Nonalcoholic Fatty Liver Disease, the Gut Microbiome, and Diet

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ABSTRACT

Nonalcoholic fatty liver disease (NAFLD) is the most common liver disorder in the world, yet the pathogenesis of the disease is not well elucidated. Due to the close anatomic and functional association between the intestinal lumen and the liver through the portal system, it is speculated that the gut microbiome may play a pivotal role in the pathogenesis of NAFLD. Furthermore, diet, which can modulate the gut microbiome and several metabolic pathways involved in NAFLD development, shows a potential tripartite relation between the gut, diet, and the liver. In this review, we summarize the current evidence that supports the association between NAFLD, the gut microbiome, and the role of diet. Adv Nutr 2017;8:240–52.

Keywords: nonalcoholic fatty liver disease, gut microbiota, diet, NAFLD, NASH, probiotics

Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver diseases in the world (1). NAFLD is defined as a liver pathologic spectrum, which is initiated by steatosis that may progress to nonalcoholic steatohepatitis (NASH). NASH manifestations beyond steatosis include necro-inflammation and fibrosis (2). The disease can progress to cirrhosis and eventual hepatocellular carcinoma (3). The reported prevalence of NAFLD is 30% in Western countries (1, 4) and 12–24% in Asia (5), which shows the global importance of understanding the disease pathology. In addition to genetic background, environmental factors such as diet and the intestinal microbiome should be considered critical factors in the development of NAFLD (6, 7).

The gut microbiome, the trillions of microbes living in the gut, may contribute to the pathogenesis of liver diseases (8, 9). Increasing evidence in recent decades shows that the gut microbiome plays a significant role in metabolism, health, and disease of the host (10), which suggests that the gut microbiome is a metabolic organ in the host. Considering that the anatomy of the liver facilitates a close interaction between the liver and gut microbiome, it is possible that gut microbial metabolites and circulating byproducts influence liver function. Indeed, it is known that slow blood flow and fenestrated endothelium in the sinusoids lead to the interaction between substances derived from the gut and hepatocytes, parenchymal cells, and hepatic immune cells (11). Similarly, dietary antigens are known to affect liver function, and because dietary components are among the strongest factors that predict the ecosystem that makes up the gut microbiome (12, 13), both diet and the microbiome need to be considered in the pathogenesis of NAFLD.

In the present review, we summarize the current evidence that supports the association between NAFLD, the gut microbiome, and dietary factors.

Current Status of Knowledge

NAFLD and the gut microbiome. The gut harbors $10^{13}$–$10^{14}$ bacteria, which is predicted to encode genes >100 times as in the human genome (14). Despite the wide microbial diversity in humans, only 4 main phyla dominate: Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria. Approximately 90% of the microbes come from the Firmicutes and Bacteroidetes phyla (14) and each has unique
functions. Major contributors of Firmicutes are as follows: Lachnospiraceae, which are composed mostly of microbes from the genera Blautia, Clostridium, Coprococcus, Eubacterium, Roseburia, and Ruminococcaceae, which are composed mostly of the genera Faecalibacterium, Oscillospira, and Ruminococcus. Major contributors of the Bacteroidetes phylum are Bacteroides, Parabacteroides, Porphyromonas, Prevotellaceae (Prevotella), and Rikenellaceae (Alistipes). The most prominent Actinobacteria are Bifidobacteriaceae (Bifidobacterium), and the most abundant Proteobacteria are the Enterobacteriaceae (Escherichia) (15, 16). Although the functions of most Firmicutes are not yet clear, there is some evidence that indicates that some members of this phylum are among the butyrate-producing bacteria that increase energy extraction from the diet (17–19). On the other hand, members of the phylum Bacteroidetes participate in carbohydrate metabolism and accomplish this by expressing enzymes similar to glycosyl transferases, glycoside hydrolases, and polysaccharide lyases (20). Understanding the functions associated with the microbial community is important, because alterations in the intestinal microbiota have been associated with host diseases, including obesity, type 2 diabetes, and NAFLD (8, 21, 22). In patients with NAFLD, the microbiome’s abundance (23) and community structure is altered (also called dysbiosis) (15, 24). The importance of the gut microbiome in the prevention and treatment of NAFLD is highlighted by the fact that fecal transplantation from mice with NAFLD into wild-type mice caused NAFLD in mice that received the microbiota (25). In addition, the alteration of microbiota can change the disease severity (26–30). Overall, these results suggest that the microbiome is important in NAFLD.

