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REVIEW

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Effects of SCFAs and TMAO on non-alcoholic fatty liver disease indicating the therapeutic benefits of plant-based diet, and supplemental prebiotics, probiotics and synbiotics

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Abstract

This review discusses the effects of short-chain fatty acids (SCFAs) and trimethylamine-N-oxide (TMAO) on metabolic diseases, focusing on non-alcoholic fatty liver disease (NAFLD) and cardiovascular disease, and suggests dietary modification as a promising therapeutic strategy. SCFAs, a product of fiber fermentation by microbiota, foster intestinal cell populations, upregulate mucin production, and secure the gut barrier. In contrast, TMAO, a microbiota-produced metabolite from choline, phosphatidylcholine, and L-carnitine, induces atherosclerosis by decreasing cholesterol clearance. An unmanageable abundance of TMAO is potentially harmful to patients with NAFLD owing to its ability to regulate the synthesis and transport of bile acids. The production of SCFAs and TMAO is strongly dependent on the microbial community; therefore, dietary modifications, such as reduction in meat intake, and prebiotic and probiotic consumption that can shape the gut microbiome are considered as promissing therapeutic approaches. This review focuses on well-known prebiotics, such as inulin, fructooligosaccharides, and β-glucan, and probiotics, such as VSL#3 mixture, *Lactobacillus rhamnosus* GG, *Bifidobacterium*, and *Lactobacillus* spp. These additives facilitate microbiota modification, gut homeostasis, intestinal barrier maintenance, and promotion of cholesterol excretion, which may protect the liver from steatosis, inflammation, and fibrosis. Controversial results from previous studies suggest that personalized approaches should be used for dietary modifications.

Keywords Bile acid, Cardiovascular disease, Non-alcoholic fatty liver disease, Prebiotics, Probiotics, Short-chain fatty acids, Synbiotics, Trimethylamine N-oxide

Non-alcoholic fatty liver disease and non-alcoholic steatohepatitis

Over the past century, excessive growth of the fast-food industry and predominance of automatic devices have resulted in sedentary lifestyles and unbalanced energy consumption, which are the crucial causes of metabolic disorders, such as obesity and diabetes. In addition, non-alcoholic fatty liver disease (NAFLD) and its progressive stage, non-alcoholic steatohepatitis (NASH) are the most important consequences of such disorders, as 30% and 3–6% of US citizens suffer from NAFLD and NASH, respectively, and 20% of total cases of NASH progress to cirrhosis, which increases the mortality rate or leads to

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liver transplantation [1]. Although there are some available non-invasive tests, including blood and urine analysis for liver damage markers, such as aspartate aminotransferase (AST) and alanine aminotransferase (ALP), only liver biopsy is accepted as reliable diagnosis to differentiate NASH from simple steatosis [1]. NAFLD is confirmed by the presence of more than 5% hepatic steatosis, and NASH is diagnosed by the simultaneous occurrence of hepatocyte ballooning degeneration and lobular inflammation (neutrophil invasion) with steatosis [2]. Notably, 4% of simple steatosis and 20% of NASH cases finally develop cirrhosis with fibrosis-including NASH as the intermediary stage [3]. No therapy has been officially approved by the US Food and Drug Administration, and controlled lifestyle, including dietary management and exercise, is recommended for patients with a low-grade condition [1]. The imbalance of energy intake, together with the disruption in energy and nutrition metabolism, are the main causes of NAFLD. Excessive and continuous consumption of saturated fatty acids (SFAs) induces liver fat accumulation with NAFLD as the ultimate consequence, while moderate unsaturated fat consumptions shows protective effects [4]. A recent study suggests that SFAs can be associated with liver steatosis by changing the structure and function of mitochondria, which ultimately disrupts the respiratory transport, produces reactive oxygen species, and damages the neighboring structures, causing apoptosis, inflammation, and scarring in the liver [4]. Besides unhealthy intake of SFAs, steatosis can be observed as a hepatic manifestation during inflammatory bowel disease (IBD), suggesting an intimate relationship between the gastrointestinal tract and liver condition [5, 6]. During IBD, the intestinal barrier is disrupted, leading to increased gut permeability and uncontrolled transport of lipopolysaccharide (LPS) to the liver via the portal vein [5, 6]. As a result of aforementioned endotoxin transport, hepatocytes are damaged and macrophages infiltrate into the liver and can lead to liver inflammation [5].

Short-Chain fatty acids

Gut microbiota plays an important role in maintaining gut homeostasis and is essential for regulating the gut and liver axes [6]. A healthy population of microbiota

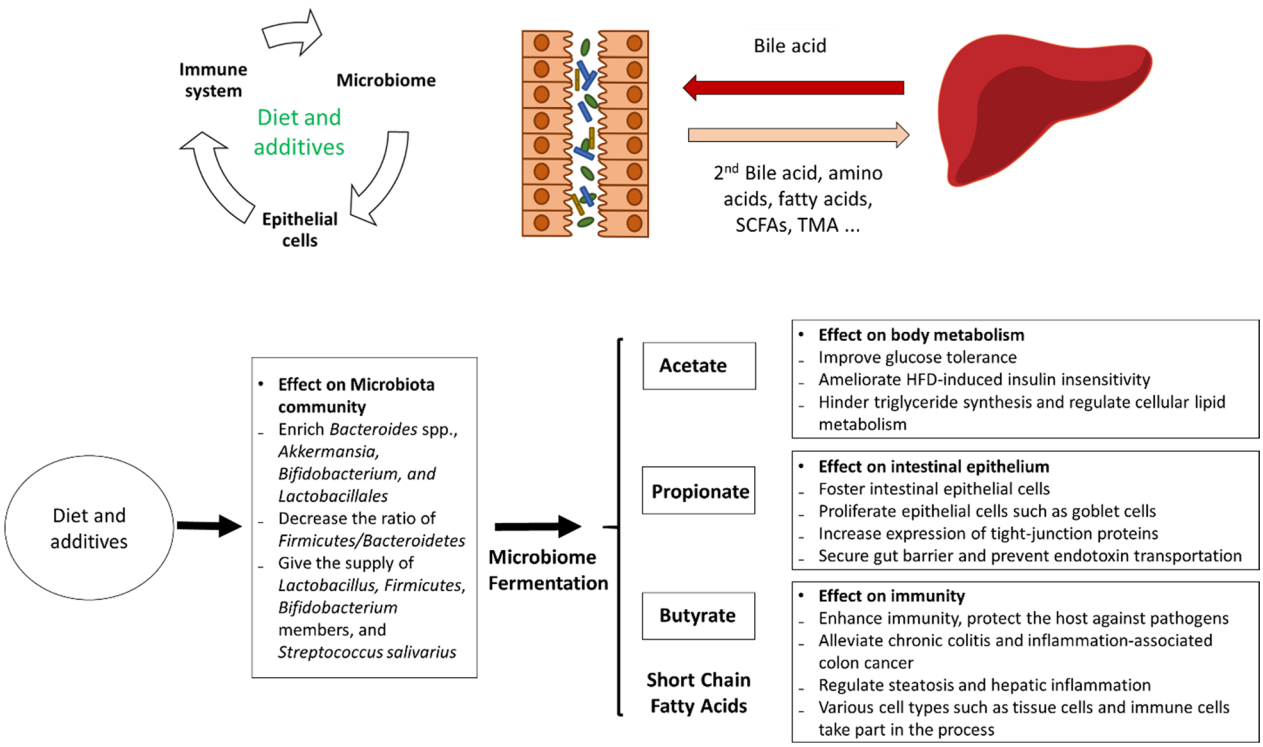

Fig. 1 Beneficial effect of SCFAs on Gut–liver axis. There is a close relationship between the liver and the gut. Humans consume food that is digested and metabolized by microbiota in the gut to produce essential metabolites, such as trimethylamine (TMA), amino acids, and fatty acids, which are transported to the liver via portal veins. Since different microbiota results in distinct products of digestion that will be transported to the liver, healthy gut microbiota is essential for a healthy liver. Diet modification and additives (prebiotics and probiotics) consumption are promising approaches to have a healthy microbiota community. SCFAs are products of the beneficial bacterial community which have various benefits toward the host consisting of improving overall metabolism, strengthening intestinal epithelium, and regulating immune response

