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Intestinally derived bacterial products stimulate development of nonalcoholic steatohepatitis

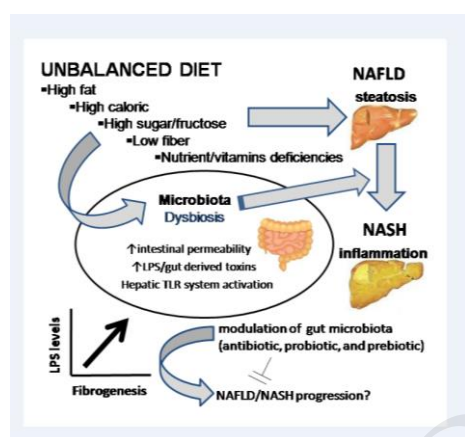
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Graphical abstract



Abstract

Fatty livers are susceptible to factors that cause inflammation and fibrosis, but fat deposition and the inflammatory response can be dissociated. While nonalcoholic fatty liver disease (NAFLD), caused by pathologic fat accumulation inside the liver, can remain stable for several years, in other cases NAFLD progresses to nonalcoholic steatohepatitis (NASH), which is characterized by fat accumulation and inflammation and is not a benign condition. In this review, we discuss the NASH host cells and microbial mechanisms that stimulate inflammation and predispose the liver to hepatocyte injury and fibrotic stages via increased lipid deposition. We highlight the interactions between intestine-derived bacterial products, such as lipopolysaccharide, and nutritional models of NAFLD and/or obese individuals. The results of modulating enteric microbiota suggest that gut-derived endotoxins may be essential determinants of fibrotic progression and regression in NASH.

Keywords: Gut microbiota; Hepatic stellate cell; Inflammation; Lipopolysaccharide; Liver fibrosis; Nonalcoholic steatohepatitis

Abbreviations

α -SMA	Alpha-smooth muscle
AC	Alcoholic cirrhosis
ALT	Alanine aminotransferase
ApoE	Apoprotein E
AST	Aspartate aminotransferase
BDL	Bile duct ligation
BMI	Body mass index
CDAA	Choline-deficient amino acid-defined
CDHF	Choline-deficient high-fat
CD14	Cluster of differentiation 14
COL I α 1	Collagen type I α 1
COX-2	Cyclooxygenase-2
CRP	C-reactive protein
DAMPs	Damage associated molecular patterns
DSS	Dextran sulfate sodium

FOS	Fructo-oligosaccharides
FXR	Farnesoid X receptor
GalN	D-galactosamine
GI	Gastrointestinal
GLP-2	Glucagon-like peptide 2
GPR43	G protein-coupled receptor 43
HC	High cholesterol
HCal	High caloric
HCV	Hepatitis C virus
HF	High fat
HFr	High fructose
HNE	4-Hydroxynonenal
HOMA	Homeostasis model assessment–estimated insulin resistance index
HSCs	Hepatic stellate cells
IL	Interleukin
iNOS	Inducible nitric oxide synthase
IR	Insulin resistance
KCs	Kupffer cells
KO	Knockout
LEC	Liver endothelial cell
LKO	Liver-specific knockout mice
LPS	Lipopolysaccharide
MCD	Methionine/choline-deficient
MDA	Malondialdehyde
MD2	TLR4/myeloid differentiation protein 2
MMP	Matrix metalloproteinase
MyD88	Myeloid differentiation primary-response gene 88
MFB	Myofibroblast
NAFLD	Nonalcoholic fatty liver disease
NASH	Nonalcoholic steatohepatitis
NF-κB	Factor nuclear <i>kappa B</i>
NLRP	Nucleotide-binding domain leucine-rich repeat protein
NLRs	NOD-like receptors
NOD	Nucleotide oligomerization domain
OFS	Oligofructose
PAMPs	Pathogen-associated molecular patterns
PPAR	Peroxisome proliferator-activated receptor
PUFAs	Polyunsaturated fatty acids
ROS	Reactive oxygen species
SCFAs	Short-chain fatty acids
SIBO	Small intestinal bacterial overgrowth
TG	Triglyceride
TGF-β	Transforming growth factor
TIMP	Tissue inhibitor of metalloproteinase
TLR	Toll-like receptor
TNF-α	Tumor necrosis factor alpha
VT	Vitamin
VSL#3	Probiotic mixture of <i>Streptococcus thermophilus</i> , <i>Lactobacillus/Bifidobacterium</i> species
WT	Wild type

1. Introduction

Nonalcoholic fatty liver disease (NAFLD), described by pathologic fat accumulation inside the liver, is well characterized. However, hepatocyte injury involved in the progression of NAFLD to nonalcoholic steatohepatitis (NASH), and particularly the inflammatory and profibrotic drivers of NASH, need further clarification. Evidence shows that pro-inflammatory stimuli, such as oxidative stress and mitochondrial dysfunction, determine the evolution of NASH [1–3]. But whether targeting lipotoxicity-mediated activation of immune cells is useful for the prevention or treatment of NASH remains unclear. Recently, new approaches using inhibition of fibrogenesis, and reversal of advanced fibrosis and cirrhosis, demonstrate that secretion of endotoxins originating from the gastrointestinal (GI) tract can play important roles in this process [4]. Bacterial products provoke the release of pro-inflammatory mediators, such as chemokines, cytokines, and reactive oxygen/nitrogen species, which contribute to deleterious effects involving inflammatory infiltration and fibrosis in the liver [5,6]. While gut microbiota are associated with different phenotypes of NAFLD in the liver where the more serious NAFLD lesions are linked with gut dysbiosis [7], many studies indicate that it is important to examine the contribution of the gut-liver axis to the pathogenesis of chronic liver diseases (Fig. 1). Here, we discuss the important roles of gut microbiota in pathogenesis of NASH and progression of liver disease.

2. Dysbiosis and progression of chronic liver injury

The liver is the main target of bacterial products derived from the intestine. Bacterial translocation augments liver injury [8–10], which proposes that the liver–gut axis plays a role in the pathogenesis of NASH. Studies using animal models indicate that gut-derived endotoxins mediate the development of NASH, while treatment with probiotics prevents histological alterations and insulin resistance (IR) associated with steatohepatitis [11,12]. Detoxification from intestine-derived toxins

elevates liver exposure to these substances; this occurs via activated macrophages, which release hepatotoxic factors by means of abnormal activation of the immune system [13]. However, studies conducted with human patients indicate that the exact mechanisms remain unclear [14,15].

2.1 Small intestinal bacterial overgrowth

Changes or imbalance in gut microbiota may be a primordial contributing factor that influences the onset of liver dysfunction via inflammation and development of fibrosis [16–22]. An imbalance of microbiota in the intestine can have serious consequences, including small intestinal bacterial overgrowth (SIBO). SIBO involves the translocation of colonic bacteria into the small intestine, which triggers disruption of intestinal homeostasis and decreased mobility of small intestine, leading to increased intestinal permeability and endotoxin absorption [23]. SIBO occurs with higher incidence in patients with NASH [24], and is accompanied by elevated concentrations of interleukin and disrupted metabolic activity of intestinal microbiota [25]. In particular, impaired intestinal motility may contribute to the development of intestinal bacterial overgrowth with late gastrointestinal transit. This can affect intestinal barrier function and lead to microbial translocation [26].

2.2 Intestinal permeability and endotoxins

While changes in gut microbiota can independently indicate the severity of NAFLD [27], increased permeability is also involved via rupture of intercellular junctions. This may be an essential factor in intestinal-barrier function [28]. Analysis of intact narrow junctions in the intestine, which prevent alterations in the intestinal flora, shows that a delicate balance is necessary to maintain intestinal functionality and to avoid immune responses against invading commensal microbes [29]. Changes in the function of intestinal tight-junction protein occludin play a major role in increased intestinal permeability. Thus, impairment of tight junctions increases intestinal permeability, which is accompanied by the translocation of microbial products from intestinal lumen to the bloodstream [30,31]. Modulators of this new gut vascular barrier are not yet determined; however, dietary factors, mucosal inflammation of any etiology (such as that involving drugs, infections, or toxins), and hypoperfusion, are currently recognized as disruptors of the intestinal epithelial wall [32].

2.3 Endotoxemia, LPS, and Toll-Like receptors

Excess levels of endotoxins, which can reach the liver through portal circulation, result from higher concentrations of endotoxins in the gut due to bacterial overgrowth, or from increased absorption of endotoxin from the gut in patients with NASH [33]. Particularly, fatty liver is severely damaged when lipopolysaccharide (LPS) from the Gram-negative intestinal microflora is present after bacterial death. The damage occurs via pattern recognition receptors, including Toll-like receptors (TLRs), when the innate immune system identifies conserved pathogen-associated molecular patterns (PAMPs) [34]. Whereas a healthy liver expresses low mRNA levels of TLRs, which results in elevated hepatic resistance to TLR ligands from the intestinal microbiome [35,36], TLR4, responsive to LPS, is expressed by Kupffer cell (KC), hepatic stellate cell (HSC), hepatocytes, biliary epithelial cells, sinusoidal endothelial cells, and hepatic dendritic cells [37]. LPS, transported via TLR4-dependent pathway, can play an important function for patients with NASH who have previous history of benign steatosis [38,39]. In fact, several studies have investigated how to counteract and reverse the pro-inflammatory and lipogenic effects of LPS [40–45]. These data imply that liver damage can occur when hepatic immune cells are exposed to TLR ligands [46–48]. Studies have also confirmed the significance of TLR4 upregulation in the liver and intestine-derived LPS in experimental models of NASH (Fig. 2). Consequently, TLRs can be viewed as key receptors regulating inflammation, increased activity of myofibroblasts. LPS-mediated TLR4 activation plays a role in fibrogenic signaling pathways [49,50]. Specifically, hepatocyte-specific deletion of myeloid differentiation primary-response gene 88 (Myd88), which is a common TLR signaling adaptor, leads to liver inflammation, steatosis, and IR [51], and KCs positive for cluster of differentiation 14 (CD14) shows an enhanced response to stimulation with low-dose LPS in simple steatosis [52]. Hence, LPS-induced intestinal permeability is related to TLR4-dependent increases via CD14 expression [53–55]. This important regulatory factor in LPS-induced inflammation enhances the effects of LPS, leading to the development of NAFLD/NASH, and can modulate metabolic activity, stimulating production of pro-inflammatory factors [56–58].

