcinoma cells) [71]. Shimada et al. [70] extended these observations to an *in vivo* model by showing that indole increases the expression of genes that promote intestinal barrier function and attenuated indicators of inflammation in a mouse model of colitis. Venkatesh *et al.* [73] showed that indole propionic acid and other indolelike molecules are important in maintaining intestinal epithelial barrier integrity.

A likely target for indole propionic acid activity is the xenobiotic sensor pregnane X receptor (*PXR*). The anti-inflammatory effects of indole propionic acid are abrogated in *PXR*-deficient mice [73] and *PXR* activity has been shown to correlate with NAFLD. Pretreatment of human primary hepatocytes with a *PXR*-ligand (rifampicin) decreased LPS-induced expression of pro-inflammatory cytokines [74]. Polymorphisms in the *PXR* gene have been associated with disease severity in NAFLD patients [75]. However, it is far from clear that activation of *PXR* is beneficial in liver disease. Bitter et al. observed that both activation and silencing of *PXR* increased steatosis in a hepatoma cell line [76], whereas Li et al. reported that lipid accumulation in human primary hepatocytes increased with rifampicin treatment [77]. Additional studies are required to clarify the crosstalk between liver *PXR* signaling and microbiota amino acid metabolism.

Wikoff et al. [18] demonstrated that indole-like molecules are depleted in germ-free mice, and that their levels in normal mice depend on an intact bacterial community. Furthermore,

indoxyl sulfate, a biotransformation product of indole, was decreased in circulation and liver of germ-free mice [18, 19]. These observations suggest that, in addition to their indirect effects through modulation of intestinal inflammation, microbiota metabolites such as indole can directly act on hepatocytes and immune cells in the liver (Figure 2). Beaumont et al. demonstrated recently that indole reduced the production of pro-inflammatory mediators from LPS treated liver slices prepared from obese and normal mice [78]. The same study also found that oral administration of indole before LPS challenge reduced liver pro-inflammatory gene upregulation *in vivo* through an NLRP3 dependent pathway in Kupffer cells [78].

In addition to indole, recent work from our lab [19] identified additional amino acid-derived metabolites that depend on microbial metabolism and are depleted in serum and liver of HFD-fed mice. Among them, tryptamine and indole-3-acetate (I3A) reduced free fatty acid and LPS induced production of pro-inflammatory cytokines in RAW 264.7 macrophages and inhibited the migration of bone marrow derived macrophages toward the chemoattractant MCP-1. In hepatocytes, I3A attenuated lipid loading induced inflammatory responses and reduced the expression of fatty acid synthase and sterol regulatory element-binding protein-1c (SRBP1c). Treatment of the hepatocytes with an aryl-hydrocarbon receptor (AhR) antagonist abrogated the effects, indicating that the metabolites' effects are mediated by this nuclear receptor. These results suggest that gut microbiota metabolites could influence hepatic inflammation by directly engaging specific host nuclear receptors.

Bile acids

Bile acids are cholesterol derivatives synthesized and conjugated in the liver, and play a critical role in digestion by facilitating absorption of dietary lipids and vitamins in the small intestine (primarily ileum). Human livers synthesize two main primary bile acids, cholic acid (CA) and chenodeoxycholic acid, which are subsequently conjugated with either glycine or taurine. The conjugated primary bile acids are then secreted into bile for release into the intestine. Most of the bile acids (> 95%) released into the ileum are reabsorbed by the enterocytes into the portal circulation to reenter the liver [79]. However, a fraction of conjugated bile acids in the ileum are deconjugated and modified by bacterial enzymes into secondary bile acids. In humans, the most abundant secondary bile acids are deoxycholic acid (DCA) and lithocholic acid. In recent years, other secondary bile acids such as ursodeoxycholic acid (UDCA) have garnered increasing attention due to their role as host cellular receptor ligands mediating gut-liver signaling. Receptors recognizing bile acids include nuclear receptors, e.g., farnesoid X receptor (FXR) [80], PXR [81], and vitamin D receptor (VDR) [82], as well as cell surface receptors, e.g., G-protein coupled bile acid receptor (GPBAR1 or TGR5) [83], sphingosine 1 phosphate receptor 2 (S1PH2) [84], and muscarinic receptors 2 and 3 [85, 86]. The remainder of this section will focus on FXR- and TGR5-mediated bile acid signaling and their metabolic and inflammatory consequences in the liver.

Ligand activation of liver FXR is essential for feedback regulation of bile acid synthesis. A recent study on dysregulation of FXR signaling in NAFLD patients found elevated bile acid production in these patients, with a disproportionate increase in bile acids antagonizing FXR

[87]. The study also found increased abundance of taurine and glycine metabolizing bacteria in NAFLD patients, suggesting an increase in secondary bile acid metabolism. It has been suggested that restoring FXR signaling could ameliorate NAFLD, for example by activating lipoprotein lipase (LPL) and repressing microsomal triglyceride transfer protein (MTTP) and reducing serum triglyceride [88, 89]. Additionally, FXR could act on the insulin/protein kinase B pathway to promote glycogen synthesis and suppress gluconeogenesis [90-92]. Taken together, these studies suggest mechanistic benefits of FXR activation for attenuating metabolic phenotypes associated with NAFLD. This idea has led to several clinical trials testing FXR agonists as therapeutics for treating NAFLD and NASH. Results from a phase 2 trial using a synthetic bile acid, obeticholic acid (OCA), showed improvement in overall NASH score, inflammation, and fibrosis. However, the trial also raised concerns regarding potentially negative side effects on the cardiovascular system [93, 94].

Studies in mice have shown that the intestinal microbiota profoundly impacts FXR signaling. Antibiotic induced dysbiosis promotes tauro- β -muricholic acid (T β -MCA) accumulation and attenuates FGF 15-mediated FXR signaling, while protecting against HFD-induced hepatic lipid accumulation and fibrosis [95, 96]. In CaCo2 cells (human colorectal adenocarcinoma cells), T β -MCA can act as FXR antagonist, even in the presence of other bile acid agonists for this receptor [97]. Other natural bile acids showing FXR antagonist activity include glyco- and tauroursodeoxycholic acid and T α -MCA [98, 99]. One possible mechanism for the protective effect of FXR antagonism is the downregulation of ceramide synthesis in the ileum [95]. In mice, ceramide induces fatty acid synthesis-related genes in the liver and promote steatosis.