consolidates the intestinal barrier and helps to maintain gut homeostasis by interacting with intestinal cells and the immune system [5, 7]. Short-chain fatty acids (SCFAs) are important products of fiber fermentation by microbiota that are known to have diverse benefits on gut-liver axes (Fig. 1). SCFAs help to secure the intestinal barrier by increasing the expression levels of tight junction proteins, such as zonula occludens-1, claudin, occludin, and E-cadherin that limits intestinal permeability and reduces the transportation of endotoxin to the liver in IBD [5]. In addition, butyrate, one of the SCFAs, is mainly utilized by intestinal epithelial cells such as enterocytes and goblet cells to proliferate and secrete lubricating mucin [5, 8]. Besides that, SCFAs increase the protein expression levels of glucagon-like peptide-1 (GLP-1) in enteroendocrine cells, improving glucose tolerance in both cell and animal models [9]. Consistent with this, Weitkunat et al. [10] demonstrated that acetate and propionate ameliorate high-fat diet (HFD)-induced insulin insensitivity and hepatic triglyceride (TG) accumulation [10]. In addition, propionate is known to specifically enhances GLP-1 levels and decreases fat accumulation in the liver by hindering triglyceride synthesis [11]. Moreover, propionate could also regulate cellular lipid metabolism via the its effect in the modulation of fasting-induced adipose factor, protein-coupled receptor 43, and histone deacetylases [12]. In a co-culture system with epithelial cells, hepatocyte-like cells show an increase in glycogen synthesis and storage following propionate treatment [13]. GLP-1 receptor expression levels are downregulated in patients with NAFLD and animal models, but treatment with butyrate can reverse this effect and induce phosphorylation of hepatic AMP-activated protein kinase (AMPK) and insulin receptor [14]. Besides butyrate, propionate is also known about its capacity in increasing the phosphorylation of AMPK and limiting a number of transcriptional factors which control the expression of gluconeogenesis enzymes like glucose-6-phosphatase and phosphoenolpyruvate carboxykinase [15]. SCFAs are also known about their capacity in immunoregulation and immunity enhancement. It is stated that the immunity is enhanced by SCFAs to extracellular bacteria (*C. rodentium* and *C. difficile*), viruses (influenza and respiratory viruses), and intracellular pathogens (*Listeria monocytogenes* and *Salmonella typhimurium*) [16–19]. Moreover, SCFAs also ameliorated chronic colitis and the development of inflammation-associated colon cancer [20, 21]. SCFAs can also be carried to the liver, where they exert beneficial effects by regulating steatosis and inflammation, as proven in both in vitro and in vivo experiments [22]. Butyrate also decreases the expression levels of tumor necrosis factor-α and increases the levels of prostaglandin E2 in a myeloid subset consisting of hepatic and Kupffer cells, suggesting the immunoregulatory and anti-inflammatory capacity of butyrate [23, 24]. Finally, there are various cell types consisting of tissue cells (epithelial cells and myeloid cells) and immune cells (T cells, B cells, and innate lymphoid cells) that take part in inducing the protective effect of SCFAs [25].

Effects of trimethylamine N-oxide on cardiovascular disease and NAFLD

According to mentioned results, it is clear that the gut has a substantial impact on the liver. On the other hand, the liver can regulate the microbiota population via secretion of bile acids (BA), potent antimicrobials [26]. Since the late 1940, BAs have been known about their impact on susceptible bacteria such genera as *Staphylococcus*, *Balantidium*, *Pneumococcus*, *Enterococcus,* and members of the phylum Spirochaetes in both bacteriostatic and bactericidal fashion [26]. Primary BAs could dose dependently disrupt bacterial membranes and non-conjugated BAs could induce a more vigorous reduction in viability than their conjugated counterparts against *Staphylococcus aureus*, several *Lactobacillus* and *Bifidobacterium* species [26]. Later on, the secondary BA is transported back to the liver, together with other substrates, such as amino acids, glucose, free fatty acids (FFAs), and trimethylamine (TMA), with the production of TMA is strongly depend on gut microbiota. FFAs and TMA are accumulated and metabolized in the liver into trimethylamine N-oxide (TMAO) by flavin-containing monooxygenases, and high levels of urinary and plasma TMAO are strongly associated with insulin resistance, NAFLD, and cardiovascular disease (CVD) [27, 28]. For decades, TMA was known as a microbiota-dependent metabolite of choline and phosphatidylcholine, until Koeth et al. [29] demonstrated that TMA is also a product of gut flora-metabolized L-carnitine in red meat [28, 29]. Importantly, the authors pointed out that the gut microbes from omnivorous participants may support a higher production of TMAO than that from participants who consume a vegan diet, emphasizing the norm that dietary habits shape the microbiome [29]. Moreover, oral administration of broad-spectrum antibiotics decreased plasma TMAO levels (lower than 100 nM) by 100-fold compared to the control, indicating the essential role of gut microbiota in TMAO synthesis [28].

Past studies have shown that the administration of L-carnitine to Apoe-/- mice leads to double atherosclerosis burden due to increased TMAO levels compared with control counterparts, while suppression of gut microbiota by antibiotics successfully prevents this risk [29]. TMAO and its precursors, such as choline, phosphatidylcholine,

and L-carnitine, can decrease reverse cholesterol transport (RCT) by 35%, which is crucial for the accumulation of cholesterol in peripheral tissues, ultimately resulting in cholesterol-related CVDs [29]. In literature, RCT is the process in which excess cholesterol from cells in peripheral tissues and macrophages in vessel walls is transported to the liver in order to be removed from the body via excretion of neutral sterols and bile acids in feces [30]. The suppression of RCT by TMAO is explained by the downregulation of BA transport and synthesis in the liver of TMAO-treated mice, while BA is the major product of cholesterol, its synthesis pathway is the main elimination mechanism of cholesterol from the blood circulation [29]. In addition, NPC1-like intracellular cholesterol transporter and ATP binding cassette subfamily G (ABCG)-5–8, which are cholesterol transporters in and out of enterocytes, respectively, were suppressed by TMAO consumption, resulting in a 26% loss of cholesterol absorption by the gut [29]. In addition, Zhao's study using rats fed a high-fat high-cholesterol (HFHC) diet demonstrated that oral treatment with TMAO lowered HFHC-induced steatohepatitis by reducing cholesterol accumulation in the liver [31]. Tan et al. (2019) revealed that the serum level of BA is significantly higher in patients with NAFLD, and that there is a positive correlation between the serum levels of TMAO and BA and the mRNA levels of cholesterol 7 alpha-hydroxylase (CYP7A1), an enzyme for cholesterol-origin BA synthesis [32]. The authors also showed that 18 weeks of treatment with TMAO led to liver dysfunction, lipogenesis, and TG accumulation in HFD-fed mice [32]. In-depth experiments showed that TMAO increases BA synthesis and shifts the proportion of liver BA toward farnesoid X receptor (FXR)-antagonistic activity [32]. Knockdown of CYP7A1 or activation of FXR by GW4064 inhibits TMAO-induced lipogenesis in palmitic acid-treated HEPG2 cells [32]. FXR is an important nuclear receptor that maintains the synthesis and transport of hepatic BA, and downregulation of FXR activation can lead to liver diseases, such as cholestasis, NAFLD, and hepatocellular carcinoma [33]. TMAO plays a crucial role in the modulation of BA metabolism and FXR activation, while BA is a key regulator of glucose and lipid metabolism, suggesting that TMAO can exert an impact on hepatic fat accumulation, cholesterol regulation, glucose, and energy homeostasis [28]. The pathogenic impact of TMAO on CVD has been confirmed; however, the roles of TMAO and BA in liver diseases, such as NAFLD and NASH, remain ambiguous. Koeth, Zhao, Wang, and Tan's studies revealed controversial results regarding the effects of TMAO in the liver (Table 1). Tan's results disagree with those of the early three groups, concluding that TMAO reduces BA synthesis and steatohepatitis, while inducing CVD. These differences in results can be explained