2.4 Increased sensitivity to endotoxin-induced injury in model of NASH

A methionine/choline-deficient (MCD) diet, widely utilized like an animal model of NASH, enhances susceptibility to the TLR4 ligand LPS [59]. Achiwa et al. [60] showed in mice lower colon length, increased incidence of mucosal changes in the ileum and colon, inflammation and, hepatic fibrosis for choline-fat-deficient diet (CDHF) linked with higher LPS concentrations in the CDHF group plus a dextran sodium sulfate (DSS)-induced colitis model. Similarly, Gabele et al. [61] have shown that combining high-fat (HF) diet and DSS leads to disrupted homeostasis between gut microbiota and the host organism. This suggests that bacterial translocation from the gut into portal circulation leads to liver damage, indicating that gut microbiota is highly involved in NAFLD and obesity. Endotoxemia causes elevated levels of tumor necrosis factor alpha (TNF- α) in steatohepatitis [11,62–64], and these findings indicate innate immune reactivity in the cells of patients with NASH [65]. However, Kirsch et al. [66] observed that while an MCD diet increases the levels of LPS, TNF- α does not appear necessary for the development of steatohepatitis. Therefore, alternative cytokines may participate of roles normally played by TNF- α , and further studies are necessary to delineate the exact cytokine response in TNF- α -knockout animal models.

3. Hepatic fat accumulation, gut derived factors, and NASH progression

Nutrient consumption is a vital factor in the evolution of NASH [67]. Alterations in the composition of intestinal barrier function are associated with development of steatosis and progression of NASH [68], while an augmented response to accumulation of hepatic fat is observed with NASH models using diet and gut-derived factors (Table 1). Choline, for example, is implicated in levels of low-density lipoprotein and dysfunction of hepatic lipid export [69]. In that way, microbiota promotes hepatic steatosis, IR, and injury via lipoperoxidation. In particular, there are interactions between choline deficiency diet and dysfunction of the GI microbiome [70]. In this model, the inflammatory response, stimulated either by LPS or by pro-inflammatory intermediates, can be efficiently counteracted by peroxisome proliferator-activated

receptor alpha-mediated transrepression of signaling pathways that involve adipose triglyceride lipase [71,72]. As a result, MCD diet and LPS causes steatohepatitis with enhanced role of hepatic pro-inflammatory mediators [73,74].

In rodents, a high-cholesterol (HC) diet, used to model steatohepatitis including an IR phenotype that closely resembles human NASH, aggravates the sensitivity to LPS and TNF- α [74]. KCs of HF-fed rats secrete pro-inflammatory cytokines and chemokines under LPS stimulation and can be reversed by inhibition of lipogenesis [75]. Infusion of LPS causes hepatic steatosis, triggering gain of liver and adipose tissue weight, liver IR, and glycemia/insulinemia, which are characteristics comparable to those obtained via HF diet. However, this did not occur in CD14 mutant mice [76], demonstrating that CD14/TLR4 signaling is essential for metabolic diseases that are induced by LPS. Moreover, while specific knockout models also show improvements in metabolic phenotypes upon administration of HF diet, the TLR5-knockout model exhibits a contradictory phenotype, with increased body weight, steatosis, and blood glucose defending against gut bacteria and diet-induced liver injury [77].

The intestinal epithelium, along with its colonizing bacteria, characterizes an initial point of connections between diet and the host immune system associated with the development of liver disease [78]. This interaction can impact the structure and composition of intestinal microbiota in which fatty liver alters bacterial capacity for energy harvest [79–82]. Although an HF diet alone causes a steatotic liver without NASH or inflammation within 10 wk of treatment [83], a HF diet for 16 weeks show markedly altered intestinal microbiota and intrahepatic cytokine profiles [84]. Similarly, a synthetic diet of HF and sucrose, administered for 5 wk and combined with stimulation by LPS, causes mild steatosis of liver cells in rats, leading to a high sensitivity of the liver to harmful factors [85]. Fukunishi et al. [86] also showed that LPS aggravate steatosis, focal necrosis, and fibrosis in the livers of Zucker rats when used twelve weeks of exposure to this synthetic diet and LPS treatment in the last 2 weeks. However, consumption of fructose with controlled caloric intake generates signs of endotoxemia and liver damage without evident modification in the microbiome [87]. This finding indicates that although fructose-induced intestinal dysbiosis can only occur in the presence of excess energy consumption, liver damage is not always connected to the microbiome.

Lipid accumulation is linked to a fibrotic KC phenotype, which is accompanied by increased release of several cytokines and chemokines, resulting in NASH [43,88]. However, fibrogenesis is dependent on TLR4 expression in HSCs more than that in other hepatic cell types, as demonstrated by experiments in chimeric mice [89]. TLR4 was shown to mediate HSC activation through increased exposure to KC-derived transforming growth factor (TGF- β). This response was observed with HSC-mediated KC chemotaxis, where HSC activation and fibrogenesis were nearly completely suppressed in KC-depleted mice. This view is supported by endotoxin signaling identified in HSCs activation (Table 2), while fibrogenic cell type in injured liver mediates key responses and inflammatory phenotype [90,91]. *In-vitro* models can offer mechanistic insights into how inflammation and lipotoxicity directly contribute to the development of NASH [92]. Tang et al. [93] investigated the importance of LPS-induced liver injury in administration of HF diet. IL-17, which mediates potent inflammatory immune responses, also causes accumulation of intracellular lipids [93]. This may play a crucial role in inflammation of NAFLD and may favor hepatic steatosis and a pro-inflammatory response in NAFLD, facilitating the transition from simple steatosis to steatohepatitis. Moreover, nucleotide oligomerization domains (NOD)-like receptors (NLRs) have been found to play a major role in NASH pathogenesis. NLR activation in response to injury, coupled with damage-associated molecular pattern molecules (DAMPs) or PAMPs, mediates the recruitment of the inflammasome, a multiprotein complex required for caspase-1 activity and initiation of inflammatory signals [94]. Nucleotide-binding domain leucine-rich repeat protein (NLRP)6 and NLRP3 inflammasomes are sensors of endogenous or exogenous PAMPs or DAMPs, and negatively control the development of NAFLD and NASH by modulating gut microbiota [95]. This suggests that dysbiosis alone can stimulate steatosis and metabolic syndrome, as demonstrated by Csak et al. [96].

The influence of gut microbiota on energy metabolism in the liver may also be mediated by short-chain fatty acids (SCFAs) including acetate (C2), propionate (C3), and butyrate (C4). SCFAs are the major metabolic products of bacterial anaerobic fermentation in the intestine. SCFAs constitute a substantial portion of essential energy, which favors the development of steatosis, increasing the distribution of SCFAs to the liver [97]. However, SCFAs also act on other cells to regulate various leukocyte roles [98] connected with G protein-coupled receptor 43 (GPR43), which affects inflammatory responses. Gpr43^{-/-} mice show systemic inflammation in various tissues [99]. Acetate can reduce liver damage induced by bacterial products. This beneficial effect of acetate is not observed in GPR43^{-/-} mice when SCFA/GPR43 signaling increases intestinal permeability [100]. Furthermore, bacteria inside the intestine can also chemically transform bile acids, thereby altering composition of bile-acid pool. While bile acids play a role as detergents to enable fat absorption, they are now documented as vital cell-signaling molecules activating several pathways [101]. Bile acids in the intestine act as strong antimicrobial agents controlling the growth of gut bacteria and microbiome composition. Bile acid molecules exert these effects by binding and activating farnesoid X receptor (FXR; nuclear hormone receptor) and G protein coupled cell surface receptor TGR5 [102,103]. FXR knockout mice receiving HF diet showed some aspects of NASH, such as hepatic steatosis and necroinflammation [104]; as well as administration of TGR5/FXR agonists alleviate NAFLD and diminish inflammation in the liver [105].

4. Antibiotics, probiotics, and prebiotics in liver disease/NASH studies

Microbiota manipulation can be considered a potentially effective therapeutic option for the treatment of NAFLD/NASH. Understanding how microbiota influences liver disease is vital in the treatment and prevention of complications such as cirrhosis, with emerging data supporting the role of antimicrobial intervention [106–108]. Numerous studies demonstrate the relationship between gut microbiota and liver diseases (Table 3), which raises the possibility of normalizing gut microbiota as a therapeutic strategy.

4.1 Rationale for use of antibiotics in patients and animal models with chronic liver disease

In patients with bacterial peritonitis, treatment with antibiotics is correlated with diminished bacterial translocation [109]. LPS levels and fibrogenesis are markedly reduced in mice treated with a combination of three nonabsorbable oral antibiotics (ampicillin, neomycin sulfate, vancomycin) and metronidazole, suggesting that intestinal flora can be the main source of increased amounts of LPS, and indicating that intestinally derived endotoxins may drive fibrogenesis [89]. Strong intestinal decontaminants reduce fibrosis, angiogenesis, and portal hypertension in a mutant mice model of intravenous ligation [110]. In particular, administration of antibiotics decreases the chance of developing spontaneous hepatic bacterial peritonitis and

hepatorenal syndrome [111]. Gut decontamination in mice fed an HF diet also reduces the levels of endotoxins, improves glucose tolerance, and decreases the levels of hepatic triglycerides [112].

Initially, NASH was a frequent complication of jejunoileal bypass surgery for morbid obesity during the 1980s and could be reversed by treatment with metronidazole [113]. Although the precise mechanism of this type of NASH is not fully understood, antibiotics appear to prevent this complication. Other examples show that antibiotics can reduce hepatic steatosis and endotoxemia in an NAFLD model of rodents fed a fructose-rich diet [114]. Of note, mucosal injury can lead to hepatic damage by increased permeability of small intestine and absorption of toxic products such as bacterial cell-wall polymers; however, antibiotics reduce the absorption of peptidoglycan-polysaccharide polymers derived from bacteria, thereby preventing hepatic injury [115]. Furthermore, recruitment of migratory macrophages is significantly suppressed by treatment with antibiotics when mice receive a new class of HF diet; this suggests that gut microbiota and related metabolites are indispensable for macrophage recruitment to the liver [116].

In accordance with these observations, a short-term treatment with 1200 mg/day rifaximin inhibits the production of endotoxins in patients with NAFLD/NASH [117]. A significant reduction in the levels of transaminases was observed in this study, although serum levels of TLR4 and IL-1, IL-6, IL-12, or TNF- α do not appear to be affected. Antibiotics decrease choline-deficient, L-amino acid-defined (CDAA)-induced liver fibrosis and HSC activation, thereby reducing intestinal permeability [118]. Membrez et al. [119] have shown that modulation of intestinal microbiota and improved glucose response in *ob/ob* mice is associated with altered expression of hepatic and intestinal genes involved in inflammation. These results highlight the role of flora in the small intestine, implying that treating SIBO may decrease LPS concentrations. However, antibiotics are not generally used to treat NASH. Antibiotics can result in unwanted side effects when administered long-term. Moreover, bacterial resistance may limit the efficacy of antibiotics and predispose patients to serious conditions, such as fungal and pathogenic bacteria overgrowth, with increased risk of morbidity and mortality [120].

4.2 Association between probiotics and clinical outcomes in hepatic injury

Numerous studies have shown the positive effects of probiotics in influencing immune responses and preventing the development of inflammatory diseases. Probiotics are live microbes that modulate intestinal microflora and increase overall health by immunoregulatory and anti-inflammatory activity; modifying permeability and epithelial function; improving intestinal barrier defense; defending against LPS-induced liver damage; exerting antioxidant activity; and improving liver-function [121–125]. This suggests that probiotics can improve the composition of gut microbiota, downregulate serum levels of LPS and liver TLR4, which delay the progression of liver disease [126].