As is the case with SCFAs and amino acid metabolites, the profile of bile acids capable of agonizing or antagonizing FXR correlates with particular bacterial groups. In mice, treatment with the antioxidant tempol improves liver steatosis and fibrosis, while increasing the ratio of T β -MCA to β -MCA and lowering the abundance of *Lactobacillus*, which covers many species with potent bile salt hydrolase activity [97, 100, 101]. Another study in mice found that treatment with the anti-diabetic compound metformin decreases the ratio of UDCA to conjugated UDCA, while lowering the abundance of *Bacteroides fragilis* [99]. This species expresses both 7 α -hydroxysteroid dehydrogenase as well as bile salt hydrolase activity towards conjugated UDCA [100, 102].

Another major bile acid receptor in the liver is TGR5. In rodents, TGR5 activation promotes whole body energy expenditure through a cAMP-dependent pathway in brown adipose tissue (BAT) and muscle [103]. In murine enteroendocrine cells, TGR5 activation induces GLP-1 secretion to promote insulin secretion from β -cells and improve glucose homeostasis [104]. Conflicting results have been reported regarding TGR5-FXR crosstalk. While one study showed that FXR activation induced TGR5 to upregulate GLP-1 secretion in mice L-cells [104], another study found that treatment with FXR antagonist glyco- or ursodeoxycholic acid or intestine-specific FXR knockout upregulated GLP-1 expression [99].

Similarly, there have been varying reports regarding the effects of FXR- and TGR5-mediated bile acid signaling on inflammatory pathways [105, 106]. A recent study in a rodent model of liver cancer showed that T β -MCA signals natural killer T (NKT) cell accumulation by

increasing chemokine CXCL16 expression. The same study found that administering *Clostridium scindens*, one of a handful of commensal species with documented 7 α -hydroxysteroid dehydrogenase activity, promoted the conversion of T β -MCA into T ω -MCA, while suppressing CXCL16 expression [107]. This suggests that the gut microbiota could influence immune cell recruitment in the liver by modulating the intestinal bile acid pool. In the intestine, bile acids can act as pro- or anti-inflammatory molecules. *In vivo* and *in vitro* studies have shown that TUDCA improves intestinal barrier function by increasing expression of tight junction proteins (TJPs) [108]. In contrast, more hydrophobic bile acids such as lithocholic acid can cause membrane damage, leading to production of reactive oxygen species (ROS) and other inflammatory signals [109].

Taken together, it is clear that bile acids are key signaling molecules linking the gut and liver to impact hepatic lipid accumulation and inflammation. The net effect of this signaling is bile acid specific and context-dependent (Table 1). Further studies are warranted to elucidate the underlying mechanisms to determine, for example, under what context FXR agonism or antagonism is beneficial in NAFLD.

CONCLUSIONS AND FUTURE DIRECTIONS

There is accumulating evidence that dysbiosis of gut microbiota contributes to the development of NAFLD. In particular, animal as well as human studies have correlated dysbiosis-associated shifts in microbiota community composition with alterations in the profile of metabolites that can directly and indirectly affect liver metabolism and inflammation in NAFLD. This review highlights three classes of such metabolites - SCFAs, amino acid derived metabolites, and bile acids. These metabolites can engage with host intestinal epithelial cells and immune cells to modulate intestinal barrier function and inflammation and indirectly affect the progression of NAFLD. Further, these metabolites are capable of crossing the epithelial barrier to enter circulation and modulate host responses in distal organs such as the liver. However, a number of unresolved issues and questions remain regarding the role of microbiota metabolites in NAFLD.

First, the metabolites reviewed here comprise an incomplete list of bioactive microbiota products that could signal receptor-mediated pathways involved in NAFLD. The intestinal metabolome is a complex milieu of dietary residues, host-derived biomolecules, and products of microbiota metabolism. Further advances in analytical technologies and chemical structure identification methods are needed to obtain a more comprehensive understanding of microbiota derived gut-liver signaling molecules. Second, functional studies on microbiota metabolites have largely focused on single metabolite or a particular organism thought to produce a metabolite of interest. However, conditions that induce NAFLD, e.g., HFD, can profoundly alter the microbiome, leading to multiple simultaneous changes in the levels of immunomodulatory metabolites. Venkatesh et al. [73] reported that indole propionic acid, indole and indole-3-acetate synergistically activate *PXR*. Whether SCFAs and tryptophan metabolites elicit a similarly synergistic response in promoting intestinal barrier and immune functions or instead compete for the same pathways remains to be elucidated. As additional mediators are identified, multiplexed approaches will be needed to more systematically assess the functional consequences of diet and other

environmental perturbations. Third, questions need to be addressed regarding translocation of the metabolites from the intestinal lumen to host tissues. What metabolite cross the epithelial barrier and under what conditions? Is the transport of a bioactive microbiota metabolite actively regulated or result from weakened barrier function? Along this vein, does an altered microbiota metabolite profile in the intestine translate into an altered systemic profile, or could an intact epithelial barrier inoculate the host's circulation from changes on the luminal side. In summary, the significant progress in identifying microbiota derived bioactive metabolites and understanding their role in the gut-liver axis strongly indicates their potential for use as therapeutic options in treating NAFLD.