Several lines of evidence have shown that NAFLD can be affected by the gut microbiome through various proposed mechanisms. The contribution of increased intestinal permeability (20), overgrowth of bacteria (15), and elevated serum LPS (31, 32) has been shown in patients with NAFLD. Overgrowth of bacteria, in particular gram-negative bacteria, increases the production of hepatotoxic products such as LPS (23). The disruption of the intestinal integrity increases intestinal permeability, which leads to bacterial translocation, and bacterial endotoxins penetrate into the portal vein, which increases the risk of NAFLD development through the activation of hepatic inflammatory cells (23, 33). Bacterial endotoxins are recognized by toll-like receptors (TLRs) on hepatocytes, which recognize several components of microbes and initiate immunologic responses (32). When bacterial LPS signals through TLR4, signaling ultimately activates NF-κB and proinflammatory cytokines (32, 34). Moreover, dysbiosis is associated with reduced synthesis and secretion of fasting-induced adipocyte factor (FIAF) also known as angiopoietin-like 4] in enterocytes, which results in increased activity of lipoprotein lipase (LPL) and the accumulation of TGs in the liver (35, 36).

Dysbiosis and inflammasome deficiency may be important in the development of hepatic steatosis and inflammation (37, 38). Inflammasomes are cytoplasmic multiprotein complexes that sense endogenous or exogenous stress- or damage-associated molecular patterns (39, 40). Upon sensing the relevant signal, they assemble, typically together with the adaptor protein, apoptosis-associated speck-like protein (ASC), into a multiprotein complex that governs caspase-1 activation and subsequent cleavage of effector proinflammatory cytokines, including pro–IL-1β and pro–IL-18, which ultimately leads to apoptosis (38). Inflammasome deficiency, large amounts of bacterial endotoxins enter the portal vein, and the liver then has to process high amounts of endotoxins, which leads to aberrant and damaging inflammatory responses that promote progression to NASH (37). Another mechanism by which the gut microbiota contributes to the development of NAFLD may be through increasing the number of ethanol-producing bacteria (e.g., Escherichia coli) (15). These bacteria produce alcohol, which could participate in the disruption of gut permeability, the generation of ROS, and consequently, liver inflammation (41, 42). However, the specific mechanisms of this hypothesis require further investigation.

The characterization of the gut microbial composition and comparing it between patients and healthy individuals is regarded as one way of identifying a healthy microbial ecosystem (43). Indeed, a few studies have examined dysbiosis in NAFLD (Table 1). Patients with NASH harbor a lower abundance of Faecalibacterium and Firmicutes (Clostridiales family, Anaerospirabacter) but a higher abundance of Parabacteroides and Allisonella in their fecal microbiome (45). Importantly, it has been reported that improved intrahepatic TG content is related to a lower abundance of Firmicutes and a higher abundance of Bacteroidetes (45). In support of this, obese individuals with NAFLD have increased members of the phylum Firmicutes [Lachnospiraceae (Dorea, Robinsoniella, and Roseburia)] (46). Yet, children and adolescents with NASH do not show similar patterns and instead have increased Bacteroidetes [Prevotellaceae (Prevotella, Porphyromonas)] and Proteobacteria [Enterobacteriaceae (Escherichia), Alcaligenaceae] and a decrease in Firmicutes [Lachnospiraceae (Blautia, Coprococcus, Eubacterium, Roseburia), Ruminococcaceae (Faecalibacterium, Oscillospira, Ruminococcus)] and Actinobacteria [Bifidobacteriaceae (Bifidobacterium)] in their fecal microbiomes (15). In support of these studies, pediatric patients with NAFLD showed an increase in Bradyrhizobium, Anaerococcus, Peptoniphilus, Propionibacterium acnes, Dorea, and Ruminococcus and a decrease in Oscillospira and Rikenellaceae (44). Although it is clear that there may be some discrepancies in what defines dysbiosis in liver disease, the frequency of disease also occurs in association with obesity and is considered a manifestation of metabolic syndrome. Thus, the dysbiosis may be related to these metabolic disturbances, considering that several studies suggested that increased Firmicutes and reduced Bacteroidetes may be a cause of obesity (17, 47). However, the reduction in Bacteroidetes is not simply a cause of obesity in patients with NASH, considering that Bacteroidetes abundance is reduced in these patients even after adjusting for BMI and fat intake (24).
Overall, the evidence suggests that the gut microbiome may have an important role in NAFLD pathology, but the studies have not identified a particular microbe involved due to the heterogeneous results. Similar to other microbiome studies, discrepancies can be due to variations in the study designs. Some of the studies, such as the study conducted by Zhu et al. (15), used patients with no history of antibiotics, probiotics, proton pump inhibitors, and histamine receptor antagonists within 3 mo before examining the fecal microbiota; however, others did not consider all of these precautions. In addition, the collection and processing of fecal samples have been shown to produce large variances and inaccuracies in the interpretation of the taxa present in the microbiota (48), which may be a contributing factor to the conflicting data found in NAFLD microbiome studies. Even so, all of these studies have evaluated the association, and well-designed studies are needed to unravel any causal relation between the gut microbes and NAFLD.