Bifidobacterium and *Lactobacillus* are extensively used as probiotics because they efficiently reduce the growth of pathogenic microorganisms. Although these probiotics are usually present in the intestine, the population of probiotic microbes is reduced under pathogenic setting. A randomized double-blind controlled study found that in adults with NAFLD, treatment with 500 million *Lactobacillus bulgaricus* and *Streptococcus thermophilus* decreased the levels of liver transaminase [127]. VSL#3, a mixture of *Streptococcus thermophilus*, *Bifidobacterium breve*, *Bifidobacterium infantis*, *Bifidobacterium longum*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei*, and *L. bulgaricus*, showed beneficial effects in hepatic injury [128,129], although Solga et al. [130] found that VSL#3 negatively affected patients with hepatic steatosis. In rats with oxidative and inflammatory injury induced via administration of HF-diet, VSL#3 blocked augmented expression of inflammatory mediators in the liver [131]. VSL#3 also prevented IR, steatohepatitis, and reduced aortic plaques in a mouse model of atherogenesis, where low status of inflammation aggravates atherosclerosis [132]. Moreover, VSL#3 improved the quality of life, and decreased both Child-Turcotte-Pugh-class and Model of End-Stage Liver Disease scores, with improvement of liver function in patients with cirrhosis [133].

In terms of NASH severity, administering probiotics to leptin-deficient *ob/ob* mice considerably decreases hepatic lipid accumulation by counteracting anti-TNF antibodies; this suggests a relationship between the enteric flora and liver injury [12]. In mouse model, *Lactobacillus casei* Shirota exerted positive effects on fructose-induced liver injury by regulating the expression of TLR4 [134], and suppressed serum LPS concentrations, reducing inflammation, and protecting against hepatic steatosis and fibrosis in liver of a MCD diet [135]. Nevertheless, few studies have investigated whether administration of probiotics can decrease hepatic fibrosis in human patients, and the majority of studies have focused on treating complications in advanced stages of the illness. In this phase, liver fibrosis is irreversible due to development of collagen crosslinks; as a result, any beneficial effects of probiotics at this stage of fibrosis would be highly improbable. Hence, while recent investigations with numerous strains of probiotics, including *Lactobacillus rhamnosus*, *Lactobacillus plantarum bulgaricus*, and *Lactobacillus casei* showed positive effects in rodent models [134–137], randomized clinical trial data collected long-term are not presently available.

4.3 Treatment with prebiotics and alterations in gut microbiota, inflammation, steatosis, and plasma levels of gut peptides

Prebiotics are indigestible carbohydrates that favorably affect the expansion and activity of gut bacteria, mainly those of *Bifidobacteria* and *Lactobacilli* [138]. Although studies show that prebiotics can potentially be used as a nutritional treatment for patients with NAFLD/NASH, high-quality clinical trials on this subject are currently lacking.

The prebiotic oligofructose (OFS) is a nondigestible oligomer of β -D-fructose, acquired by enzymatic hydrolysis of inulin or oligofructose removed from chicory root [139]. OFS is readily fermented in the colon, triggering selective production of *Bifidobacterium* [140]. In mice that are administered a polyunsaturated fatty acid-depleted diet to induce hepatic steatosis, OFS protects against steatosis, which is accompanied by decreased oxidation of fatty acids [141]. In patients with NASH, property of *B. longum* and the synthetic fructooligosaccharides (FOS) associated with lifestyle alteration *versus* lifestyle alteration only included metabolic and anti-inflammatory effects [142]. Daubioul et al. [143] reported improved activity of hepatic enzymes in patients with NASH receiving dietary fructans. Ferolla et al. [144] observed a reduction in steatosis, body weight, body mass index (BMI), waist circumference, and uric acid levels in patients with NASH administered guar gum and inulin (1×10^8 CFU of *L. reuteri*) twice daily for 3 months; however, no effects on gut permeability or SIBO were observed.

Products of butyrate fermentation are energetic substrates for colonocytes. In this metabolic cascade, trophic influences on mucosa can increase the ratio of propionate to acetate in rats administered high fructose (HFr) diet [145]; this can reduce lipogenesis because propionate decreases lipogenesis while acetate stimulates it. These data show that in carbohydrate fermentation, administration of HFr diet decreases the levels of pooled SCFA, but, supplementation with OFS affects fructose absorption. Especially, prebiotics can influence the production of the gut trophic hormone, glucagon-like peptide-2, which

modulates lipid and LPS absorption via its effects on intestinal permeability [146]. This may positively impact the integrity of gut barrier.

5. Conclusions and perspectives

Studies indicate that gut microbiota play a central role in the fibrotic processes of NAFLD, and increased levels of endotoxins have been found to exacerbate NASH evolution. However, the complexity of NAFLD/NASH, as well as differences between individuals, renders it difficult to conduct population studies. There is no effective treatment for NASH, and the efficacy of new therapeutic approaches has not been assessed in human patients due to variations in methodology, and dietary variations that may lead to different fibro-inflammatory phenotypes. Indeed, persistent exposure to gut-derived bacterial endotoxins may contribute to hepatic hemodynamic regulation and immune responses. Nevertheless, the connections between metabolic pathways of the gut microbial system and the immune system have not yet been well delineated. Further studies are needed to address whether the course of NASH can be altered by modulating the gut microbiota. Determining exactly how the gut microbiome participates in NASH will certainly advance our knowledge and development of treatment options.

Declarations of interest

None

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References

- Day CP, James OP (1998) Steatohepatitis: a tale of two "hits"? *Gastroenterology* 114:842–845.
- Chung MY, Yeung SF, Park HJ, Volek JS, Bruno RS (2010) Dietary α - and γ -tocopherol supplementation attenuates lipopolysaccharide-induced oxidative stress and inflammatory-related responses in an obese mouse model of nonalcoholic steatohepatitis. *J Nutr Biochem* 21:1200–1206.
- Han YH, Kim HJ, Kim EJ, Kim KS, Hong S, Park HG, Lee MO (2014) ROR α decreases oxidative stress through the induction of SOD2 and GPx1 expression and thereby protects against nonalcoholic steatohepatitis in mice. *Antioxid & Redox Signaling* 21:2083–2094.
- Thomsen KL, Hebbard L, Glavind E, Clouston A, Vilstrup H, George J, Grønbaek H (2014) Nonalcoholic steatohepatitis weakens the acute phase response to endotoxin in rats. *Liver Int* 34:1584–1592.
- Sakaguchi S, Takahashi S, Sasaki T, Kumagai T, Nagata K (2011) Progression of alcoholic and nonalcoholic steatohepatitis: common metabolic aspects of innate immune system and oxidative stress. *Drug Metab Pharmacokinet* 26:30–46.
- Imajo K, Yoneda M, Ogawa Y, Wada K, Nakajima A (2014) Microbiota and nonalcoholic steatohepatitis. *Semin Immunopathol* 36:115–132.
- Boursier J, Mueller O, Barret M, Machado M, Fizanne L, Araujo-Perez F, Guy CD, Seed PC, Rawls JF, David LA, Hunault G, Oberti F, Calès P, Diehl AM (2016) The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. *Hepatology* 63:764–775.
- Moschen AR, Kaser S, Tilg H (2013) Non-alcoholic steatohepatitis: a microbiota-driven disease. *Trend Endocrinol Metab* 24:537–545.
- Quigley EM, Stanton C, Murphy EF (2013) The gut microbiota and the liver. Pathophysiological and clinical implications. *J Hepatol* 58:1020–1027.
- Mao JW, Tang HY, Zhao T, Tan XY, Bi J, Wang BY, Wang YD (2015) Intestinal mucosal barrier dysfunction participates in the progress of nonalcoholic fatty liver disease. *Int J Clin Exp Pathol* 8:3648–3658.
- Yang SQ, Lin HZ, Lane MD, Clemens M, Diehl AM (1997) Obesity increases sensitivity to endotoxin liver injury: implications for the pathogenesis of steatohepatitis. *Proc Natl Acad Sci USA* 94:2557–2562.
- Li Z, Yang S, Lin H, Huang J, Watkins PA, Moser AB, Desimone C, Song XY, Diehl AM (2003) Probiotics and antibodies to TNF inhibit inflammatory activity and improve nonalcoholic fatty liver disease. *Hepatology* 37:343–350.
- Mehal WZ. (2013) The Gordian Knot of dysbiosis, obesity and NAFLD. *Nat Rev Gastroenterol Hepatol* 10:637–644.
- du Plessis J, Korf H, van Pelt J, Windmolders P, Vander Elst I, Verrijken A, Hubens G, Van Gaal L, Cassiman D, Nevens F, Francque S, van der Merwe S (2016) Pro-Inflammatory cytokines but not endotoxin-related parameters associate with disease severity in patients with NAFLD. *PLoS One* 11:e016604.
- Kitabatake H, Tanaka N, Fujimori N, Komatsu M, Okubo A, Kakegawa K, Kimura T, Sugiura A, Yamazaki T, Shibata S, Ichikawa Y, Yoshita S, Umemura T, Matsumoto A, Koinuma M, Sano K, Aoyama T, Tanaka E (2017) Association between endotoxemia and histological features of nonalcoholic fatty liver disease. *World J Gastroenterol* 23:712–722.
- Mouzaki M, Comelli EM, Arendt BM, Bonengel J, Fung SK, Fischer SE, McGilvray ID, Allard JP (2013) Intestinal microbiota in patients with nonalcoholic fatty liver disease. *Hepatology* 58:120–127.
- Del Chierico F, Gnani D, Vernocchi P, Petrucca A, Alisi A, Dallapiccola B, Nobili V, Lorenza P (2014) Meta-omic platforms to assist in the understanding of NAFLD gut microbiota alterations: tools and applications. *Int J Mol Sci* 15:684–711.
- Goel A, Gupta M, Aggarwal R (2014) Gut microbiota and liver disease. *J Gastroenterol Hepatol* 29:1139–1148.