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Abbreviations

DCA	deoxycholic acid
FXR	farnesoid X receptor
HFD	high-fat-diet
LPS	lipopolysaccharide
MCA	muricholic acid
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
SCFAs	short chain fatty acids
TUDCA	tauroursodeoxycholic acid
UDCA	ursodeoxycholic acid
GLP-1	glucagon-like peptide-1

REFERENCES

- [1]. Day CP, Non-alcoholic fatty liver disease: a massive problem, *Clinical medicine* 11(2) (2011) 176–8. [PubMed: 21526706]
- [2]. Estes C, Razavi H, Loomba R, Younossi Z, Sanyal AJ, Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease, *Hepatology* 67(1) (2018) 123–133. [PubMed: 28802062]
- [3]. Tiniakos DG, Vos MB, Brunt EM, Nonalcoholic fatty liver disease: pathology and pathogenesis, *Annual review of pathology* 5 (2010) 145–71.
- [4]. Calzadilla Bertot L, Adams LA, The Natural Course of Non-Alcoholic Fatty Liver Disease, *International journal of molecular sciences* 17(5) (2016).
- [5]. Day CP, James OF, Hepatic steatosis: innocent bystander or guilty party?, *Hepatology* 27(6) (1998) 1463–6. [PubMed: 9620314]

- [6]. Ma KL, Ruan XZ, Powis SH, Chen Y, Moorhead JF, Varghese Z, Inflammatory stress exacerbates lipid accumulation in hepatic cells and fatty livers of apolipoprotein E knockout mice, *Hepatology* 48(3) (2008) 770–81. [PubMed: 18752326]
- [7]. Tilg H, Moschen AR, Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis, *Hepatology* 52(5) (2010) 1836–46. [PubMed: 21038418]
- [8]. Ley RE, Backhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI, Obesity alters gut microbial ecology, *Proceedings of the National Academy of Sciences of the United States of America* 102(31) (2005) 11070–5. [PubMed: 16033867]
- [9]. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI, An obesity-associated gut microbiome with increased capacity for energy harvest, *Nature* 444(7122) (2006) 1027–31. [PubMed: 17183312]
- [10]. Henao-Mejia J, Elinav E, Jin C, Hao L, Mehal WZ, Strowig T, et al. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity, *Nature* 482(7384) (2012) 179–85. [PubMed: 22297845]
- [11]. Le Roy T, Llopis M, Lepage P, Bruneau A, Rabot S, Bevilacqua C, et al. Intestinal microbiota determines development of non-alcoholic fatty liver disease in mice, *Gut* 62(12) (2013) 1787–94. [PubMed: 23197411]
- [12]. Brandl K, Schnabl B, Intestinal microbiota and nonalcoholic steatohepatitis, *Curr Opin Gastroenterol* 33(3) (2017) 128–133. [PubMed: 28257306]
- [13]. Boursier J, Mueller O, Barret M, Machado M, Fizanne L, Araujo-Perez F, et al. The Severity of Nonalcoholic Fatty Liver Disease Is Associated With Gut Dysbiosis and Shift in the Metabolic Function of the Gut Microbiota, *Hepatology* 63(3) (2016) 764–775. [PubMed: 26600078]
- [14]. Schnabl B, Brenner DA, Interactions between the intestinal microbiome and liver diseases, *Gastroenterology* 146(6) (2014) 1513–24. [PubMed: 24440671]
- [15]. Bluemel S, Williams B, Knight R, Schnabl B, Precision medicine in alcoholic and nonalcoholic fatty liver disease via modulating the gut microbiota, *American journal of physiology. Gastrointestinal and liver physiology* 311(6) (2016) G1018–G1036. [PubMed: 27686615]
- [16]. Saltzman ET, Palacios T, Thomsen M, Vitetta L, Intestinal Microbiome Shifts, Dysbiosis, Inflammation, and Non-alcoholic Fatty Liver Disease, *Frontiers in microbiology* 9 (2018) 61. [PubMed: 29441049]
- [17]. Postler TS, Ghosh S, Understanding the Holobiont: How Microbial Metabolites Affect Human Health and Shape the Immune System, *Cell metabolism* 26(1) (2017) 110–130. [PubMed: 28625867]
- [18]. Wikoff WR, Anfora AT, Liu J, Schultz PG, Lesley SA, Peters EC, et al. Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites, *Proceedings of the National Academy of Sciences of the United States of America* 106(10) (2009) 3698–703. [PubMed: 19234110]
- [19]. Krishnan S, Ding Y, Saedi N, Choi M, Sridharan GV, Sherr DH, et al. Gut Microbiota-Derived Tryptophan Metabolites Modulate Inflammatory Response in Hepatocytes and Macrophages, *Cell reports* 23(4) (2018) 1099–1111. [PubMed: 29694888]
- [20]. Bibbo S, Ianiro G, Dore MP, Simonelli C, Newton EE, Cammarota G, Gut Microbiota as a Driver of Inflammation in Nonalcoholic Fatty Liver Disease, *Mediators of inflammation* (2018).
- [21]. Mokhtari Z, Gibson DL, Hekmatdoost A, Nonalcoholic Fatty Liver Disease, the Gut Microbiome, and Diet, *Adv Nutr* 8(2) (2017) 240–252. [PubMed: 28298269]
- [22]. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, et al. Diversity of the human intestinal microbial flora, *Science* 308(5728) (2005) 1635–8. [PubMed: 15831718]
- [23]. Mouzaki M, Comelli EM, Arendt BM, Bonengel J, Fung SK, Fischer SE, et al. Intestinal microbiota in patients with nonalcoholic fatty liver disease, *Hepatology* 58(1) (2013) 120–7. [PubMed: 23401313]
- [24]. Wigg AJ, Roberts-Thomson IC, Dymock RB, McCarthy PJ, Grose RH, Cummins AG, The role of small intestinal bacterial overgrowth, intestinal permeability, endotoxaemia, and tumour necrosis factor α in the pathogenesis of non-alcoholic steatohepatitis, *Gut* 48(2) (2001) 206–11. [PubMed: 11156641]