Table 1 Effects of trimethylamine-N-oxide on different states of cholesterol

References	Subject	Treatment	Results
Koeth, Robert A 2013 [29]	Apoe − / − mice	L-carnitin, choline, trimethylamine-N-oxide (TMAO) supplemented diets	↓ Reverse cholesterol transport ↓ Bile acid synthesis and transport ↓ Bile acid pool size ↓ Steatohepatitis ↑ Atherosclerosis ↑ RNA and protein levels in macrophage scavenger receptors involved in cardiovascular disease (CVD) ↑ Macrophage foam cell formation
Zhao, Z. H 2019 [31]	Rats fed high-fat high-cholesterol (HFHC) diet	TMAO (120 mg/kg/day) oral gavage for 8 weeks	
Wang, Z 2011 [28]	Apoe − / − mice	Choline (0.5%, 1.0%) or TMAO (0.12%) added in chow diet	
Tan, Xuying 2019 [32]	1. Mice fed high-fat diet (HFD) 2. HepG2 cells treated with palmatic acid	1. TMAO (18% w/v) in drinking water for 18 weeks 2. TMAO (at 0.1, 0.2, 0.3, and 0.4 mm) for 24 h	• Correlations between non-alcoholic fatty liver disease (NAFLD) and TMAO, bile acid pool size, and hepatic mRNA cholesterol 7 alpha-hydroxylase (CYP7A1) • Knockdown of CYP7A1 or activation of the farnesoid X receptor (FXR) blocks TMAO-induced lipogenesis ↑ Bile acid synthesis ↑ Proportion of FXR antagonistic species ↑ Lipogenesis and triglyceride (TG) accumulation ↑ Liver dysfunction

CVD cardiovascular disease, *CYP7A1* cholesterol 7 alpha-hydroxylase, *FXR* farnesoid X receptor, *HFD* high-fat diet, *NAFLD* non-alcoholic fatty liver disease, *TG* triglyceride, *TMAO* trimethylamine-N-oxide, *AKT* protein kinase B, *ALT* alanine aminotransferase, *AMPK* AMP-activated protein kinase, *ASC* apoptosis-associated speck-like protein, *AST* aspartate transaminase, *BAMBI* BMP and activin membrane-bound inhibitor, *BMI* body mass index, *CFU* colony-forming unit, *CYP7A1* cholesterol 7 alpha-hydroxylase, *ERK* extracellular signal-regulated kinase, *FXR* farnesoid X Receptor, *GLP* Glucagon-like peptide, *HDL-C* high-density lipoprotein cholesterol, *HFD* high-fat diet, *IL* interleukin, *JNK* c-Jun N-terminal kinase, *LDL* low density lipoprotein, *LDL-C* low-density lipoprotein cholesterol, *LGG* Lactobacillus rhamnosus GG, *LPS* lipopolysaccharide, *MCD* methionine-choline deficient, *NAFLD* non-alcoholic fatty liver disease, *NASH* nonalcoholic steatohepatitis, *NF-κB* Nuclear factor kappa B, *NLRP3* NLR family pyrin domain-containing 3, *PPAR* peroxisome proliferator-activated receptor, *SREBP-1c* sterol regulatory element binding transcription factor 1 isoform c, *TG* Triglycerides, *TLR* toll-like receptor, *TNF-α* tumor necrosis factor alpha, *US* ultrasound

A positive or upward effect is denoted by (↑), a negative or downward effect is denoted by (↓)

by the high cholesterol level models used to investigate CVD in Koeth, Zhao, and Wang's studies, unlike the use of a normal HFD-fed model by Tan. These data suggest that the effect of TMAO could vary and strongly depend on the plasma levels of other substrates, such as cholesterol. However, based on Tan's results, high plasma TMAO levels were observed in patients with NAFLD together with liver dysfunction and TG accumulation in TMAO-treated HFA-fed mice, suggesting the pathogenic potential of TMAO in NAFLD. The conclusion is also supported by a cohort study conducted by Jose L. Flores-Guerrero et al. (2021) which investigated the association between TMAO level and mortality rate in people with NAFLD [34]. The result of the study demonstrated that a high level of TMAO was associated with an increase in the risk of all-cause mortality in patients with NAFLD while the relationship was not observed in patients without NAFLD [34]. Jose's and Tan's studies suggest that TMAO could be the key factor that can exacerbate the severity of NAFLD which finally ends up increasing the rate of mortality.