19. Sharma M, Mitnala S, Vishnubhotla RK, Mukherjee R, Reddy DN, Rao PN (2015) The riddle of nonalcoholic fatty liver disease: progression from nonalcoholic fatty liver to nonalcoholic steatohepatitis. *J Clin Exp Hepatol* 5:147–158.
20. Wieland A, Frank DN, Harnke B, Bambha K (2015) Systematic review: microbial dysbiosis and nonalcoholic fatty liver disease. *Aliment Pharmacol Therap* 42:1051–1063.
21. Fukui H (2015) Gut Microbiota and host reaction in liver diseases. *Microorganisms* 3:759–791.
22. Abdou RM, Zhu L, Baker RD, Baker SS (2016) Gut microbiota of nonalcoholic fatty liver disease. *Dig Dis Sci* 61:1268–1281.
23. Sekirov I, Russell SL, Antunes LC, Finlay BB (2010) Gut microbiota in health and disease. *Physiol Rev* 90:859–904.
24. Wigg AJ, Roberts-Thomson IC, Dymock RB, McCarthy PJ, Grose RH, Cummins AG (2001) The role of small intestinal bacterial overgrowth, intestinal permeability, endotoxaemia, and tumour necrosis factor alpha in the pathogenesis of non-alcoholic steatohepatitis. *Gut* 48:206–211.
25. Shanab AA, Scully P, Crosbie O, Buckley M, O'Mahony L, Shanahan F, Gazareen S, Murphy E, Quigley EM (2011) Small intestinal bacterial overgrowth in nonalcoholic steatohepatitis: association with toll-like receptor 4 expression and plasma levels of interleukin 8. *Dig Dis Sci* 56:1524–1534.
26. Ilan Y (2012) Leaky gut and the liver: a role for bacterial translocation in nonalcoholic steatohepatitis. *World J Gastroenterol* 18:2609–2618.
27. Chiu CC, Ching YH, Li YP, Liu JY, Huang YT, Huang YW, Yang SS, Huang WC, Chuang HL (2017) Nonalcoholic Fatty Liver Disease Is Exacerbated in High-Fat Diet-Fed Gnotobiotic Mice by Colonization with the Gut Microbiota from Patients with Nonalcoholic Steatohepatitis. *Nutrients* 9(11):pii:E1220.
28. Miele L, Valenza V, La Torre G, Montalto M, Cammarota G, Ricci R, Mascianà R, Forgione A, Gabrieli ML, Perotti G, Vecchio FM, Rapaccini G, Gasbarrini G, Day CP, Grieco A (2009) Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. *Hepatology* 49:1877–1887.
29. Bashiardes S, Shapiro H, Rozin S, Shibolet O, Elinav E (2016) Non-alcoholic fatty liver and the gut microbiota. *Mol Metab* 5:782–794.
30. Brun P, Castagliuolo I, Di Leo V, Buda A, Pinzani M, Palù G, Martines D (2007) Increased intestinal permeability in obese mice: new evidence in the pathogenesis of nonalcoholic steatohepatitis. *Am J Physiol Gastrointest Liver Physiol* 292:G518–G525.
31. Dai X, Wang B (2015) Role of gut barrier function in the pathogenesis of nonalcoholic fatty liver disease. *Gastroenterol Res Pract* 287348.
32. Wiest R, Albillos A, Trauner M, Bajaj JS, Jalan R (2017) Targeting the gut-liver axis in liver disease. *J Hepatol* 67(5):1084–1103.
33. Farhadi A, Gundlapalli S, Shaikh M, Frantzides C, Harrell L, Kwasny MM, Keshavarzian A (2008) Susceptibility to gut leakiness: a possible mechanism for endotoxaemia in nonalcoholic steatohepatitis. *Liver Int* 28:1026–1033.
34. Akira S, Takeda K, Kaisho T (2001) Toll-like receptors: critical proteins linking innate and acquired immunity. *Nat Immunol* 2:675–680.
35. Soares JB, Pimentel-Nunes P, Roncon-Albuquerque R, Leite-Moreira A (2010) The role of lipopolysaccharide/toll-like receptor 4 signalling in chronic liver diseases. *Hepatol Int* 4:659–672.
36. Miura K, Ohnishi H (2014) Role of gut microbiota and Toll-like receptors in nonalcoholic fatty liver disease. *World J Gastroenterol* 20:7381–7391.
37. Mencin A, Kluwe J, Schwabe RF (2009) Toll-like receptors as targets in chronic liver diseases. *Gut* 58:704–720.
38. Valenti L, Fraconzani AL, Fargion S (2009) The immunopathogenesis of alcoholic and nonalcoholic steatohepatitis: two triggers for one disease? *Semin immunopathol* 31:359–369.
39. Sharifnia T, Antoun J, Verriere TG, Suarez G, Wattacheril J, Wilson KT, Peek RM Jr, Abumrad NN, Flynn CR (2015) Hepatic TLR4 signaling in obese NAFLD. *Am J Physiol Gastrointest Liver Physiol* 309:G270–G278.
40. Gao Y, Song LX, Jiang MN, Ge GY, Jia YJ (2008) Effects of traditional chinese medicine on endotoxin and its receptors in rats with non-alcoholic steatohepatitis. *Inflammation* 31:121–132.
41. Namisaki T, Noguchi R, Moriya K, Kitade M, Aihara Y, Douhara A, Nishimura N, Takeda K, Okura Y, Kawaratani H, Takaya H, Seki K, Yoshiji H (2016) Beneficial effects of combined ursodeoxycholic acid and angiotensin-II type 1 receptor blocker on hepatic fibrogenesis in a rat model of nonalcoholic steatohepatitis. *J Gastroenterol* 51:162–172.
42. Kessoku T, Imajo K, Honda Y, Kato T, Ogawa Y, Tomeno W, Kato S, Mawatari H, Fujita K, Yoneda M, Nagashima Y, Saito S, Wada K, Nakajima A (2016) Resveratrol ameliorates fibrosis and inflammation in a mouse model of nonalcoholic steatohepatitis. *Sci Rep* 6:22251.
43. Chanda D, Li J, Oligschlaeger Y, Jeurissen ML, Houben T, Walenbergh SM, Shiri-Sverdlov R, Neumann D (2016) MSP is a negative regulator of inflammation and lipogenesis in ex vivo models of non-alcoholic steatohepatitis. *Exp Mol Med* 48:e258.
44. Cabrera D, Wree A, Povero D, Solís N, Hernandez A, Pizarro M, Moshage H, Torres J, Feldstein AE, Cabello-Verrugio C, Brandan E, Barrera F, Arab JP, Arrese M (2017) Andrographolide ameliorates inflammation and fibrogenesis and attenuates inflammasome activation in experimental non-alcoholic steatohepatitis. *Sci Rep* 7:3491.
45. Shu X, Wang M, Xu H, Liu Y, Huang J, Yao Z, Zhang L (2017) Extracts of *Salvia-Nelumbinis Naturalis* ameliorate nonalcoholic steatohepatitis via inhibiting gut-derived endotoxin mediated TLR4/NF- κ B activation. *Evid Based Complement Alternat Med* 9208314.
46. Rivera CA, Adegboyega P, van Rooijen N, Tagalicud A, Allman M, Wallace M (2007) Toll-like receptor-4 signaling and Kupffer cells play pivotal roles in the pathogenesis of non-alcoholic steatohepatitis. *J Hepatol* 47:571–579.
47. Seki E, Brenner DA (2008) Toll-like receptors and adaptor molecules in liver disease: update. *Hepatology* 48:322–335.
48. Csak T, Velayudham A, Hritz I, Petrasek J, Levin I, Lippai D, Catalano D, Mandrekar P, Dolganiuc A, Kurt-Jones E, Szabo G (2011) Deficiency in myeloid differentiation factor-2 and toll-like receptor 4 expression attenuates nonalcoholic steatohepatitis and fibrosis in mice. *Am J Physiol Gastrointest Liver Physiol* 300:G433G441.
49. Yang L, Miura K, Zhang B, Matsushita H, Yang YM, Liang S, Song J, Roh YS, Seki E (2017) TRIF differentially regulates hepatic steatosis and inflammation/fibrosis in mice. *Cell Mol Gastroenterol Hepatol* 3:469–483.
50. Lee SJ, Kang JH, Choi SY, Kwon OS (2013) PKC δ as a regulator for TGF β 1-induced α -SMA production in a murine nonalcoholic steatohepatitis model. *PLoS One* 8:e55979.