- [25]. Zhu LX, Baker SS, Gill C, Liu WS, Alkhouri R, Baker RD, et al. Characterization of Gut Microbiomes in Nonalcoholic Steatohepatitis (NASH) Patients: A Connection Between Endogenous Alcohol and NASH, *Hepatology* 57(2) (2013) 601–609. [PubMed: 23055155]
- [26]. Wang B, Jiang X, Cao M, Ge J, Bao Q, Tang L, et al. Altered Fecal Microbiota Correlates with Liver Biochemistry in Nonobese Patients with Non-alcoholic Fatty Liver Disease, *Scientific reports* 6 (2016) 32002. [PubMed: 27550547]
- [27]. Ley RE, Gut microbiota in 2015: Prevotella in the gut: choose carefully, *Nature reviews. Gastroenterology & hepatology* 13(2) (2016) 69–70. [PubMed: 26828918]
- [28]. Jegatheesan P, Beutheu S, Freese K, Waligora-Dupriet AJ, Nubret E, Butel MJ, et al. Preventive effects of citrulline on Western diet-induced non-alcoholic fatty liver disease in rats, *Br J Nutr* 116(2) (2016) 191–203. [PubMed: 27197843]
- [29]. Anderson JM, Van Itallie CM, Physiology and function of the tight junction, *Cold Spring Harb Perspect Biol* 1(2) (2009) a002584. [PubMed: 20066090]
- [30]. Jiang W, Wu N, Wang X, Chi Y, Zhang Y, Qiu X, et al. Dysbiosis gut microbiota associated with inflammation and impaired mucosal immune function in intestine of humans with non-alcoholic fatty liver disease, *Scientific reports* 5 (2015) 8096. [PubMed: 25644696]
- [31]. Briskey D, Heritage M, Jaskowski LA, Peake J, Gobe G, Subramaniam VN, et al. Probiotics modify tight-junction proteins in an animal model of nonalcoholic fatty liver disease, *Therap Adv Gastroenterol* 9(4) (2016) 463–72.
- [32]. Farhadi A, Gundlapalli S, Shaikh M, Frantzides C, Harrell L, Kwasny MM, et al. Susceptibility to gut leakiness: a possible mechanism for endotoxaemia in non-alcoholic steatohepatitis, *Liver international : official journal of the International Association for the Study of the Liver* 28(7) (2008) 1026–33. [PubMed: 18397235]
- [33]. Alisi A, Carpino G, Oliveira FL, Panera N, Nobili V, Gaudio E, The Role of Tissue Macrophage-Mediated Inflammation on NAFLD Pathogenesis and Its Clinical Implications, *Mediators of inflammation* 2017 (2017) 8162421. [PubMed: 28115795]
- [34]. McKay DM, Baird AW, Cytokine regulation of epithelial permeability and ion transport, *Gut* 44(2) (1999) 283–9. [PubMed: 9895392]
- [35]. Al-Sadi R, Boivin M, Ma T, Mechanism of cytokine modulation of epithelial tight junction barrier, *Front Biosci (Landmark Ed)* 14 (2009) 2765–78. [PubMed: 19273235]
- [36]. Chen P, Starkel P, Turner JR, Ho SB, Schnabl B, Dysbiosis-induced intestinal inflammation activates tumor necrosis factor receptor I and mediates alcoholic liver disease in mice, *Hepatology* 61(3) (2015) 883–94. [PubMed: 25251280]
- [37]. Csak T, Velayudham A, Hritz I, Petrasek J, Levin I, Lippai D, et al. Deficiency in myeloid differentiation factor-2 and toll-like receptor 4 expression attenuates nonalcoholic steatohepatitis and fibrosis in mice, *American journal of physiology. Gastrointestinal and liver physiology* 300(3) (2011) G433–41. [PubMed: 21233280]
- [38]. Luck H, Tsai S, Chung J, Clemente-Casares X, Ghazarian M, Revelo XS, et al. Regulation of obesity-related insulin resistance with gut anti-inflammatory agents, *Cell metabolism* 21(4) (2015) 527–42. [PubMed: 25863246]
- [39]. Aranha MM, Cortez-Pinto H, Costa A, da Silva IB, Camilo ME, de Moura MC, et al. Bile acid levels are increased in the liver of patients with steatohepatitis, *European journal of gastroenterology & hepatology* 20(6) (2008) 519–25. [PubMed: 18467911]
- [40]. Zhao Y, Wu J, Li JV, Zhou NY, Tang H, Wang Y, Gut microbiota composition modifies fecal metabolic profiles in mice, *J Proteome Res* 12(6) (2013) 2987–99. [PubMed: 23631562]
- [41]. Cummings JH, Macfarlane GT, Role of intestinal bacteria in nutrient metabolism, *JPEN. Journal of parenteral and enteral nutrition* 21(6) (1997) 357–65. [PubMed: 9406136]
- [42]. Vinolo MA, Rodrigues HG, Festuccia WT, Crisma AR, Alves VS, Martins AR, et al. Tributyrin attenuates obesity-associated inflammation and insulin resistance in high-fat-fed mice, *American journal of physiology. Endocrinology and metabolism* 303(2) (2012) E272–82. [PubMed: 22621868]
- [43]. Weitkunat K, Stuhlmann C, Postel A, Rumberger S, Fankhanel M, Woting A, et al. Short-chain fatty acids and inulin, but not guar gum, prevent diet-induced obesity and insulin resistance

through differential mechanisms in mice, *Scientific reports* 7(1) (2017) 6109. [PubMed: 28733671]