Bile acid and the activation of FXR

BA is synthesized from cholesterol in the liver via various pathways, with CYP7A1 is the rate-limiting enzyme in the main pathway [33]. After being formed by CYP7A1, 7α-hydroxycholesterol is converted to cholic acid (CA) or chenodeoxycholic acid (CDCA), and the proportion of CA and CDCA differs between species, such as humans and mice [33]. In humans, primary BA consists CA and CDCA, which finally forms a BA pool comprise 40% CA, 40% CDCA (potent FXR agonist), and 20% deoxycholic acid (DCA), whereas in mice, primary BAs are CA and muricholic acid (MCA; FXR antagonist) which is the product of 6-hydroxylation of CDCA [33]. Moreover, the DCA level is higher in humans owing to the presence of CYP2A12 in mice, the enzyme catalyzes the 7α-rehydroxylation of deoxycholic acid (DCA) and lithocholic acid (LCA) back to CA. Finally, primary BA was subjected to final conjugation using BA-CoA: amino acid N-acyltransferase with glycine and taurine, with mainly glycine in humans and taurine in rodents [33]. Conjugated BA is transported to the gut, where it is deconjugated by bacterial bile salt hydrolases. Furthermore, the microbiota enzyme, 7α-dehydroxylase, in the large intestine converts CA to DCA, and CDCA to LCA. Subsequently, LCA is excreted together with feces, while DCA can be reabsorbed via passive diffusion. In the mouse liver, DCA can be rehydroxylated back to CA using CYP2A12, suggesting that differences in species should be considered when choosing the model for the investigation of human BA signaling. The effects of BA on the intestinal tract include lipid and lipid-soluble nutrient absorption, cholesterol removal, microbiota modification, and regulation of energy homeostasis. FXR plays the most important role in maintaining BA homeostasis, and its operating mechanism and structure have been well described in a previous study [33]. FXR is mainly expressed in the liver, ileum, kidneys, and adrenal glands, and either free or conjugated BAs, mostly CDCA, can activate FXR, however, the responses induced by the activation of FXR are different in a tissue-dependent manner [33]. While activation of FXR in both the intestine and liver suppresses the expression of classical BA synthesis enzymes such as CYP7A1 and CYP8B1, and regulates the enterohepatic circulation of BAs, the response with lower degree is observed in hepatic FXR activation [33]. FXR knockout mice suffer from fatty liver, increased levels of circulating FFAs, serum glucose, and insulin resistance [35], while overexpression of FXR exerts the opposite effects [36] suggesting that FXR activation is required for the recovery of metabolic homeostasis. Importantly, FXR activation is beneficial in liver inflammation and fibrosis, since the activation leads to a decrease in inflammatory cell infiltration by suppressing the monocyte chemoattractant protein-1, and FXR knockout results in strong hepatic inflammation via liver necrosis and upregulation of inducible nitric oxide synthase, cyclooxygenase-2, and interferon-γ [37]. Moreover, FXR suppresses hepatic inflammation by regulating cholestasis and the production and transportation of toxic BA [33]. Activation of the mammalian target of rapamycin by secondary BA in the liver is suggested to be responsible for hepatic carcinogenesis during NASH [38, 39]. The FXR agonist, GW4064, markedly reduces liver injury in cholestatic models via a decrease in alanine transaminase (ALT) and aspartate transaminase (AST) serum levels, necrosis, inflammation, and bile duct proliferation [40, 41]. However, another study demonstrated that FXR knockout confers protection against obstructive cholestasis by reducing mortality and liver injury [42]. Because of the powerful impact of FXR, some FXR-targeting drugs, such as obeticholic acid (OCA; steroidal FXR agonist) and tropifexor (non-steroidal agonist), are being developed to treat NASH [43–45]. A trial in which patients with cirrhotic NASH received 25 mg OCA daily for 72 weeks showed improved biochemical and histological features [43]. Another study using 2,065 patients with NASH divided into three groups treated with 10 mg OCA, 25 mg OCA, and placebo for 18 months showed improvement in fibrosis and NASH stage without worsening fibrosis [44]. In an animal study using a mouse model, reduction in oxidative stress, steatosis, inflammation, and fibrosis was observed following treatment with tropifexor [45].

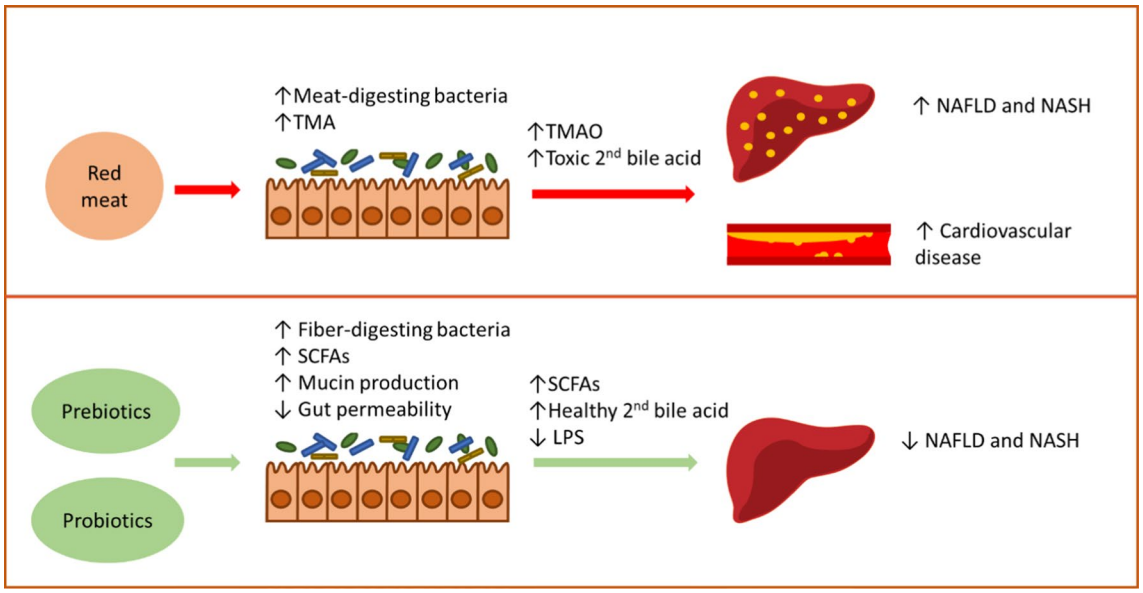

Fig. 2 Dietary modification affects gut and liver health. Dietary modifications and supplementation of additives can improve the gut health by shaping the gut toward a healthy population that produces beneficial metabolites, such as short-chain fatty acids (SCFAs). SCFAs can then be utilized by intestinal cells to produce essential substances, such as lubricating mucus and tight-junction proteins, which are required to maintain the intestinal barrier and proper bowel movement. Meanwhile, excessive intake of red meat containing a high amount of choline and L-carnitine can increase the trimethylamine-N-oxide (TMAO) levels, leading to cardiovascular disease (CVD), non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), and cirrhosis

Dietary modification approaches

Since dietary modification is the most advocated approach for NAFLD, it is necessary to determine which kind of dietary treatment can improve the gut and liver health (Fig. 2). Because of the important role of gut microbiota in regulating the gut and liver axes, three types of additives known to modulate the gut microbiota have received vast attention: prebiotics, probiotics, and synbiotics.

Prebiotics

Dietary fiber is comprised of non-digestible forms of carbohydrates which are usually in the form of polysaccharides (≥ 10 monomers) and could be easily found in plant-based food [46]. Although human is unable to digest and absorb polysaccharides, the microbiota in our gut could handle the task effectively. As the result of the fiber fermentation by gut microbiome, dietary fibers are broken down in the form of SCFAs consisting of acetate, propionate, and butyrate [5]. Those metabolites could be utilized by human cells and have a vast impact on our body by securing intestinal epithelium, regulating overall metabolism, and taking part in immunoregulation [5]. Owing to the benefits of SCFAs, the sources of SCFAs as fibers are recommended for metabolic conditions, such as obesity, diabetes, and IBD (Table 2) [5, 47]. For instance, universal prebiotic fibers, including fructooligosaccharides (FOS), galacto-oligosaccharides, and inulin, have been recently used in many investigations in order to ameliorate IBD [48]. Prebiotic fiber, such as FOS, galactooligosaccharides, and inulin, exert beneficial effects against obesity, IBD, and NAFLD [47, 49]. A study in which C57BL/6 mice were fed a chow diet, choline-deficient high-fat diet (CDHFD), or high-fat high-cholesterol diet (HFHCD) for 16 weeks compared the effects of soluble fibers (inulin) and insoluble fibers (cellulose) on NASH development [50]. Both CDHFD and HFHCD induced NASH in mice via hepatic steatosis and necrosis inflammation, whereas treatment with inulin significantly ameliorated the development of NASH [50]. In comparison with the cellulose-treated group, inulin markedly enriched *Bacteroides uniformis*, *Bacteroides acidifaciens*, and *Parabacteroides distasonis*, which are beneficial in reducing CDHFD-induced NASH. In stool and serum of inulin-treated mice, the authors found a marked increase in pentadecanoic acid, an inulin metabolite produced by *P. distasonis*, which has protective effects against fat accumulation, inflammation, and oxidative stress in the liver [50]. Another group used C57BL/6 mice fed a normal diet and HFD, with or without inulin, for 14 weeks [51]. Inulin successfully ameliorated HFD-induced outcomes, such as body and liver weight gain, increased AST, ALT, TG, and total Cho levels, and reduced plasma interleukin (IL)-10 levels [51]. In