51. Duparc T, Plovier H, Marrachelli VG, Van Hul M, Essaghir A, Ståhlman M, Matamoros S, Geurts L, Pardo-Tendero MM, Druart C, Delzenne NM, Demoulin JB, van der Merwe SW, van Pelt J, Bäckhed F, Monleon D, Everard A, Cani PD (2017) Hepatocyte MyD88 affects bile acids, gut microbiota and metabolome contributing to regulate glucose and lipid metabolism. *Gut* 66:620–632.
52. Imajo K, Fujita K, Yoneda M, Nozaki Y, Ogawa Y, Shinohara Y, Kato S, Mawatari H, Shibata W, Kitani H, Ikejima K, Kirikoshi H, Nakajima N, Saito S, Maeyama S, Watanabe S, Wada K, Nakajima A (2012) Hyperresponsivity to low-dose endotoxin during progression to nonalcoholic steatohepatitis is regulated by leptin-mediated signaling. *Cell Metab* 16:44–54.
53. Kawaratani H, Tsujimoto T, Kitazawa T, Kitade M, Yoshiji H, Uemura M, Fukui H (2008) Innate immune reactivity of the liver in rats fed a choline-deficient L-amino-acid-defined diet. *World J Gastroenterol* 14:6655–6661.
54. Machado MV, Cortez-Pinto H (2012) Gut microbiota and nonalcoholic fatty liver disease. *Ann Hepatol* 11:440–449.
55. Guo S, Al-Sadi R, Said HM, Ma TY (2013) Lipopolysaccharide causes an increase in intestinal tight junction permeability in vitro and in vivo by inducing enterocyte membrane expression and localization of TLR4 and CD14. *Am J Pathol* 182:375–387.
56. Pradere JP, Troeger JS, Dapito DH, Mencin AA, Schwabe RF (2010) Toll-like receptor 4 and hepatic fibrogenesis. *Semin Liver Dis* 30:232–244.
57. Tilg H, Moschen AR (2010) Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. *Hepatology* 52:1836–1846.
58. Ogawa Y, Imajo K, Yoneda M, Kessoku T, Tomeno W, Shinohara Y, Kato S, Mawatari H, Nozaki Y, Fujita K, Kirikoshi H, Maeda S, Saito S, Wada K, Nakajima A (2013) Soluble CD14 levels reflect liver inflammation in patients with nonalcoholic steatohepatitis. *PLoS One* 8:e65211.
59. Szabo G, Velayudham A, Romics L Jr, Mandrekar P (2005) Modulation of non-alcoholic steatohepatitis by pattern recognition receptors in mice: the role of toll-like receptors 2 and 4. *Alcohol Clin Exp Res* 29:140S–145S.
60. Achiwa K, Ishigami M, Ishizu Y, Kuzuya T, Honda T, Hayashi K, Hirooka Y, Katano Y, Goto H (2016) DSS colitis promotes tumorigenesis and fibrogenesis in a choline-deficient high-fat diet-induced NASH mouse model. *Biochem Biophys Res Commun* 470:15–21.
61. Gäbele E, Dostert K, Hofmann C, Wiest R, Schölmerich J, Hellerbrand C, Obermeier F (2011) DSS induced colitis increases portal LPS levels and enhances hepatic inflammation and fibrogenesis in experimental NASH. *J Hepatol* 55:1391–1399.
62. Kudo H, Takahara T, Yata Y, Kawai K, Zhang W, Sugiyama T (2009) Lipopolysaccharide triggered TNF-alpha-induced hepatocyte apoptosis in a murine nonalcoholic steatohepatitis model. *J Hepatol* 51:168–175.
63. Alisi A, Manco M, Devito R, Piemonte F, Nobili V (2010) Endotoxin and plasminogen activator inhibitor-1 serum levels associated with nonalcoholic steatohepatitis in children. *J Pediatr Gastroenterol Nutr* 50:645–649.
64. Ceccarelli S, Panera N, Mina M, Gnani D, De Stefanis C, Crudele A, Rychlicki C, Petrini S, Bruscalupi G, Agostinelli L, Stronati L, Cucchiara S, Musso G, Furlanello C, Svegliati-Baroni G, Nobili V, Alisi A (2015) LPS-induced TNF- α factor mediates pro-inflammatory and pro-fibrogenic pattern in non-alcoholic fatty liver disease. *Oncotarget* 6:41434–41452.
65. Duman DG, Ozdemir F, Birben E, Keskin O, Ekşioglu-Demiralp E, Celikel C, Kalayci O, Kalayci C (2007) Effects of pentoxifylline on TNF-alpha production by peripheral blood mononuclear cells in patients with nonalcoholic steatohepatitis. *Dig Dis Sci* 52:2520–2524.
66. Kirsch R, Clarkson V, Verdonk RC, Marais AD, Shephard EG, Ryffel B, de la M Hall P (2006) Rodent nutritional model of steatohepatitis: effects of endotoxin (lipopolysaccharide) and tumor necrosis factor alpha deficiency. *J Gastroenterol Hepatol* 21:174–182.
67. Delarue J, Lallès JP (2016) Nonalcoholic fatty liver disease: Roles of the gut and the liver and metabolic modulation by some dietary factors and especially long-chain n-3 PUFA. *Mol Nutr Food Res* 60:147–159.
68. Matsushita N, Osaka T, Haruta I, Ueshiba H, Yanagisawa N, Omori-Miyake M, Hashimoto E, Shibata N, Tokushige K, Saito K, Tsuneda S, Yagi J (2015) Effect of lipopolysaccharide on the progression of non-alcoholic fatty liver disease in high caloric diet-fed mice. *Scand J Immunol* 83:109–118.
69. Dumas ME, Barton RH, Toye A, Cloarec O, Blancher C, Rothwell A, Fearnside J, Tatoud R, Blanc V, Lindon JC, Mitchell SC, Holmes E, McCarthy MI, Scott J, Gauguier D, Nicholson JK (2006) Metabolic profiling reveals a contribution of gut microbiota to fatty liver phenotype in insulin-resistance mice. *Proc Natl Acad Sci USA* 103:12511–12516.
70. Spencer MD, Hamp TJ, Reid RW, Fischer LM, Zeisel SH, Fodor AA (2011) Association between composition of the human gastrointestinal microbiome and development of fatty liver with choline deficiency. *Gastroenterology* 140:976–986.
71. Pawlak M, Baugé E, Bourguet W, De Bosscher K, Lalloyer F, Tailleux A, Lebherz C, Lefebvre P, Staels B (2014) The transrepressive activity of peroxisome proliferator-activated receptor alpha is necessary and sufficient to prevent liver fibrosis in mice. *Hepatology* 60:1593–1596.
72. Jha P, Claudel T, Baghdasaryan A, Mueller M, Halilbasic E, Das SK, Lass A, Zimmermann R, Zechner R, Hoefler G, Trauner M (2014) Role of adipose triglyceride lipase (PNPLA2) in protection from hepatic inflammation in mouse models of steatohepatitis and endotoxemia. *Hepatology* 59:858–869.
73. Okubo H, Kushiyama A, Sakoda H, Nakatsu Y, Iizuka M, Taki N, Fujishiro M, Fukushima T, Kamata H, Nagamachi A, Inaba T, Nishimura F, Katagiri H, Asahara T, Yoshida Y, Chonan O, Encinas J, Asano T (2016) Involvement of resistin-like molecule β in the development of methionine-choline deficient diet-induced non-alcoholic steatohepatitis in mice. *Sci Rep* 6:20157.
74. Anavi S, Hahn-Obercyger M, Margalit R, Madar Z, Tirosh O (2013) A novel antihypoglycemic role of inducible nitric oxide synthase in liver inflammatory response induced by dietary cholesterol and endotoxemia. *Antioxid Redox Signal* 19:1889–1901.
75. Leroux A, Ferrere G, Godie V, Cailleux F, Renoud ML, Gaudin F, Naveau S, Prévot S, Makhzami S, Perlemuter G, Cassard-Doulier AM (2012) Toxic lipids stored by Kupffer cells correlates with their pro-inflammatory phenotype at an early stage of steatohepatitis. *J Hepatol* 57:141–149.

76. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, Neyrinck AM, Fava F, Tuohy KM, Chabo C, Waget A, Delmée E, Cousin B, Sulpice T, Chamontin B, Ferrières J, Tanti JF, Gibson GR, Casteilla L, Delzenne NM, Alessi MC, Burcelin R (2007) Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 56:1761–1772.
77. Etienne-Mesmin L, Vijay-Kumar M, Gewirtz AT, Chassaing B (2016) Hepatocyte Toll-Like Receptor 5 promotes bacterial clearance and protects mice against high-fat diet-induced liver disease. *Cell Mol Gastroenterol Hepatol* 2:584–604.
78. Xie G, Wang X, Liu P, Wei R, Chen W, Rajani C, Hernandez BY, Alegado R, Dong B, Li D, Jia W (2016) Distinctly altered gut microbiota in the progression of liver disease. *Oncotarget* 7:19355–19366.
79. Ley RE, Bäckhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI (2005) Obesity alters gut microbial ecology. *Proc Nat Acad Sci USA* 102:11070–11075.
80. Ley RE, Turnbaugh PJ, Klein S, Gordon JI (2006) Microbial ecology: human gut microbes associated with obesity. *Nature* 444:1022–1023.
81. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI (2006) An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 444:1027–1031.
82. Cao Y, Pan Q, Cai W, Shen F, Chen GY, Xu LM, Fan JG (2016) Modulation of gut microbiota by Berberine improves steatohepatitis in high-fat diet-fed BALB/C mice. *Arch Iran Med* 19:197–203.
83. Liang W, Lindeman JH, Menke AL, Koonen DP, Morrison M, Havekes LM, van den Hoek AM, Kleemann R (2014) Metabolically induced liver inflammation leads to NASH and differs from LPS- or IL-1 β -induced chronic inflammation. *Lab Invest* 94:491–502.
84. Zhou D, Pan Q, Shen F, Cao HX, Ding WJ, Chen YW, Fan JG (2017) Total fecal microbiota transplantation alleviates high-fat diet-induced steatohepatitis in mice via beneficial regulation of gut microbiota. *Sci Rep* 7:1529.
85. Guo JH, Han DW, Li XQ, Zhang Y, Zhao YC (2014) The impact of small doses of LPS on NASH in high sucrose and high fat diet induced rats. *Eur Rev Med Pharmacol Sci* 18:2742–2747.
86. Fukunishi S, Nishio H, Fukuda A, Takeshita A, Hanafusa T, Higuchi K, Suzuki K (2009) Development of fibrosis in nonalcoholic steatosis through combination of a synthetic diet rich in disaccharide and low-dose lipopolysaccharides in the livers of Zucker (fa/fa) rats. *J Clin Biochem Nutr* 45:322–328.
87. Kavanagh K, Wylie AT, Tucker KL, Hamp TJ, Gharraibeh RZ, Fodor AA, Cullen JM (2013) Dietary fructose induces endotoxemia and hepatic injury in calorically controlled primates. *Am J Clin Nutr* 98:349–357.
88. Kobori M, Ni Y, Takahashi Y, Watanabe N, Sugiura M, Ogawa K, Nagashimada M, Kaneko S, Naito S, Ota T (2014) β -Cryptoxanthin alleviates diet-induced nonalcoholic steatohepatitis by suppressing inflammatory gene expression in mice. *PLoS One* 9:e98294.
89. Seki E, De Minicis S, Osterreicher CH, Kluwe J, Osawa Y, Brenner DA, Schwabe RF (2007) TLR4 enhances TGF-beta signaling and hepatic fibrosis. *Nat Med* 13:1324–1332.
90. Guo J, Friedman SL (2010) Toll-like receptor 4 signaling in liver injury and hepatic fibrogenesis. *Fibrogenesis Tissue Repair* 3:21.
91. Marra F, Lotersztajn S (2013) Pathophysiology of NASH: perspectives for a targeted treatment. *Curr Pharm Des* 19:5250–5269.
92. Boeckmans J, Natale A, Buyl K, Rogiers V, De Kock J, Vanhaecke T, Rodrigues RM (2018) Human-based systems: Mechanistic NASH modelling just around the corner? *Pharmacol Res* 134:257–267.
93. Tang Y, Bian Z, Zhao L, Liu Y, Liang S, Wang Q, Han X, Peng Y, Chen X, Shen L, Qiu D, Li Z, Ma X (2011) Interleukin-17 exacerbates hepatic steatosis and inflammation in non-alcoholic fatty liver disease. *Clin Exp Immunol* 166:281–290.
94. Caligiuri A, Gentilini A, Marra F (2016) Molecular pathogenesis of NASH. *Int J Mol Sci* 20:17.
95. Henaoui-Mejia J, Elinav E, Jin C, Hao L, Mehal WZ, Strowig T, Thaïss CA, Kau AL, Eisenbarth SC, Jurczak MJ, Camporez JP, Shulman GI, Gordon JI, Hoffman HM, Flavell RA (2012) Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. *Nature* 482:179–185.
96. Csak T, Ganz M, Pespisa J, Kodys K, Dolganiuc A, Szabo G (2011) Fatty acid and endotoxin activate inflammasomes in mouse hepatocytes that release danger signals to stimulate immune cells. *Hepatology* 54:133–144.
97. Liu W, Baker RD, Bhatia T, Zhu L, Baker SS (2016) Pathogenesis of nonalcoholic steatohepatitis. *Cell Mol Life Sci* 73:1969–1987.
98. Vernia P, Marcheggiano A, Caprilli R, Frieri G, Corrao G, Valpiani D, Di Paolo MC, Paoluzi P, Torsoli A (1995) Short-chain fatty acid topical treatment in distal ulcerative colitis. *Aliment Pharmacol Therap* 9:309–313.
99. Maslowski KM, Vieira AT, Ng A, Kranich J, Sierro F, Yu D, Schilter HC, Rolph MS, Mackay F, Artis D, Xavier RJ, Teixeira MM, Mackay CR (2009) Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature* 461:1282–1286.
100. Zhu L, Baker RD, Baker SS (2015) Gut microbiome and nonalcoholic fatty liver diseases. *Pediatr Res* 77:245–251.
101. Copple BL, Li T (2016) Pharmacology of bile acid receptors: Evolution of bile acids from simple detergents to complex signaling molecules. *Pharmacol Res* 104:9–21.
102. Zhu Y, Li F, Guo GL (2011) Tissue-specific function of farnesoid X receptor in liver and intestine. *Pharmacol Res* 63(4):259–265.
103. Brandi G, De Lorenzo S, Candela M, Pantaleo MA, Bellentani S, Tovoli F, Saccoccio G, Biasco G (2017) Microbiota, NASH, HCC and the potential role of probiotics. *Carcinogenesis* 38:231–240.
104. Kong B, Luyendyk JP, Tawfik O, Guo GL (2009) Farnesoid X receptor deficiency induces nonalcoholic steatohepatitis in low-density lipoprotein receptor-knockout mice fed a high-fat diet. *J Pharmacol Exp Ther* 328:116–122.
105. McMahan RH, Wang XX, Cheng LL, Krisko T, Smith M, El Kasmi K, Pruzanski M, Adorini L, Golden-Mason L, Levi M, Rosen HR (2013) Bile acid receptor activation modulates hepatic monocyte activity and improves nonalcoholic fatty liver disease. *J Biol Chem* 288:11761–11770.
106. Haque TR, Barritt AS 4th (2016) Intestinal microbiota in liver disease. *Best Pract Res Clin Gastroenterol* 30:133–142.
107. Federico A, Dallio M, Godos J, Loguercio C, Salomone F (2016) Targeting gut-liver axis for the treatment of nonalcoholic steatohepatitis: translational and clinical evidence. *Transl Res* 167:116–124.
108. Fiorucci S, Biagioli M, Distrutti E (2018) Future trends in the treatment of non-alcoholic steatohepatitis. *Pharmacol Res* 134:289–298.