- [44]. Ferreira CM, Vieira AT, Vinolo MA, Oliveira FA, Curi R, Martins Fdos S, The central role of the gut microbiota in chronic inflammatory diseases, *Journal of immunology research* 2014 (2014) 689492. [PubMed: 25309932]
- [45]. Kanauchi O, Suga T, Tochiwara M, Hibi T, Naganuma M, Homma T, Asakura H, et al. Treatment of ulcerative colitis by feeding with germinated barley foodstuff: first report of a multicenter open control trial, *Journal of gastroenterology* 37 Suppl 14 (2002) 67–72. [PubMed: 12572869]
- [46]. Frank DN, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR, Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases, *Proceedings of the National Academy of Sciences of the United States of America* 104(34) (2007) 13780–5. [PubMed: 17699621]
- [47]. Machiels K, Joossens M, Sabino J, De Preter V, Arijis I, Eeckhaut V, et al. A decrease of the butyrate-producing species *Roseburia hominis* and *Faecalibacterium prausnitzii* defines dysbiosis in patients with ulcerative colitis, *Gut* 63(8) (2014) 1275–83. [PubMed: 24021287]
- [48]. Sokol H, Seksik P, Furet JP, Firmesse O, Nion-Larmurier I, Beaugerie L, et al. Low counts of *Faecalibacterium prausnitzii* in colitis microbiota, *Inflamm Bowel Dis* 15(8) (2009) 1183–9. [PubMed: 19235886]
- [49]. Macia L, Tan J, Vieira AT, Leach K, Stanley D, Luong S, et al. Metabolite-sensing receptors GPR43 and GPR109A facilitate dietary fibre-induced gut homeostasis through regulation of the inflammasome, *Nature communications* 6 (2015) 6734.
- [50]. Kalina U, Koyama N, Hosoda T, Nuernberger H, Sato K, Hoelzer D, et al. Enhanced production of IL-18 in butyrate-treated intestinal epithelium by stimulation of the proximal promoter region, *Eur J Immunol* 32(9) (2002) 2635–43. [PubMed: 12207348]
- [51]. Wrzosek L, Miquel S, Noordine ML, Bouet S, Joncquel Chevalier-Curt M, Robert V, et al. *Bacteroides thetaiotaomicron* and *Faecalibacterium prausnitzii* influence the production of mucus glycans and the development of goblet cells in the colonic epithelium of a gnotobiotic model rodent, *BMC Biol* 11 (2013) 61. [PubMed: 23692866]
- [52]. Singh N, Thangaraju M, Prasad PD, Martin PM, Lambert NA, Boettger T, et al. Blockade of dendritic cell development by bacterial fermentation products butyrate and propionate through a transporter (Slc5a8)-dependent inhibition of histone deacetylases, *The Journal of biological chemistry* 285(36) (2010) 27601–8. [PubMed: 20601425]
- [53]. Chang PV, Hao L, Offermanns S, Medzhitov R, The microbial metabolite butyrate regulates intestinal macrophage function via histone deacetylase inhibition, *Proceedings of the National Academy of Sciences of the United States of America* 111(6) (2014) 2247–52. [PubMed: 24390544]
- [54]. Vinolo MA, Rodrigues HG, Hatanaka E, Sato FT, Sampaio SC, Curi R, Suppressive effect of short-chain fatty acids on production of proinflammatory mediators by neutrophils, *J Nutr Biochem* 22(9) (2011) 849–55. [PubMed: 21167700]
- [55]. Kim M, Qie Y, Park J, Kim CH, Gut Microbial Metabolites Fuel Host Antibody Responses, *Cell host & microbe* 20(2) (2016) 202–14. [PubMed: 27476413]
- [56]. Arpaia N, Campbell C, Fan X, Dikiy S, van der Veeken J, deRoos P, et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation, *Nature* 504(7480) (2013) 451–5. [PubMed: 24226773]
- [57]. Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells, *Nature* 504(7480) (2013) 446–50. [PubMed: 24226770]
- [58]. Singh N, Gurav A, Sivaprakasam S, Brady E, Padia R, Shi H, et al. Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis, *Immunity* 40(1) (2014) 128–39. [PubMed: 24412617]
- [59]. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly YM, et al. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis, *Science* 341(6145) (2013) 569–73. [PubMed: 23828891]

- [60]. Svegliati-Baroni G, Saccomanno S, Rychlicki C, Agostinelli L, De Minicis S, Candelaresi C, et al. Glucagon-like peptide-1 receptor activation stimulates hepatic lipid oxidation and restores hepatic signalling alteration induced by a high-fat diet in nonalcoholic steatohepatitis, *Liver international : official journal of the International Association for the Study of the Liver* 31(9) (2011) 1285–97. [PubMed: 21745271]
- [61]. Tolhurst G, Heffron H, Lam YS, Parker HE, Habib AM, Diakogiannaki E, et al. Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2, *Diabetes* 61(2) (2012) 364–71. [PubMed: 22190648]
- [62]. den Besten G, Lange K, Havinga R, van Dijk TH, Gerding A, van Eunen K, et al. Gut-derived short-chain fatty acids are vividly assimilated into host carbohydrates and lipids, *American journal of physiology. Gastrointestinal and liver physiology* 305(12) (2013) G900–10. [PubMed: 24136789]
- [63]. Alves-Bezerra M, Cohen DE, Triglyceride Metabolism in the Liver, *Compr Physiol* 8(1) (2017) 1–8. [PubMed: 29357123]
- [64]. Weidemann MJ, Hems R, Williams DL, Spray GH, Krebs HA, Gluconeogenesis from propionate in kidney and liver of the vitamin B12-deficient rat, *The Biochemical journal* 117(1) (1970) 177–81. [PubMed: 5420952]
- [65]. Sellmann C, Jin CJ, Degen C, De Bandt JP, Bergheim I, Oral Glutamine Supplementation Protects Female Mice from Nonalcoholic Steatohepatitis, *J Nutr* 145(10) (2015) 2280–6. [PubMed: 26246326]
- [66]. Sellmann C, Baumann A, Brandt A, Jin CJ, Nier A, Bergheim I, Oral Supplementation of Glutamine Attenuates the Progression of Nonalcoholic Steatohepatitis in C57BL/6J Mice, *J Nutr* 147(11) (2017) 2041–2049. [PubMed: 28931589]
- [67]. Lin Z, Cai F, Lin N, Ye J, Zheng Q, Ding G, Effects of glutamine on oxidative stress and nuclear factor- κ B expression in the livers of rats with nonalcoholic fatty liver disease, *Exp Ther Med* 7(2) (2014) 365–370. [PubMed: 24396406]
- [68]. Sellmann C, Degen C, Jin CJ, Nier A, Engstler AJ, Hasan Alkhatib D, et al. Oral arginine supplementation protects female mice from the onset of non-alcoholic steatohepatitis, *Amino Acids* 49(7) (2017) 1215–1225. [PubMed: 28434046]
- [69]. Jegatheesan P, Beutheu S, Ventura G, Sarfati G, Nubret E, Kapel N, et al. Effect of specific amino acids on hepatic lipid metabolism in fructose-induced non-alcoholic fatty liver disease, *Clin Nutr* 35(1) (2016) 175–82. [PubMed: 25736031]
- [70]. Shimada Y, Kinoshita M, Harada K, Mizutani M, Masahata K, Kayama H, et al. Commensal bacteria-dependent indole production enhances epithelial barrier function in the colon, *PloS one* 8(11) (2013) e80604. [PubMed: 24278294]
- [71]. Bansal T, Alaniz RC, Wood TK, Jayaraman A, The bacterial signal indole increases epithelial-cell tight-junction resistance and attenuates indicators of inflammation, *Proceedings of the National Academy of Sciences of the United States of America* 107(1) (2010) 228–33. [PubMed: 19966295]
- [72]. Ritze Y, Bardos G, Hubert A, Bohle M, Bischoff SC, Effect of tryptophan supplementation on diet-induced non-alcoholic fatty liver disease in mice, *Br J Nutr* 112(1) (2014) 1–7. [PubMed: 24708895]
- [73]. Venkatesh M, Mukherjee S, Wang H, Li H, Sun K, Benechet AP, et al. Symbiotic bacterial metabolites regulate gastrointestinal barrier function via the xenobiotic sensor PXR and Toll-like receptor 4, *Immunity* 41(2) (2014) 296–310. [PubMed: 25065623]
- [74]. Sun M, Cui W, Woody SK, Staudinger JL, Pregnane X Receptor Modulates the Inflammatory Response in Primary Cultures of Hepatocytes, *Aliment Pharmacol Ther* 43(3) (2015) 335–343.
- [75]. Sookoian S, Castano GO, Burgueno AL, Gianotti TF, Rosselli MS, Pirola CJ, The nuclear receptor PXR gene variants are associated with liver injury in nonalcoholic fatty liver disease, *Pharmacogenetics and genomics* 20(1) (2010) 1–8. [PubMed: 19940802]
- [76]. Bitter A, Rümmele P, Klein K, Kandel BA, Rieger JK, Nüssler AK, et al. Pregnane X receptor activation and silencing promote steatosis of human hepatic cells by distinct lipogenic mechanisms, *Hepatology* 61(3) (2015) 2089–2103.