Table 2 Prebiotics-incorporated studies on non-alcoholic fatty liver disease/non-alcoholic steatohepatitis

Prebiotic	References	Subject	Treatment	Results
Inulin	Wei, Wenchao 2021 [50]	C57BL/6 mice fed choline-deficient HFD and HFHC diet	10% w/w of the diet for 16 weeks	↑ *Bacteroides* and *Parabacteroides distasonis* ↑ Pentadecanoic acid in stool and serum ↓ NASH
	Bao,Ting 2020 [51]	C57BL/6 mice fed HFD	5 g/kg/daily oral administration for 14 weeks	↑ *Akkermansia* and *Bifidobacterium* ↓ *Firmicutes/Bacteroidetes* ratio ↓ HFD-induced increase in body and liver weights ↓ Total Cho, TG, AST, ALT serum levels ↓ Hepatic steatosis and inflammation
	Aoki, Ryo 2021 [52]	C57BL/6 mice fed high-fat, high-fructose, and high-cholesterol diet	10% w/w of the diet for 20 weeks	↑ *Bacteroides* and *Blautia* ↑ SCFAs, especially acetate ↓ Hepatic steatosis and fibrosis Require the acetate receptor, FFAR2
	Wang, Rui 2022 [54]	C57BL/6 mice fed HFD	5 g/kg/daily oral administration for 14 weeks	↑ Excretion of abnormal bile acid via activation of FXR–FGF-15 signaling ↑ Bile acid de novo synthesis ↓ NAFLD
	Chambers, Edward S 2019 [53]	Adults with NAFLD	20 g/d of inulin	↑ Colonic acetic acid ↑ Intrahepatocellular lipids
FOS	Takai, Atsuko 2020 [55]	C57BL/6 J mice were subcutaneously injected with monosodium glutamate	5% FOS via drinking water for 18 weeks	↑ Fecal and serum concentrations of SCFAs ↓ Hepatic steatosis and inflammation ↓ Hepatocyte ballooning, crown-like structures, and M1 macrophages infiltration
	Borges Haubert, N. J 2015 [56]	Rats fed high-carbohydrate diet	10/100 g diet for 20 d	↓ Total mean fat in liver and heart tissue ↓ Hepatic and cardiac steatosis
	Matsumoto, K 2017 [57]	C57BL/6 J mice a methionine–choline-deficient diet	5% FOS in drinking water for 3 weeks	Maintains healthy microbiota ↑ Fecal SCFAs and IgA concentration ↓ Hepatic steatosis and inflammation ↓ $CD14^+$ Kupffer cells and TLR-4 expression
β-glucan	Vu, Vuong 2021 [5]	C57BL/6 J mice fed HFD and 3% DSS in drinking water	3 g/kg diet *Schizophyllum commune* β-glucan for 12 weeks	Maintains healthy microbiota ↑ Fecal SCFAs ↑ Gut barrier against DSS ↓ Hepatic steatosis and inflammation
	You, S 2013 [58]	Intraperitoneal (i.p.) injection of 1.5 mg/kg body weight per day for 6 weeks	Goat β-glucan 1, 5, and 10% added to diet	↓ Serum endotoxin and glucose levels, and insulin resistance caused by LPS ↓ Plasma AST and ALT levels ↓ Hepatic steatosis, inflammation, and fibrosis
	Aoki, Shiho 2015 [59]	Specific pathogen-free C57BL/6 N mice fed on HFD	*Aureobasidium pullulans* β-glucan was given orally for 16 weeks	↓ HFD-induced high cholesterol, ALT, and TG levels ↑ CYP7A1 gene expression
	Ikewaki, Nobunao 2022 [60]	STAM C57BL/6 J mice model of NASH	1 mg/kg oral administration of *Aureobasidium pullulans* β-glucan for 8 weeks	↓ Hepatic steatosis, inflammation, and fibrosis ↓ NAFLD
	Huang, T 2020 [61]	C57 BL/6 J male mice fed MCD diet	10, 30, and 100 mg/kg/day oral gavage of baker's yeast β-glucan for 8 weeks	↓ Serum ALT and AST levels ↓ Hepatic steatosis and inflammation

ALT alanine aminotransferase, *AST* aspartate transaminase, *CYP7A1* cholesterol 7 alpha-hydroxylase, *DSS* Dextran sulfate sodium, *FFAR2* free fatty acid receptor 2, *FGF* fibroblast growth factor, *FOS* fructooligosaccharides, *FXR* farnesoid X Receptor, *HFD* high-fat diet, *HFHC* high-fat high-cholesterol, *LPS* lipopolysaccharide, *MCD* methionine-choline deficient, *NAFLD* non-alcoholic fatty liver disease, *NASH* nonalcoholic steatohepatitis, *SCFAs* short-chain fatty acids, *TG* Triglycerides, *TLR* toll-like receptor

A positive or upward effect is denoted by (↑), a negative or downward effect is denoted by (↓), and an insignificant effect is denoted by (−)