109. Runyon BA (2007) A pill a day can improve survival in patients with advanced cirrhosis. *Gastroenterology* 133:1029–1031.
110. Zhu Q, Zou L, Jagavelu K, Simonetto DA, Huebert RC, Jiang ZD, DuPont HL, Shah VH (2012) Intestinal decontamination inhibits TLR4 dependent fibronectin-mediated cross-talk between stellate cells and endothelial cells in liver fibrosis in mice. *J Hepatol* 56:893–899.
111. Fernández J, Navasa M, Planas R, Montoliu S, Monfort D, Soriano G, Vila C, Pardo A, Quintero E, Vargas V, Such J, Ginès P, Arroyo V (2007) Primary prophylaxis of spontaneous bacterial peritonitis delays hepatorenal syndrome and improves survival in cirrhosis. *Gastroenterology* 133:818–824.
112. Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O, Geurts L, Naslain D, Neyrinck A, Lambert DM, Muccioli GG, Delzenne NM (2008) Changes in gut microbiota control metabolic endotoxemia induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes* 57:1470–1481.
113. Drenick EJ, Fisler J, Johnson D (1982) Hepatic steatosis after intestinal bypass. Prevention and reversal by metronidazole, irrespective of protein calorie malnutrition. *Gastroenterology* 82:535–548.
114. Bergheim I, Weber S, Vos M, Krämer S, Volynets V, Kaserouni S, McClain CJ, Bischoff SC (2008) Antibiotics protect against fructose induced hepatic lipid accumulation in mice: role of endotoxin. *J Hepatol* 48:983–992.
115. Lichtman SN, Keku J, Schwab JH, Sartor RB (1991) Hepatic injury associated with small bowel bacterial overgrowth in rats is prevented by metronidazole and tetracycline. *Gastroenterology* 100:513–519.
116. Yamada S, Kamada N, Amiya T, Nakamoto N, Nakaoka T, Kimura M, Saito Y, Ejima C, Kanai T, Saito H (2017) Gut microbiota-mediated generation of saturated fatty acids elicits inflammation in the liver in murine high-fat diet-induced steatohepatitis. *BMC Gastroenterol* 29:136.
117. Gangarapu V, Ince AT, Baysal B, Kayar Y, Kılıç U, Gök Ö, Uysal Ö, Şenturk H (2015) Efficacy of rifaximin on circulating endotoxins and cytokines in patients with nonalcoholic fatty liver disease. *Eur J Gastroenterol Hepatol* 27:840–845.
118. Douhara A, Moriya K, Yoshiji H, Noguchi R, Namisaki T, Kitade M, Kaji K, Aihara Y, Nishimura N, Takeda K, Okura Y, Kawaratani H, Fukui H (2015) Reduction of endotoxin attenuates liver fibrosis through suppression of hepatic stellate cell activation and remission of intestinal permeability in a rat non-alcoholic steatohepatitis model. *Mol Med Rep* 11:1693–1700.
119. Membrez M, Blancher F, Jaquet M, Bibiloni R, Cani PD, Burcelin RG, Corthesy I, Macé K, Chou CJ (2008) Gut microbiota modulation with norfloxacin and ampicillin enhances glucose tolerance in mice. *FASEB J* 22:2416–2426.
120. Fernández J, Tandon P, Mensa J, Garcia-Tsao G (2016) Antibiotic prophylaxis in cirrhosis: Good and bad. *Hepatology* 63(6):2019–2031.
121. Adawi D, Kasravi FB, Molin G, Jeppsson B (1997) Effect of *Lactobacillus* supplementation with and without arginine on liver damage and bacterial translocation in an acute liver injury model in the rat. *Hepatology* 25:642–647.
122. Loguercio C, De Simone T, Federico A, Terracciano F, Tuccillo C, Di Chicco M, Carteni M (2002) Gut-liver axis: a new point of attack to treat chronic liver damage? *Am J Gastroenterol* 97:2144–2146.
123. Osman N, Adawi D, Ahrné S, Jeppsson B, Molin G (2007) Endotoxin- and D-galactosamine-induced liver injury improved by the administration of *Lactobacillus*, *Bifidobacterium* and blueberry. *Dig Liv Dis* 39:849–856.
124. Ewaschuk J, Endersby R, Thiel D, Diaz H, Backer J, Ma M, Churchill T, Madsen K (2007) Probiotic bacteria prevent hepatic damage and maintain colonic barrier function in a mouse model of sepsis. *Hepatology* 46:841–85.
125. Kelishadi R, Farajian S, Mirlolhi M (2013) Probiotics as a novel treatment for non-alcoholic fatty liver disease; a systematic review on the current evidences. *Hepat Mon* 13:e7233.
126. Xue L, He J, Gao N, Lu X, Li M, Wu X, Liu Z, Jin Y, Liu J, Xu J, Geng Y (2017) Probiotics may delay the progression of nonalcoholic fatty liver disease by restoring the gut microbiota structure and improving intestinal endotoxemia. *Sci Rep* 7:45176.
127. Aller R, De Luis DA, Izaola O, Conde R, Gonzalez Sagrado M, Primo D, De La Fuente B, Gonzalez J (2011) Effect of a probiotic on liver aminotransferases in nonalcoholic fatty liver disease patients: a double blind randomized clinical trial. *Eur Rev Med Pharmacol Sci* 9:1090–1095.
128. Loguercio C, Federico A, Tuccillo C, Terracciano F, D'Auria MV, De Simone C, Del Vecchio Blanco C (2005) Beneficial effects of a probiotic VSL#3 on parameters of liver dysfunction in chronic liver diseases. *J Clin Gastroenterol* 39:540–543.
129. Velayudham A, Dolganiuc A, Ellis M, Petrasek J, Kodys K, Mandrekar P, Szabo G (2009) VSL#3 probiotic treatment attenuates fibrosis without changes in steatohepatitis in a diet-induced nonalcoholic steatohepatitis model in mice. *Hepatology* 49:989–997.
130. Solga SF, Buckley G, Clark JM, Horska A, Diehl AM (2008) The effect of a probiotic on hepatic steatosis. *J Clin Gastroenterol* 42(10):1117–1119.
131. Esposito E, Iacono A, Bianco G, Autore G, Cuzzocrea S, Vajro P, Canani RB, Calignano A, Raso GM, Meli R (2009) Probiotics reduce the inflammatory response induced by a high-fat diet in the liver of young rats. *J Nutr* 139:905–911.
132. Mencarelli A, Cipriani S, Renga B, Bruno A, D'Amore C, Distrutti E, Fiorucci S (2012) VSL#3 resets insulin signaling and protects against NASH and atherosclerosis in a model of genetic dyslipidemia and intestinal inflammation. *PLoS One* 7:e45425.
133. Dhiman RK, Rana B, Agrawal S, Garg A, Chopra M, Thumburu KK, Khattri A, Malhotra S, Duseja A, Chawla YK (2014) Probiotic VSL#3 reduces liver disease severity and hospitalization in patients with cirrhosis: a randomized, controlled trial. *Gastroenterology* 147:1327–1337.
134. Wagnerberger S, Spruss A, Kanuri G, Stahl C, Schröder M, Vetter W, Bischoff SC, Bergheim I (2012) *Lactobacillus casei* Shirota protects from fructose-induced liver steatosis: a mouse model. *J Nutr Biochem* 24:531–538.
135. Okubo H, Sakoda H, Kushiyama A, Fujishiro M, Nakatsu Y, Fukushima T, Matsunaga Y, Kamata H, Asahara T, Yoshida Y, Chonan O, Iwashita M, Nishimura F, Asano T (2013) *Lactobacillus casei* strain Shirota protects against nonalcoholic steatohepatitis development in a rodent model. *Am J Physiol Gastrointest Liver Physiol* 305: G911–G918.
136. Ritze Y, Bárdos G, Claus A, Ehrmann V, Bergheim I, Schwiertz A, Bischoff SC (2014) *Lactobacillus rhamnosus* GG protects against non-alcoholic fatty liver disease in mice. *PLoS One* 27;9(1):e80169.