- [77]. Li L, Li H, Garzel B, Yang H, Sueyoshi T, Li Q, et al. SLC13A5 Is a Novel Transcriptional Target of the Pregnane X Receptor and Sensitizes Drug-Induced Steatosis in Human Liver, 87(4) (2015) 674–682.
- [78]. Beaumont M, Neyrinck AM, Olivares M, Rodriguez J, de Rocca Serra A, Roumain M, et al. The gut microbiota metabolite indole alleviates liver inflammation in mice, FASEB J (2018) fj201800544. [PubMed: 29906245]
- [79]. Wostmann BS, Intestinal bile acids and cholesterol absorption in the germ free rat, J Nutr 103(7) (1973) 982–90. [PubMed: 4716197]
- [80]. Parks DJ, Blanchard SG, Bledsoe RK, Chandra G, Consler TG, Kliewer SA, et al. Bile acids: natural ligands for an orphan nuclear receptor, Science 284(5418) (1999) 1365–8. [PubMed: 10334993]
- [81]. Staudinger JL, Goodwin B, Jones SA, Hawkins-Brown D, MacKenzie KI, LaTour A, et al. The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity, Proceedings of the National Academy of Sciences of the United States of America 98(6) (2001) 3369–74. [PubMed: 11248085]
- [82]. Makishima M, Lu TT, Xie W, Whitfield GK, Domoto H, Evans RM, et al. Vitamin D receptor as an intestinal bile acid sensor, Science 296(5571) (2002) 1313–6. [PubMed: 12016314]
- [83]. Sato H, Macchiarulo A, Thomas C, Gioiello A, Une M, Hofmann AF, et al. Novel potent and selective bile acid derivatives as TGR5 agonists: biological screening, structure-activity relationships, and molecular modeling studies, J Med Chem 51(6) (2008) 1831–41. [PubMed: 18307294]
- [84]. Studer E, Zhou X, Zhao R, Wang Y, Takabe K, Nagahashi M, et al. Conjugated bile acids activate the sphingosine-1-phosphate receptor 2 in primary rodent hepatocytes, Hepatology 55(1) (2012) 267–76. [PubMed: 21932398]
- [85]. Sheikh Abdul Kadir SH, Miragoli M, Abu-Hayyeh S, Moshkov AV, Xie Q, Keitel V, et al. Bile acid-induced arrhythmia is mediated by muscarinic M2 receptors in neonatal rat cardiomyocytes, PloS one 5(3) (2010) e9689. [PubMed: 20300620]
- [86]. Raufman JP, Chen Y, Cheng K, Compadre C, Compadre L, Zimniak P, Selective interaction of bile acids with muscarinic receptors: a case of molecular mimicry, Eur J Pharmacol 457(2-3) (2002) 77–84. [PubMed: 12464352]
- [87]. Jiao N, Baker SS, Chapa-Rodriguez A, Liu W, Nugent CA, Tsompana M, et al. Suppressed hepatic bile acid signalling despite elevated production of primary and secondary bile acids in NAFLD, Gut 67(10) (2018) 1881–1891. [PubMed: 28774887]
- [88]. Kast HR, Nguyen CM, Sinal CJ, Jones SA, Laffitte BA, Reue K, et al. Farnesoid X-activated receptor induces apolipoprotein C-II transcription: a molecular mechanism linking plasma triglyceride levels to bile acids, Molecular endocrinology 15(10) (2001) 1720–8. [PubMed: 11579204]
- [89]. Hirokane H, Nakahara M, Tachibana S, Shimizu M, Sato R, Bile acid reduces the secretion of very low density lipoprotein by repressing microsomal triglyceride transfer protein gene expression mediated by hepatocyte nuclear factor-4, Journal of Biological Chemistry 279(44) (2004) 45685–45692. [PubMed: 15337761]
- [90]. Renga B, Mencarelli A, Vavassori P, Brancaleone V, Fiorucci S, The bile acid sensor FXR regulates insulin transcription and secretion, Biochimica et biophysica acta 1802(3) (2010) 363–72. [PubMed: 20060466]
- [91]. Yamagata K, Daitoku H, Shimamoto Y, Matsuzaki H, Hirota K, Ishida J, et al. Bile acids regulate gluconeogenic gene expression via small heterodimer partner-mediated repression of hepatocyte nuclear factor 4 and Foxo1, The Journal of biological chemistry 279(22) (2004) 23158–65. [PubMed: 15047713]
- [92]. Staybrook KR, Bramlett KS, Savkur RS, Ficorilli J, Cook T, Christe ME, et al. Regulation of carbohydrate metabolism by the farnesoid X receptor, Endocrinology 146(3) (2005) 984–91. [PubMed: 15564327]
- [93]. Neuschwander-Tetri BA, Loomba R, Sanyal AJ, Lavine JE, Van Natta ML, Abdelmalek MF, et al. N.C.R. Network, Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-

- alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial, *Lancet* 385(9972) (2015) 956–65. [PubMed: 25468160]
- [94]. Carr RM, Reid AE, FXR agonists as therapeutic agents for non-alcoholic fatty liver disease, *Curr Atheroscler Rep* 17(4) (2015) 500. [PubMed: 25690590]
- [95]. Jiang C, Xie C, Li F, Zhang L, Nichols RG, Krausz KW, et al. Intestinal farnesoid X receptor signaling promotes nonalcoholic fatty liver disease, *The Journal of clinical investigation* 125(1) (2015) 386–402. [PubMed: 25500885]
- [96]. Janssen AWF, Houben T, Katiraei S, Dijk W, Boutens L, van der Bolt N, et al. Modulation of the gut microbiota impacts nonalcoholic fatty liver disease: a potential role for bile acids, *Journal of lipid research* 58(7) (2017) 1399–1416. [PubMed: 28533304]
- [97]. Li F, Jiang C, Krausz KW, Li Y, Albert I, Hao H, et al. Microbiome remodelling leads to inhibition of intestinal farnesoid X receptor signalling and decreased obesity, *Nature communications* 4 (2013) 2384.
- [98]. Sayin SI, Wahlstrom A, Felin J, Jantti S, Marschall HU, Bamberg K, et al. Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist, *Cell metabolism* 17(2) (2013) 225–35. [PubMed: 23395169]
- [99]. Sun L, Xie C, Wang G, Wu Y, Wu Q, Wang X, et al. Gut microbiota and intestinal FXR mediate the clinical benefits of metformin, *Nature medicine* 24(12) (2018) 1919–1929.
- [100]. Yao L, Seaton SC, Ndousse-Fetter S, Adhikari AA, DiBenedetto N, Mina AI, et al. A selective gut bacterial bile salt hydrolase alters host metabolism, *Elife* 7 (2018).
- [101]. Joyce SA, MacSharry J, Casey PG, Kinsella M, Murphy EF, Shanahan F, et al. Regulation of host weight gain and lipid metabolism by bacterial bile acid modification in the gut, *Proceedings of the National Academy of Sciences of the United States of America* 111(20) (2014) 7421–6. [PubMed: 24799697]
- [102]. Bennett MJ, McKnight SL, Coleman JP, Cloning and characterization of the NAD-dependent 7 α -Hydroxysteroid dehydrogenase from *Bacteroides fragilis*, *Curr Microbiol* 47(6) (2003) 475–84. [PubMed: 14756531]
- [103]. Watanabe M, Houten SM, Matakai C, Christoffolete MA, Kim BW, Sato H, et al. Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation, *Nature* 439(7075) (2006) 484–9. [PubMed: 16400329]
- [104]. Pathak P, Liu H, Boehme S, Xie C, Krausz KW, Gonzalez F, et al. Farnesoid X receptor induces Takeda G-protein receptor 5 cross-talk to regulate bile acid synthesis and hepatic metabolism, *The Journal of biological chemistry* 292(26) (2017) 11055–11069. [PubMed: 28478385]
- [105]. Hollman DA, Milona A, van Erpecum KJ, van Mil SW, Anti-inflammatory and metabolic actions of FXR: insights into molecular mechanisms, *Biochimica et biophysica acta* 1821(11) (2012) 1443–52. [PubMed: 22820415]
- [106]. Chiang JY, Bile acid metabolism and signaling, *Compr Physiol* 3(3) (2013) 1191–212. [PubMed: 23897684]
- [107]. Ma C, Han M, Heinrich B, Fu Q, Zhang Q, Sandhu M, et al. Gut microbiome-mediated bile acid metabolism regulates liver cancer via NKT cells, *Science* 360(6391) (2018).
- [108]. Wang W, Zhao J, Gui W, Sun D, Dai H, Xiao L, et al. Tauroursodeoxycholic acid inhibits intestinal inflammation and barrier disruption in mice with non-alcoholic fatty liver disease, *Br J Pharmacol* 175(3) (2018) 469–484. [PubMed: 29139555]
- [109]. Perez MJ, Briz O, Bile-acid-induced cell injury and protection, *World J Gastroenterol* 15(14) (2009) 1677–89. [PubMed: 19360911]
- [110]. Zhang L, Xie C, Nichols RG, Chan SH, Jiang C, Hao R, et al. Patterson, Farnesoid X Receptor Signaling Shapes the Gut Microbiota and Controls Hepatic Lipid Metabolism, *mSystems* 1(5) (2016).
- [111]. Jiang C, Xie C, Lv Y, Li J, Krausz KW, Shi J, et al. Intestine-selective farnesoid X receptor inhibition improves obesity-related metabolic dysfunction, *Nature communications* 6 (2015) 10166.
- [112]. Islam KB, Fukiya S, Hagio M, Fujii N, Ishizuka S, Ooka T, et al. Bile acid is a host factor that regulates the composition of the cecal microbiota in rats, *Gastroenterology* 141(5) (2011) 1773–81. [PubMed: 21839040]

- [113]. Parseus A, Sommer N, Sommer F, Caesar R, Molinaro A, Stahlman M, et al. Microbiota-induced obesity requires farnesoid X receptor, *Gut* 66(3) (2017) 429–437. [PubMed: 26740296]
- [114]. Yamada S, Takashina Y, Watanabe M, Nagamine R, Saito Y, Kamada N, et al. Bile acid metabolism regulated by the gut microbiota promotes non-alcoholic steatohepatitis-associated hepatocellular carcinoma in mice, *Oncotarget* 9(11) (2018) 9925–9939. [PubMed: 29515780]
- [115]. Sun R, Yang N, Kong B, Cao B, Feng D, Yu X, et al. Orally Administered Berberine Modulates Hepatic Lipid Metabolism by Altering Microbial Bile Acid Metabolism and the Intestinal FXR Signaling Pathway, *Molecular pharmacology* 91(2) (2017) 110–122. [PubMed: 27932556]

Chemical compounds studied in this article

Propionate (PubMed CID: 104745); Butyrate (PubMed CID: 104775); Acetate (PubMed CID: 175); Tributyrin (PubMed CID: 6050); Tryptophan (PubMed CID: 6305); Indole (PubMed CID: 798); Indole-3-acetic Acid (PubMed CID: 802); Tryptamine (PubMed CID: 1150); Cholic Acid (PubMed CID: 221493); Taurine (PubMed CID: 1123)

Figure 1. The effects of SCFAs in NAFLD.