addition, pro-inflammatory markers were decreased concomitant with reduced macrophages and Toll-like receptor (TLR)-4^{+} macrophages in the liver owing to inulin consumption [51]. In addition, treatment with inulin resulted in the enrichment of *Akkermansia* and *Bifidobacterium,* while decreasing the ratio of *Firmicutes/Bacteroidetes,* together with increased SCFA production [51]. Aoki et al. [52] have proven that inulin alleviates the phenotypes of NAFLD/NASH, such as hepatic steatosis and fibrosis, which are attributed to enrichment in *Bacteroides* and *Blautia*, and increased production of SCFAs, especially acetate [52]. Administration of acetate producing substrate; resistant starch and acetate producing *Bacteroides acidifaciens* and *Blautia producta* suppressed NAFLD development in germ-free mice [52]. Moreover, the deficiency of FFA receptor 2, an acetate receptor, completely inhibits inulin-induced benefits via hepatic hypertrophy, inflammation, and hypercholesterolemia [52]. However, a clinical trial with two groups, either receiving 20 g/day of inulin or inulin-propionate ester, demonstrated that inulin-fermented acetate could increase the accumulation of intra hepatocellular lipids, while an increase in colonic propionate could ameliorate this effect [53]. An anomaly in accumulation of BA, despite the downregulation of BA signaling pathways, was observed in patients with NAFLD, while it is known that the key function of BA synthesis is to maintain the healthy cholesterol level by induce the excretion of superfluous cholesterol [54]. The above evidence suggests that the excessive amount of BA could be the reason for the downregulation of BA synthesis and ultimately leads to high level of cholesterol in NAFLD cases [54]. Wang et al. [54] demonstrated that inulin ameliorated HFD-induced NAFLD in mice by improving BA excretion via activation of FXR–fibroblast growth factor 15 signaling and recovery of BA de novo synthesis [54]. In addition to inulin, FOS have been widely investigated as a prebiotic treatment for NAFLD and other metabolic disorders. Takai et al. [55] induced NAFLD in C57BL/6 J mice via subcutaneous injection of monosodium glutamate (MSG) and treatment with 5% FOS in drinking water for 18 weeks [55]. The induction of MSG leads to hepatic steatosis, hepatocyte hypertrophy, inflammation, and macrophage infiltration, and upregulated mRNAs are involved in fatty acid synthesis [55]. Mice in the FOS-treated group showed reduced MSG-induced NAFLD via blunted mRNA levels of lipid metabolism enzymes, reduced hepatocyte ballooning, crown-like formation, and M1 macrophages in epididymal fat [55]. The beneficial effect of FOS is attributed to the increased concentration of SCFAs, containing n-butyric, propionic, and acetic acids, in fecal samples and propionic acid in serum [55]. Another study demonstrated that treatment with choline (3 g/100 g diet) and FOS (10 g/100 g diet) reduced the total mean fat in the liver and heart tissues of NAFLD mice by 0.2 and 1.7 g, respectively [56]. FOS administration suppressed both plasma levels of cholesterol and triacylglycerides compared to the standard diet-treated mice and mice before treatment [56]. Matsumoto et al. [57] conducted a study with three groups of mice fed different diets, including a control diet, methionine–choline-deficient (MCD) diet, and an MCD diet plus 5% FOS. The MCD diet fostered *Clostridium* cluster XI and subcluster XIVa populations and suppressed *Lactobacillales* spp., whereas the supplementation of FOS in the MCD diet helped maintain a healthy population of microbiota [57]. In comparison to MCD-fed mice, mice fed the FOS-supplemented MCD diet showed reduced hepatic steatosis, inflammation, CD14 + Kupffer cells, and TLR4 expression, while increasing the fecal SCFAs and intestinal immunoglobulin A (IgA) levels [57]. The authors suggested that MCD-induced microbiota dysbiosis, which results in decreased production of SCFAs, interrupts tight junction protein expression and increases the gut permeability, followed by the translocation of pathogen-associated molecular patterns (PAMPs) to the liver. Subsequently, PAMPs recognized by TLR4 in Kupffer cells activate the cells to secrete inflammatory cytokines together with hepatocyte steatosis, which causes NAFLD/NASH [57]. FOS is known to foster populations of *Bifidobacterium* and *Lactobacillales*, which are SCFA-producing strains, thereby increasing SCFAs production and securing the intestinal barrier [57]. β-glucan, another source of fiber, which is compatible with FOS, has beneficial effects on microbiota modification, intestinal barrier maintenance, and liver condition improvement [5]. A study demonstrated that *Schizophyllum commune* (a species of fungus in the genus *Schizophyllum*)-derived β-glucan successfully secures the intestinal barrier in dextran sodium sulfate (DSS) -induced ulcerative colitis (UC) mice by fostering SCFA-producing bacteria and increasing SCFA production [5]. Hence, β-glucan significantly ameliorates DSS-induced colitis and colitis-associated NAFLD by reducing hepatic steatosis, hepatitis, and inflammatory cell infiltration [5]. LPS is a bacterial toxin, whose translocation to the liver leads to hepatic steatosis and inflammation. A study induced NASH in mice using intraperitoneal injections of LPS at a dose of 1.5 mg/kg to investigate the protective effects of oat-derived β-glucan at different doses up to 10% in a specific pathogen-free diet [58]. The results showed that β-glucan dose independently reduced the serum endotoxin and glucose levels, and insulin resistance caused by LPS [58]. Plasma AST and ALT levels also decreased due to β-glucan consumption concomitant with reduced hepatic inflammation and fibrosis observed

in hematoxylin and eosin-stained hepatic sections [58]. In addition, the effect of *Aureobasidium pullulans* (yeast-like fungus)-derived β-glucan has been investigated [59]. Oral administration of fungal β-glucan for 16 weeks significantly inhibited HFD-induced hepatic steatosis and elevated cholesterol, TG, and ALT serum levels [59]. Treatment with β-glucan leads to an increase in CYP7A1 expression and plays an important role in cholesterol excretion via BA synthesis [59]. Moreover, other sources of β-glucan, such as *A. pullulans* and baker's yeast, demonstrated beneficial effects in NAFLD [60, 61]. Compared with conventional drugs, prebiotics are easily accessible and have minimal side effects. Furthermore, abundant sources of prebiotics with distinct benefits confer a wide range of choices for personalized therapy.

Probiotics

Besides prebiotics, probiotics that produce SCFAs, such as *Lactobacillus*, *Firmicutes* members, *F. prausnitzii, Bifidobacterium*, and *Streptococcus salivarius*, have also been suggested as a promising therapeutic (Table 3) [5, 62–64]. A meta-analysis of 25 studies demonstrated a reduced body mass index (BMI) of 0.54, 0.51, and 0.13 kg/m^2 as the result of prebiotic, probiotic, and synbiotic treatments, respectively [49]. Amelioration of liver injury was also observed via treatment with additives observed via reduced ALT and AST serum levels [49]. Importantly, a subgroup analysis demonstrated that microbial therapies reduced BMI by 0.55 kg/m^2, ALT by 11.74 U/L, and AST by 8.56 U/L in patients with NAFLD, but not those without NAFLD [49]. The different effects of additives in patients with and without NAFLD suggest the need for a disease background to observe the efficiency; however, a study suggests that despite the lack of change at the basal state, prebiotics exert protective effects against common metabolic diseases [8]. Previous trials have clearly shown the beneficial effects of VSL#3, the most widely known probiotic mixture containing four strains of *Lactobacillus (L. acidophilus, L. plantarum, L. casei, and L. delbrueckii subsp. Bulgaricus), Bifidobacterium* (*B. breve*, *B. infantis*, and *B. longum*), and *Streptococcus salivarius* subsp. *Thermophilus,* on NAFLD via a reduction in BMI and liver injury markers [65]. A clinical study investigated the effect of a VSL#3 mixture on obese children with NASH and found that treatment with VSL#3 for 4 months led to a reduced BMI and NAFLD [65]. Moreover, treatment with the mixture resulted in the upregulation of GLP-1 and activated GLP-1 levels in the serum [65]. By applying VSL#3 to MCD diet-induced NASH mice, Velayudham et al. [66] indicated that VSL#3 mixture shows a lack of effect on reducing MCD-induced hepatic steatosis and inflammation [66]. In contrast, liver fibrosis caused by MCD was ameliorated by VSL#3, due to the downregulation of hepatic collagen and alpha-smooth muscle actin levels, together with an increase in the expression levels of BMP and activin membrane bound inhibitor, a transforming growth factor-beta pseudoreceptor [66]. The insufficient effect of VSL#3 was also observed in patients with NAFLD, showing insignificant improvement in the serum levels of biomarkers of cardiovascular risk and liver injury [67]. In contrast, Chong et al. [67] demonstrated that treatment with VSL#3 significantly reduced the levels of TGs, high-sensitivity C-reactive protein, transaminases, and gamma-glutamyltransferases, which are biomarkers for the occurrence of hepatic injury and inflammation [68]. Despite the amelioration of hepatic steatosis and inflammation, BMI, circumference, and plasma glucose, total Cho, LDL-C, HDL-C, and adiponectin levels remained unchanged after treatment with VSL#3 [68]. Besides VSL#3, *Lactobacillus rhamnosus* GG (LGG) is one of the most well-studied probiotics. A screening study indicated that LGG is one of the seven strains isolated from traditional Chinese fermented food and healthy human feces that exhibits a cholesterol-suppressing effect [69]. Moreover, LGG is the strain that survives in 0.3% bile salt and has the best cell adhesion abilities [69]. Treatment with LGG and *Lactobacillus plantarum* resulted in decreased serum lipid levels by increasing hepatic CYP7A1 and LDL receptor mRNA levels and peroxisome proliferator-activated receptor (PPAR)-α protein levels, while decreasing PPAR-γ and sterol regulatory element binding transcription factor 1 isoform c (SREBP-1C) protein levels [69]. Moreover, administration of 5×10^7 CFU/g body weight of LGG to high-fructose diet-induced NAFLD in C57BL/J6 mice restored the intestinal tight junction protein expression [70]. Therefore, treatment with LGG leads to reduced LPS, hepatic inflammatory marker, and ALT serum levels, and fat accumulation [70]. A clinical trial investigated the effect of LGG on obese children with persistent hypertransaminasemia and bright ultrasonographic (US) liver image [71]. The results showed a decrease in ALT and antipeptidoglycan-polysaccharide antibodies in treated individuals despite a constant BMI, z-score, and US liver parameters [71]. *Bifidobacterium* spp. and *Lactobacillu* spp. were also investigated to determine if they affected liver conditions, such as NAFLD and NASH. A clinical trial investigated the relationship between NAFLD/NASH-related metabolic parameters and fecal bacteria, focusing on *Bifidobacteria* and *Lactobacilli* [72]. The trial indicated that three *Bifidobacterium* spp. (*B. longum*, *B. bifidum*, and *B. adolescentis*) and five *Lactobacillus* spp. (*L. zeae*, *L. vaginalis, L. brevis, L. ruminis,* and *L.*