137. Manzhaliy E, Virchenko O, Falalyeyeva T, Beregova T, Stremmel W (2017) Treatment efficacy of a probiotic preparation for non-alcoholic steatohepatitis: A pilot trial. *J Dig Dis* 18(12):698–703.
138. Roberfroid MB (2000) Prebiotics and probiotics: are they functional foods? *Am J Clin Nutr* 71:1682S–1687S.
139. Parnell JA, Raman M, Rioux KP, Reimer RA (2012) The potential role of prebiotic fibre for treatment and management of non-alcoholic fatty liver disease and associated obesity and insulin resistance. *Liver Int* 32:701–711.
140. Daubioul CA, Taper HS, De Wispelaere LD, Delzenne NM (2000) Dietary oligofructose lessens hepatic steatosis, but does not prevent hypertriglyceridemia in obese Zucker rats. *J Nutr* 130:1314–1319.
141. Pachikian BD, Essaghir A, Demoulin JB, Catry E, Neyrinck AM, Dewulf EM, Sohet FM, Portois L, Clerbaux LA, Carpentier YA, Possemiers S, Bommer GT, Cani PD, Delzenne NM (2013) Prebiotic approach alleviates hepatic steatosis: Implication of fatty acid oxidative and cholesterol synthesis pathways. *Mol Nutr Food Res* 7:347–359.
142. Malaguarnera M, Vacante M, Antic T, Giordano M, Chisari G, Acquaviva R, Mastrojeni S, Malaguarnera G, Mistretta A, Li Volti G, Galvano F (2012) *Bifidobacterium longum* with fructo-oligosaccharides in patients with non alcoholic steatohepatitis. *Dig Dis Sci* 57:545–553.
143. Daubioul CA, Horsmans Y, Lambert P, Danse E, Delzenne NM (2005) Effects of oligofructose on glucose and lipid metabolism in patients with nonalcoholic steatohepatitis: results of a pilot study. *Eur J Clin Nutr* 59:723–726.
144. Ferolla SM, Couto CA, Costa-Silva L, Armiliato GN, Pereira CA, Martins FS, Ferrari Mde L, Vilela EG, Torres HO, Cunha AS, Ferrari TC (2016) Beneficial effect of synbiotic supplementation on hepatic steatosis and anthropometric parameters, but not on gut permeability in a population with nonalcoholic steatohepatitis. *Nutrients* 28:8(7).
145. Busserolles J, Gueux E, Rock E, Demigné C, Mazur A, Rayssiguier Y (2003) Oligofructose protects against the hypertriglyceridemic and pro-oxidative effects of a high fructose diet in rats. *J Nutr* 133:1903–1908.
146. Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O, Geurts L, Naslain D, Neyrinck A, Lambert DM, Muccioli GG, Delzenne NM (2009) Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. *Gut* 58:1091–1103.
147. Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR (1999) Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 94(9):2467–2474.
148. Paik YH, Schwabe RF, Bataller R, Russo MP, Jobin C, Brenner DA (2003) Toll-like receptor 4 mediates inflammatory signaling by bacterial lipopolysaccharide in human hepatic stellate cells. *Hepatology* 37:1043–1055.
149. Wang F, Liu S, DU T, Chen H, Li Z, Yan J (2014) NF- κ B inhibition alleviates carbon tetrachloride-induced liver fibrosis via suppression of activated hepatic stellate cells. *Exp Ther Med* 8(1):95–99.
150. Zhao WX, Wang L, Yang JL, Li LZ, Xu WM, Li T (2014) Caffeic acid phenethyl ester attenuates pro-inflammatory and fibrogenic phenotypes of LPS-stimulated hepatic stellate cells through the inhibition of NF- κ B signaling. *Int J Mol Med* 33(3):687–694.
151. Sprenger H, Kaufmann A, Garn H, Lahme B, Gerns D, Gressner AM (1999) Differential expression of monocyte chemoattractant protein-1 (MCP-1) in transforming rat hepatic stellate cells. *J Hepatol* 30(1):88–94.
152. Kumar S, Wang J, Shanmukhappa SK, Gandhi CR (2017) Toll-like receptor 4-independent carbon tetrachloride-induced fibrosis and lipopolysaccharide-induced acute liver injury in mice: Role of hepatic stellate cells. *Am J Pathol* 187:1356–1367.
153. Dornas W, Glaise D, Bodin A, Sharanek A, Burban A, Le Guillou D, Robert S, Dutertre S, Aninat C, Corlu A, Lagente V (2018) Endotoxin regulates matrix genes increasing reactive oxygen species generation by intercellular communication between palmitate-treated hepatocyte and stellate cell. *J Cell Physiol* 234(1):122–133.

Figure 1 Involvement of the gut-liver axis in the pathogenesis of NAFLD/NASH progression. Articles were selected based on a PubMed search (<http://www.ncbi.nlm.nih.gov/pubmed/>) using the terms “microbiota,” “LPS,” “NASH,” and “NAFLD” from 1997 to 2017; the search was limited to articles written in English. Articles were selected based on general review of the literature. If an article contained a reference that had not been detected in the search or general reading, this reference was obtained. **A**, Reviews^{5,6,8,9,13,17–22,26,29,31,32,35,36,38,47,54,56,57,67,90,91,94,97,100,103,106,107,125,138} **B**, *In vitro* studies^{30,39,41–44,49,53,62,64,65,73–75,89,93,110,116,132} **C**, Rodent studies^{2,4,10–12,27,30,40–46,48–53,59–62,66,68,73–75,78,82,84–86,88,89,93,95,96,110,116,118,126,129,131,132,135,136} **D**, Human studies^{7,14–16,24,25,28,33,39,52,58,63,64,96,117,122,137,142–144}

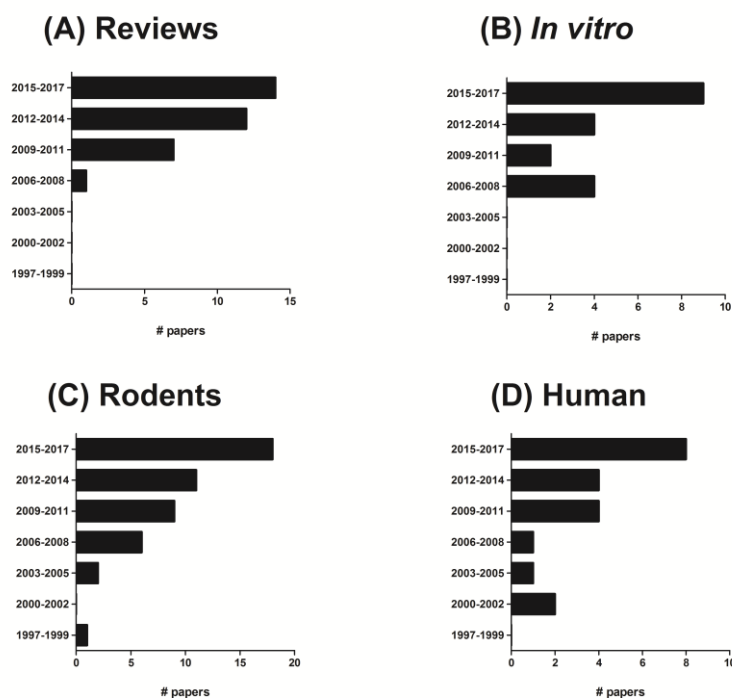


Figure 2 Changes in TLR4 mRNA levels in the liver of animal models of NASH. TLR4 expression is upregulated in the different models of NASH found in the literature. The expression of the target gene was normalized as ratio of target gene/control mRNA; columns represent the mean \pm SEM or SD. [Figure abbreviations: BDL, Bile duct ligation; CDAA, Choline deficiency amino acid; DSS, Dextran sodium sulfate; HCal, High caloric; HF, High fat; HFr, High fructose; LPS, Lipopolysaccharide; MCD, Methionine/choline deficiency].

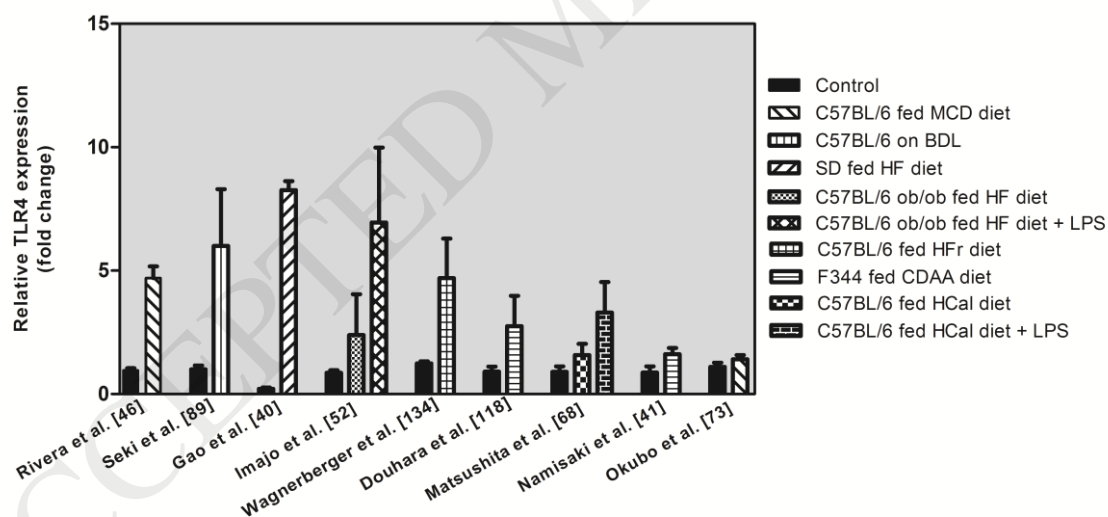


Table 1 Hepatic triglyceride levels and NAS score after treatment with endotoxin or gene deletion of pathways LPS-related during the genesis of steatohepatitis

	Groups used in NASH study				
	Control	LPS treatment or models of LPS-deficient signaling pathways related "A"		Nutritional model of steatohepatitis development "B"	A-B Interaction
	Hepatic triglyceride levels				
TNF- α knockout mice receiving MCD diet [66]	46.0 \pm 14.4 μ g/mg protein	23.5 \pm 41.1 (KO)	TNF- α	112.4 \pm 80.7 μ g/mg protein	100.3 \pm 31.2 (KO) TNF- α

		$\mu\text{g}/\text{mg protein}$		$\mu\text{g}/\text{mg protein}$
TLR-4 mutant C3H/HeJ mice receiving MCD diet [46]	34.0 \pm 4.0 mg/mg protein	36.0 \pm 6.0 TLR4 (KO) mg/mg protein	96.0 \pm 14.0 mg/mg protein	46.0 \pm 8.0 TLR4 (KO) mg/mg protein
Zucker (fa/fa) rats receiving a diet rich in disaccharide and LPS [86]	-	94.53 \pm 5.4 mg/dL	169.47 \pm 3.59 mg/dL	227.72 \pm 11.36 mg/dL
MD-2-deficient animals C57BL/6 receiving MCD diet [48]	10 \pm 0.62 mg/g liver	6.87 \pm 1.25 MD-2 (KO) mg/g liver	27.5 \pm 5 mg/g liver	15 \pm 0.625 MD-2 (KO) mg/g liver
TLR4-deficient animals C57BL/6 receiving MCD diet [48]	8.12 \pm 1.25 mg/g liver	6.87 \pm 0.3 TLR4 (KO) mg/g liver	18.75 \pm 1.87 mg/g liver	10.62 \pm 1.25 TLR4 (KO) mg/g liver
APOE3L.CETP mice receiving HF diet and LPS [83]	46 \pm 0.7 $\mu\text{g}/\text{mg protein}$	-	123 \pm 7.6 $\mu\text{g}/\text{mg protein}$	169 \pm 15.3 $\mu\text{g}/\text{mg protein}$
Hepatocyte-specific Myd88 deleted mice receiving HF diet [51]	83.8 \pm 6.4 mmol/mg protein	103.2 \pm 6.3 (LKO) mmol/mg protein	122.5 \pm 12.9 mmol/mg protein	141.9 \pm 12.6 (LKO) mmol/mg protein
	NAS score*			
C57BL/6J mice (WT) receiving HF diet and LPS [52]	0.34 \pm 0.4	0.57 \pm 0.5	2.07 \pm 0.6	4.73 \pm 0.8

*NAS score is based on an observed progressive increase in steatosis, ballooning, and lobular inflammation according to Brunt et al. [149].