SCFAs affect the progression of NAFLD indirectly through modulating intestinal barrier integrity and immune responses. They also directly promote or protect against NAFLD through GLP signaling or lipid and glucose metabolism. DCs - dendritic cells; Tregs - regulatory T cells.

Figure 2. The effect of tryptophan derived metabolites in NAFLD.

Indole and other amino acid-derived molecules protect host against NAFLD through improving intestinal epithelial barrier integrity. In addition, these metabolites can also translocate from the intestinal lumen to the liver to affect hepatic lipid metabolism and inflammation directly. AhR - Aryl hydrocarbon receptor.

Table 1:

Profile of Bile Acids and Gut Bacteria in Fatty Liver Disease

Model	Treatment	Liver/Metabolic Phenotype*	Bile Acid Profile**	Changes in Gut Microbiota	Ref.
Male C57BL/6 mice fed HFCFD for 18 weeks	Guar gum, (fermentable dietary fiber, resistant starch as control)	↑ Fibrosis ↑ Inflammation ↑ Neutrophils ↓ TGs ↓ Liver weight	↑ Plasma bile acids (CA, TCA, αMCA, TβMCA, DCA, TDCA, UDCA, HDCA, THDCA)	↑ <i>Bifidobacterium</i> ↑ <i>Prevotella</i> ↑ <i>Akkermansia</i> ↓ <i>Oscillospira</i> ↓ SMB 5 3 (family <i>Clostridiaceae</i>)	[96]
Male C57BL/6J mice fed HFD for 12 weeks	TUDCA (1000 mg/kg)	↓ Liver weight ↓ TG ↓ Inflammation ↓ Ballooning ↓ Non-alcoholic fatty liver disease activity score ↓ Intestinal inflammation		↑ <i>Bacteroidetes</i> ↑ <i>Verrucomicrobia</i> ↓ <i>Firmicutes</i>	[108]
Male C57BL/6N fed HFD for 8 weeks	Gly-MCA (10 mg/kg)	↓ TG ↓ Fibrosis	Intestine and liver: ↑ Tα-MCA ↑ Tβ-MCA ↑ TCDCA	↑ <i>Bacteroides</i> ↑ <i>Oscillibacter</i> ↑ <i>Barnesiella</i> ↓ <i>Lactobacillus</i> ↓ <i>Enterorhabdus</i> ↓ <i>Clostridium IV</i>	[110, 111]
Male WKAH/HkmSlc rats fed a control diet for 10 days	CA (5 mmol/kg)	↓ Serum adiponectin ↓ Epididymal and retroperitoneal AT mass	↑ Fecal bile acids (CA, DCA, 7oxo-DCA, TCA)	↑ <i>Allobaculum</i> (increase in middle dose 1.25 mmol/kg) ↑ <i>Blautia</i> ↓ <i>Bacteroides</i>	[112]
Male C57BL/6N mice fed HFD for 14-17 weeks	Tempol (250 mg/kg)	↓ TG ↓ Fibrosis ↓ Liver weight ↑ Insulin sensitivity ↑ Glucose tolerance	↑ Taurine conjugated bile acids (CA, α/β-MCA, DCA, CDCA, UDCA) ↓ De-conjugated bile acids in fecal material (β/ω/γ-MCA, DCA, UDCA HDCA)	↓ <i>Firmicutes</i> ↑ <i>Bacteroidetes</i> ↑ <i>Proteobacteria</i>	[95, 97]
Male C57BL/6 mice fed HFD for 10 weeks	FXR knockout (WT as control)	↑ Insulin sensitivity ↓ TG ↓ Macrophages (crown-like structures) in AT	Serum: ↑ Tβ-MCA ↓ Tω-MCA ↓ THDCA Cecum: ↑ β-MCA	↑ <i>Bacteroidetes</i> ↓ <i>Firmicutes</i> ↓ <i>Proteobacteria</i>	[113]
Male Swiss Webster mice raised or male C57BL/6 mice on control diet (chow) for 9 to 16 weeks	Raised under GERM-FREE conditions (Conventional as control)	↓ Gallbladder weight (Swiss Model) ↑ Gallbladder cholesterol (Swiss Model)	↑ Tβ-MCA ↑ Tα-MCA ↑ TCA ↓ CA ↓ β-MCA Complete depletion of secondary bile acids		[98]
C57BL/6J mice fed STHD-01 for 41 weeks	Antibiotic (1 g/L ceftazidime, 1 g/L metronidazole)	↓ Inflammation ↓ Tumor count	Liver% : ↑ TUDCA ↓ TDCA Fecal%: ↑ Tβ-MCA ↑ TCA ↑ TUDCA ↓ DCA ↓ β-MCA	↑ <i>Enterococcus</i> ↓ <i>Clostridium</i> ↓ <i>Prevotella</i> ↓ <i>Bacteroides</i>	[114]
Male C57BL/6J mice fed HFD for 8 weeks	Berberine (150 mg/kg) Sodium carboxymethyl cellulose as control	↓ TG (liver and serum) ↑ Glucose tolerance ↑ Insulin Sensitivity	↑ TCA ↓ Tβ-MCA (serum)	Reduced BSH activity	[115]

HFCFD, high-fat/high-cholesterol/high-fructose diet; STHD-01, steatohepatitis-inducing high-fat diet; HCC, hepatocellular carcinoma; CA, cholic acid; TCA, taurocholic acid; CDCA, chenodeoxycholic acid; TDCA, taurodeoxycholic acid; TCDCA, taurochenodeoxycholic acid; MCA, muricholic acid; DCA, Deoxycholic acid; UDCA, ursodeoxycholic acid; HDCA, hyodeoxycholic acid; THDCA, tauroursodeoxycholic acid; TUDCA, tauroursodeoxycholic acid; BSH, bile salt hydrolase; GF, germ-free; TG, triglyceride; AT, adipose tissue

* Phenotype refers to liver unless otherwise noted

** Bile acid profile in intestine, unless otherwise noted