Table 3 Probiotics-incorporated studies on non-alcoholic fatty liver disease/non-alcoholic steatohepatitis

Probiotic	References	Subject	Treatment	Results
VSL#3	Alisi, A. 2014 [65]	Obese children with NASH	1 sachet/day for < 10-year-old and 2 for > 10-year-old children in VSL#3 provided by VSL Pharmaceuticals Inc (Towson, MD, USA) for 4 months	↓ BMI ↑ GLP-1 and activated GLP-1 ↓ NASH
	Velayudham, A 2009 [66]	C57BL/6 mice fed MCD diet	VSL#3 (450 billion colonies/in 1 L of drinking water) for 10 weeks	(-) MCD-induced hepatic steatosis and inflammation (-) MCD-induced serum endotoxin and TLR4 signaling components expression ↓ Liver fibrosis ↓ Hepatic collagen and a-sma ↑ BAMBI, TGF-β pseudoreceptor
	Chong, Pui Lin 2021 [67]	Patients with NAFLD	2 sachets VSL#3 probiotic twice daily for 10 weeks	(-) Biomarkers of cardiovascular risk and liver injury
	Derosa,Giuseppe 2022 [68]	Patients with NAFLD	2 sachets VSL#3 per day for 3 months	(-) BMI, circumference, plasma glucose, total cholesterol, LDL-C, HDL-C, and adiponectin ↓ TG and high-sensitivity C-reactive protein ↓ Transaminases and gamma-glutamyl transferase ↓ Hepatic steatosis and inflammation
Lactobacillus rhamnosus GG (LGG)	Ritze, Yvonne 2014 [70]	C57BL/J6 mice fed HFD	LGG (5.2×10^7 CFU/g body weight) daily in water and diet	↑ Beneficial bacteria in the distal small intestine ↓ Portal LPS, hepatic TNF-α, IL-8R, and IL-1β mRNA expression levels ↓ Hepatic steatosis and portal ALT levels
	Mei, Lu 2015 [69]	Sprague–Dawley rats fed HFD	LGG and *Lactobacillus plantarum* (2×10^{10} CFU/mL in 0.9% NaCl per day) for 5 months	↓ Serum lipid levels and hepatic steatosis ↑ CYP7A1, LDL receptor, FXR, and PPAR-α levels ↓ PPAR-γ and SREBP-1c levels ↑ Intestinal tight-junction protein levels
	Vajro, P 2011 [71]	Obese children with persisting hypertransaminasemia and US bright liver	LGG (12×10^9 CFU/day) for 8 weeks	↓ ALT and antipeptidoglycan-polysaccharide antibodies (-) BMI, visceral fat, US bright liver parameters

Table 3 (continued)

Probiotic	References	Subject	Treatment	Results
Bifidobacterium and/or *Lactobacillus*	Yan, Yan 2020 [73]	Male Wistar rats fed HFD	*Bifidobacterium animalis* subsp. *lactis* V9 (1×10^9 CFU oral gavage) for 4 weeks	↓ HFD-induced serum ALT, AST, and glucose levels and hepatic steatosis ↓ Hepatic TG, FFA, and transcription of SREBP-1c and FAS ↓ inflammatory cytokine, hepatic TLR4, TLR9, NLRP3, and ASC mRNA, and activation of ERK, JNK, AKT, and NF-κB levels ↑ Glycogen levels ↑ Phosphorylated AMPK and PPAR-α
	Lee, N. Y 2021 [74]	C57BL/6 J mice fed a western diet	*L. acidophilus, L. fermentum, L. paracasei,* and *L. plantarum* (10^9 CFU/g diet) for 8 weeks	↓ Liver/body weight ratio ↓ Cholesterol levels ↓ Hepatic steatosis
	Lee, N. Y 2020 [75]	C57BL/6 J mice fed a western diet	*L. bulgaricus, L. casei, L. helveticus,* and *P. pentosaceus* KID7 (10^9 CFU/g diet) for 8 weeks	↓ Liver/body weight ratio ↓ Cholesterol levels ↓ Hepatic steatosis and inflammation

AKT protein kinase B, *ALT* alanine aminotransferase, *AMPK* AMP-activated protein kinase, *ASC* apoptosis-associated speck-like protein, *AST* aspartate transaminase, *BAMBI* BMP and activin membrane-bound inhibitor, *BMI* body mass index, *CFU* colony-forming unit, *CYP7A1* cholesterol 7 alpha-hydroxylase, *ERK* extracellular signal-regulated kinase, *FXR* farnesoid X Receptor, *GLP* Glucagon-like peptide, *HDL-C* high-density lipoprotein cholesterol, *HFD* high-fat diet, *IL* interleukin, *JNK* c-Jun N-terminal kinase, *LDL* low density lipoprotein, *LDL-C* low-density lipoprotein cholesterol, *LGG* Lactobacillus rhamnosus GG, *LPS* lipopolysaccharide, *MCD* methionine-choline deficient, *NAFLD* non-alcoholic fatty liver disease, *NASH* nonalcoholic steatohepatitis, *NF-κB* Nuclear factor kappa B, *NLRP3* NLR family pyrin domain-containing 3, *PPAR* peroxisome proliferator-activated receptor, *SREBP-1c* sterol regulatory element binding transcription factor 1 isoform c, *TG* Triglycerides, *TLR* toll-like receptor, *TNF-α* tumor necrosis factor alpha, *US* ultrasound