[Table abbreviations: HF, High fat; KO, Knockout; LKO, Liver-specific knockout mice; LPS, Lipopolysaccharide; MCD, Methionine/choline-deficient; MD2, TLR4/myeloid differentiation protein 2; MyD88, Myeloid differentiation primary-response gene 88; NASH, Nonalcoholic steatohepatitis; TLR, Toll-like receptor; TNF- α , Tumor necrosis factor alpha; WT, Wild type].

Table 2 Studies showing causal-relationship between endotoxin and development of fibrosis via HSC activation

Key mediators	Findings
TLR4	Mice with TLR4 ^{-/-} resident cells show reduced fibrosis and HSC activation [39] TLR4-mutant HSCs show reduced fibrogenesis in response to LPS [89] LPS-TLR4 pathway regulates fibronectin production in HSCs [110]
NF- κ B	LPS-induced NF- κ B but is blocked by preincubation with anti-TLR4 or Polymyxin [148] NF- κ B inhibitor suppresses HSC activation by downregulating the expression of collagen I and α -SMA [149] LPS-induces activation of NF- κ B in HSCs [152,153]
TGF- β	Antibiotics inhibit HSC activation and liver fibrosis via regulation of TGF- β and collagen expression [118] LPS stimulation enhances TGF- β 1-induced mRNA expression of COL I α 1 in HSCs [150]
Cytokines	HSCs from genetically obese mice with endotoxemia developed a pro-inflammatory phenotype [30] LPS-induces pro-inflammatory pattern in HSCs [64] MFB after stimulation with TNF- α are highly LPS-responsive and release large amounts of MCP-1 [151]

[Table abbreviations: COL I α 1, Collagen type I α 1; HSCs, Hepatic stellate cells; LPS, Lipopolysaccharide; MMP, Matrix metalloproteinase; MFB, Myofibroblast;
NF- κ B, Factor nuclear *kappa B*; α -SMA, Alpha-smooth muscle; TGF- β , Transforming growth factor;
TIMP, Tissue inhibitor of metalloproteinase;
TLR, Toll-like receptor; TNF- α , Tumor necrosis factor alpha].

Table 3 Summary of evidence supporting the beneficial effects of microbiota modulation in NAFLD/NASH

Reference	Treatment	Model	Relevant findings
Animal studies			
Zhu et al. [110]	Rifaximin	TLR4 mutant mice after BDL <i>In vitro</i> studies evaluated the effect of bacterial products and TLR agonist on HSC and LEC	Portal pressure, fibrosis, and angiogenesis were reduced dependent on the LPS/TLR4 pathway LPS promoted myofibroblastic

			activation (<i>in vitro</i>)
Yamada et al. [116]	Ceftazidime plus Metronidazole	Mice were fed with a new class of HF diet	Modifying the composition of the gut microbiota and related metabolic activities prevented NASH and activation of liver macrophages
Douhara et al. [118]	Polymyxin and neomycin	Rats on CDAA diet	Reduced hepatic fibrosis and HSC activation Improved intestinal permeability
Seki et al. [89]	Ampicillin, neomycin sulfate, metronidazole, and vancomycin	BDL TLR4-mutant mice	Decreased fibrosis, macrophage infiltration, and TLR4 expression
Bergheim et al. [114]	Polymyxin B and neomycin	Mice had free access to solutions containing 30% glucose, fructose, sucrose, or water sweetened with artificial sweetener	Reduced hepatic lipid accumulation
Cani et al. [112]	Ampicillin and neomycin	Wild type and <i>ob/ob</i> mice were fed a HF, carbohydrate-free diet	Reduced endotoxemia and improved glucose tolerance
Osman et al. [123]	<i>Lactobacillus plantarum</i> and <i>Bifidobacterium infantis</i> with and without blueberry	Acute liver injury was induced in rats with GalN and endotoxins	Reduced inflammation, improved barrier functions, and antioxidant activity
Li et al. [12]	VSL#3	<i>ob/ob</i> controls fed an HF diet	Improved liver histology and decreased levels of hepatic fatty acid, alanine aminotransferase, IR, fatty acid β -oxidation
Velayudham et al. [129]	VSL#3	MCD diet-induced mouse model of NASH	Modulated liver fibrosis but did not protect from inflammation and steatosis. Upregulated serum endotoxin and expression of TLR4 signaling components, including CD14 and MD2, MyD88, NF- κ B, and modulated collagen expression and impaired TGF- β signaling
Esposito et al. [131]	VSL#3	Rats fed a HF liquid diet (71% of energy)	Reduced TNF- α levels, MMP-2 and MMP-9 activities, and expression of iNOS, COX-2, PPAR- α Modulated the NF- κ B pathway
Mencarelli et al. [132]	VSL#3	ApoE ^{-/-} mice on administration of 0.2% of dextran sulfate sodium	Reversed IR, prevented inflammation and steatohepatitis, and reduced the extent of aortic plaques. Conditioned media obtained from cultures caused transactivation of PPAR receptor-c, FX and vit D receptor
Ewaschuk et al. [124]	VSL#3	Mice were injected with LPS and D-GalN in the presence and absence of the PPAR gamma inhibitor GW9662	Prevented the breakdown in intestinal barrier function, reduced bacterial translocation, and attenuated liver injury with involvement of a PPAR gamma-dependent mechanism
Xue et al. [126]	<i>Bifidobacterium infantis</i> , <i>Lactobacillus acidophilus</i> , and <i>Bacillus cereus</i>	HF and sucrose diet induced liver disease in a rodent model	Ameliorated dysbiosis, decreased inflammatory cytokines, attenuated increased serum liver enzymes and glycometabolic biomarkers
Wagnerberger et al. [134]	<i>Lactobacillus casei</i> Shirota	A mouse model of fructose-induced steatosis	Attenuated the induction of TLR-4 signalling cascade, formation of ROS and expression of TNF- α . Increased activity of PPAR gamma in the liver
Okubo et al. [135]	<i>Lactobacillus casei</i>	MCD diet induced NASH in a	Increased the population of <i>L. casei</i>

	Shirota	rodent model	subgroup and of other lactic acid bacteria, suppressed NASH with reduced serum LPS levels
Ritze et al. [136]	<i>Lactobacillus rhamnosus</i> GG	Experimental NAFLD was induced in mice by a HFr diet via the drinking water	Improved intestinal barrier, reduced LPS levels in portal venous blood, attenuated inflammation, and inhibited steatosis
Pachikian et al. [141]	Dietary FOS	C57Bl/6J mice fed an n-3 PUFA-depleted diet	Reverted steatosis via stimulation of the fatty acid oxidative pathway, inhibition of the cholesterol synthesis, improvement of hepatic insulin sensitivity, and increased production of a gut-derived hormone
Cani et al. [146]	OFS	<i>ob/ob</i> mice	Increased intestinal proglucagon mRNA, GLP-2 and modulated gut barrier function and inflammation
Busserolles et al. [145]	OFS	Rats were fed a HFr diet	Prevented TG accumulation in the liver, hyperlipemia, and lower plasma of vit E/TG
Clinical studies			
Fernández et al. [111]	Norfloxacin	Patients with cirrhosis and low protein ascitic levels with advanced liver failure or impaired renal function	Decreased incidence of spontaneous bacterial peritonitis, hepatorenal syndrome and increased 1-year probability of survival
Drenick et al. [113]	Metronidazole	Patients after intestinal bypass surgery	Reduction in hepatic steatosis. This improvement was evident despite concurrent progressive decreases in serum albumin levels
Solga et al. [130]	VSL#3	4 adult human patients with NAFLD in an open label pilot trial	There were no significant differences in any of the blood assays or clinical parameters, or in reduction in hepatic steatosis
Loguercio et al. [128]	VSL#3	NAFLD, AC, HCV-positive patients with chronic hepatitis with or without liver cirrhosis	Improved plasma levels of MDA and 4-HNE in NAFLD and AC, whereas cytokines (TNF- α , IL-6, and IL-10) were improved only in AC. Improved routine liver damage tests and plasma S-NO levels in all groups
Dhiman et al. [133]	VSL#3	Patients with cirrhosis	Reduced the risk of hospitalization, as well as Child-Turcotte-Pugh and model for end-stage liver disease scores
Aller et al. [127]	<i>Lactobacillus bulgaricus</i> and <i>Streptococcus thermophilus</i>	Patients with NASH	Improved liver aminotransferases levels. Anthropometric variables and cardiovascular risk factors remained unaffected
Manzhali et al. [137]	A cocktail containing <i>Lactobacillus casei</i> , <i>Lactobacillus rhamnosus</i> , <i>Lactobacillus bulgaricus</i> , <i>Bifidobacterium longum</i> , <i>Streptococcus thermophilus</i> and FOS	Patients with NASH fed a low-fat/low-calorie diet	Reduced serum ALT, BMI, and serum cholesterol
Malaguarnera et al. [142]	<i>Bifidobacterium longum</i> with FOS	Patients with NASH and lifestyle modification	Reduced levels of TNF- α , CRP, AST, HOMA-IR, endotoxin Decreased steatosis, and the NASH index

Daubioul et al. [143]	OFS	Patients with NASH	Decreased serum AST after 8 weeks, and insulin level after 4 weeks
Ferolla et al. [144]	<i>Lactobacillus reuteri</i> with guar gum and inulin	Patients with NASH	Reduced steatosis, weight, BMI and waist circumference but did not improve intestinal permeability or LPS levels

[Table abbreviations: AC, Alcoholic liver cirrhosis; ALT, Alanine aminotransferase; ApoE, Apolipoprotein E; AST, Aspartate aminotransferase; BDL, Bile duct ligation; BMI, Body mass index; CDAA, Choline-deficient amino acid-defined; CD14, Cluster of differentiation 14; COX-2, Cyclooxygenase-2; CRP, C-reactive protein; FOS, Fructo-oligosaccharides; FX, Farnesoid X; GalN, D-galactosamine; GLP-2, Glucagon-like peptide 2; HCV, Hepatitis C virus; HF, High fat; HFr, High fructose; HOMA, Homeostasis model assessment-estimated insulin; HNE, *4-Hydroxynonenal*; HSCs, Hepatic stellate cells; iNOS, Inducible nitric oxide synthase; IL, Interleukin; IR, Insulin resistance; LEC, Liver endothelial cells; LPS, Lipopolysaccharide; MCD, Methionine/choline-deficient; MD2, TLR4/myeloid differentiation protein 2; MDA, Malondialdehyde; MMP, Matrix metalloproteinase; MyD88, Myeloid differentiation primary-response gene 88; NAFLD, Nonalcoholic fatty liver disease; NASH, Nonalcoholic steatohepatitis; NF- κ B, Factor nuclear *kappa B*; OFS, Oligofructose; PPAR, Peroxisome proliferator-activated receptor; PUFAs, Polyunsaturated fatty acids; ROS, Reactive oxygen species; TG, Triglyceride; TGF- β , Transforming growth factor; TLR, Toll-like receptor; TNF- α , Tumor necrosis factor alpha; VIT, Vitamin; VSL#3, Probiotic mixture of *Streptococcus thermophilus*, *Lactobacillus/Bifidobacterium* species].