A positive or upward effect is denoted by (↑), a negative or downward effect is denoted by (↓), and an insignificant effect is denoted by (−)

mucosae) are usually detected in metagenomic analyses [72]. The metagenomic results indicated that a higher proportion of *Lactobacillus* spp. was observed in fecal samples of NAFLD, NASH, and obese children, especially *L. mucosae*, whereas an abundant population of *Bifidobacterium* spp. was observed in healthy individuals [72]. These results suggest that the uplifting population of *Bifidobacterium* members can be promising targets for gut microbiota modification [72]. A study in 2020 demonstrated that *Bifidobacterium animalis* subsp. *Lactis* V9 (V9) treatment ameliorated hepatic steatosis and inflammation in HFD-fed male Wistar rats [73]. The reduction in hepatic steatosis is attributed to the effect on de novo lipid synthesis regulation by reducing the transcription of SREBP-1c and fatty acid synthase (FAS), whereas hepatic phosphorylated-AMPK and PPAR-α expression levels were upregulated by V9 [73]. V9 anti-inflammatory effect was observed via decreased expression levels of inflammatory cytokines and receptors, together with the suppression of AMPK and TLR-NF-κB pathways, compared to HFD-fed rats [73]. Besides *Bifidobacterium*, *Lactobacillus* has also received adequate attention as a therapeutic probiotic [74, 75]. Lee et al. [75] demonstrated that treatments with four *Lactobacillus* strains, *L. acidophilus*, *L. fermentum*, *L. paracasei,* and *L. plantarum*, have beneficial effects on NAFLD [74]. Administration of *L. acidophilus* led to a decreased liver/body weight ratio, while reduced cholesterol levels were observed in mice treated with *L. acidophilus*, *L. fermentum*, and *L. plantarum* [74]. Furthermore, *L. acidophilus*-, *L. plantarum*-, and *L. paracasei*-supplemented diets induced the amelioration of hepatic steatosis [74]. Before this study, the aforementioned group had already revealed the benefits of *L. bulgaricus*, *L. casei*, *L. helveticus*, and *Pediococcus pentosaceus* KID7, including reduced liver/body ratio and improvement in hepatic steatosis and inflammation, in western diet-induced conditions [75].

Synbiotics

Notably, prebiotics and probiotics can be administered together to have a synergistic effect, and supplementation with prebiotics can prevent nutrient competition between fiber-ingesting indigenous and administered beneficial strains (Table 4) [5]. As one of the most well-investigated prebiotics, the benefits of combining inulin with other probiotics have been widely studied. A clinical study conducted in 2018 by Bakhshimoghaddam et al. consolidated the protective effects of inulin and *Bifidobacterium* spp. against NAFLD [76]. Patients consuming synbiotic yogurt daily for 24 weeks had lower risk of NAFLD and serum levels of ALT, AST, ALP, and

Table 4 Synbiotics-incorporated studies on non-alcoholic fatty liver disease/non-alcoholic steatohepatitis

Synbiotic	References	Subject	Treatment	Results
Inulin + Probiotics	Bakhshimoghaddam, F 2018 [76]	Patients with NAFLD	1.5 g inulin and 10^8 CFU of *Bifidobacterium animalis*/mL in 300 g yogurt for 24 weeks	↓ NAFLD grade ↓ ALT, AST, ALP, γ-glutamyl-transferase levels
	Javadi, Leila 2017, 2018 [77, 78]	Patients with NAFLD	*Bifidobacterium longum* and *Lactobacillus acidophilus* (2×10^7 CFU/day) and inulin (10 g/day)	↓ BMI, AST, TNF-α, high-sensitive C-reactive protein levels ↑ Serum levels of total antioxidant capacity
FOS + Probiotics	Asgharian, Atefe 2016 [79]	Patients with NAFLD	500 mg capsule per day of FOS from seven strains of probiotics for 8 weeks	↓ NAFLD grade (-), and AST and ALT levels
	Mofidi, Fatemeh 2017 [80]	Patients with NAFLD with normal or low BMI	125 mg FOS and 2×10^8 seven strains of probiotics per day for 28 weeks	↓ Hepatic steatosis and fibrosis ↓ Serum levels of fasting blood sugar, TG, and inflammatory mediators
	Scorletti, E 2020 [81]	Patients with NAFLD	FOS 4 g/twice a day and *Bifidobacterium animalis* subsp. *Lactis* BB-12 (10^{10} CFU/day)	↑ *Bifidobacterium* and *Faecalibacterium* ↓ *Oscillibacter* and *Alistipes* (-) liver fat, liver fibrosis markers
β-glucan + Probiotics	Vu, Vuong 2021 [5]	C57BL/6 J mice fed HFD	3 g β-glucan and 15 g of bacterial concoction in 1 kg diet	↓ DSS-induced UC ↓ UC-associated hepatic steatosis, inflammation, (-) body weight, and AST and ALT levels

ALP alkaline phosphatase, *ALT* alanine aminotransferase, *AST* aspartate transaminase, *BMI* body mass index, *CFU* colony-forming unit, *DSS* dextran sulfate sodium, *FOS* fructooligosaccharide, *HFD* high-fat diet, *NAFLD* non-alcoholic fatty liver disease, *TNF-α* tumor necrosis factor alpha, *UC* Ulcerative colitis

A positive or upward effect is denoted by (↑), a negative or downward effect is denoted by (↓), and an insignificant effect is denoted by (−)

γ-glutamyl-transferase in comparison with the conventional yogurt and control groups [76]. Both separate and combined treatments of inulin with *B. longum* and *L. acidophilus* in patients with NAFLD showed decreased BMI and AST serum levels, and increased total antioxidant capacity [77, 78]. In the co-treatment group, patients showed a significant decrease in high-sensitivity C-reactive protein levels compared to the placebo and single-treatment groups [77]. In addition to inulin, the combination of FOS and probiotics also has beneficial effects on NAFLD [79]. A clinical trial was conducted with 80 patients with NAFLD who were daily administered synbiotic capsules containing seven species of probiotic bacteria and FOS for 8 weeks [79]. The ultrasound grade of NAFLD was decreased in synbiotic-treated patients despite unchanged ALT and AST serum levels [79]. Mofidi et al. [80] conducted a clinical trial to investigate the combined effects of FOS and probiotics in patients with NAFLD with normal or low BMI [80]. After 28 weeks of intervention, reduction in hepatic steatosis and fibrosis, along with low serum levels of fasting blood sugar, TG, and inflammatory mediators were observed in the synbiotic-treated group [80]. In another study, 104 patients with NAFLD participated in a double-blind and placebo-controlled phase 2 trial for 12 months and were administered a synbiotic or a placebo [81]. Patients treated with FOS and probiotics showed insignificant changes in hepatic fat and liver fibrosis markers despite the modification of gut microbiota by fostering *Bifidobacterium* and *Faecalibacterium*, while suppressing *Oscillibacter* and *Alistipes* [81]. Besides inulin and FOS, the co-administration of β-glucan with other probiotics is worth investigating. In 2021, a study using HFD-induced obese mice with UC induction using 3% DSS revealed the close relationship between UC and NAFLD, in which colitis-associated gut permeability led to the transport of endotoxins and caused hepatic steatosis and inflammation [5]. The study demonstrated that treatment with β-glucan together with probiotics (VSL#3 and LGG) outperformed the separate treatment groups (β-glucan or probiotics) in protecting the liver from UC-induced NAFLD via hepatic steatosis and inflammation using hematoxylin and eosin-stained liver sections [5]. Several clinical trials using a combination of prebiotics, such as inulin and FOS, and probiotics, such as *Bifidobacterium, Lactobacillus,* and *Streptococcus, showed* decreased BMI and serum levels of AST and ALT [82–84]. However, many trials have indicated that the treatment with additives is inefficient and has variable benefits, suggesting that individual genetic traits also affect the treatment [79, 85, 86].

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Author contributions
VV and YMK collected information and wrote the review article. MJC conceptualized and reviewed the article. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declarations

Competing interests
The authors declare that they have no competing interests.

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