**Diet and the gut microbiome.** Dietary factors are strong predictors of the gut microbiota composition (49–51). In fact, it has been projected that dietary factors play a more important role in shaping the gut microbiota composition than do genetic factors (52). To understand the role of nutrition, the gut microbiome, and NAFLD, we summarized the experimental studies that evaluated this potential relation (Table 2).

**Energy homeostasis and the gut microbiota.** An imbalance between energy intake and expenditure is regarded as the main cause of increased hepatic lipogenesis. Increased lipogenesis and reduced FA oxidation result in increased susceptibility to hepatic steatosis and the subsequent consequences (63). Several studies indicated that an increase in the relative abundance of the members of Firmicutes, and especially of some Clostridium clusters, is involved in hepatic lipogenesis.

### TABLE 1 Intestinal microbiota composition in patients with NAFLD

<table>
<thead>
<tr>
<th>Study, year (ref)</th>
<th>Subjects</th>
<th>Method</th>
<th>Change in microbiota</th>
</tr>
</thead>
<tbody>
<tr>
<td>Del Chierico et al., 2016 (44)</td>
<td>Pediatric NAFLD, NASH, or obesity (n = 61); healthy subjects (n = 54)</td>
<td>16S rRNA pyrosequencing</td>
<td>NASH vs. healthy controls: ↑Bradyrhizobium, Anaerococcus, Peptoniphilus, Propionibacterium acnes, Dorea, Ruminococcus ↓Oscillapora and Rikenellaceae</td>
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<tr>
<td>Mouzaki et al., 2013 (24)</td>
<td>NASH (n = 22); SS (n = 11); healthy controls (n = 17)</td>
<td>qPCR</td>
<td>NASH vs. both SS and healthy controls: ↓Percentage of Bacteroidetes (Bacteroidetes to total bacteria counts) NASH vs. SS: ↑Clostridium coccoides</td>
</tr>
<tr>
<td>Wong et al., 2013 (45)</td>
<td>NASH (n = 16); healthy controls (n = 22)</td>
<td>16S rRNA pyrosequencing</td>
<td>NASH vs. healthy controls: ↑Enterococci, ↑Anaeroplasma, ↑Parabacteroides, ↑Allisonella Improvement in intrahepatic TG content was associated with a reduction in the abundance of Firmicutes (R² = 0.4820, P = 0.0028) and an increase in Bacteroidetes (R² = 0.4366, P = 0.0053)</td>
</tr>
<tr>
<td>Raman et al., 2013 (46)</td>
<td>Obese NAFLD patients (n = 30); healthy controls (n = 30)</td>
<td>16S rRNA pyrosequencing</td>
<td>Obese NAFLD vs. healthy controls: ↑Lactobacillus species ↑Firmicutes (Lachnospiraceae; genera: Dorea, Robisonella, and Roseburia) ↓One member of phylum Firmicutes (Ruminococcaceae; genus, Oscillibacter) ↓A member of phylum Bacteroides (Porphyromonadaceae)</td>
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<tr>
<td>Zhu et al., 2013 (15)</td>
<td>NASH children (n = 22); obese children (n = 25); healthy children (n = 16)</td>
<td>16S rRNA pyrosequencing</td>
<td>NASH vs. healthy controls: ↑Proteobacteria [Enterobacteriaceae (Escherichia), Alcaligenaceae] ↑Bacteroidetes [Prevotellaceae (Prevotella), Porphyromonas], ↓Rikenellaceae (Alistipes) ↓Actinobacteria [Bifidobacteriaceae (Bifidobacterium)] ↓Firmicutes [Lachnospiraceae (Blautia, Coprooccus, Eubacterium, Roseburia), Ruminococcaceae (Faecalibacterium, Oscillobia, Ruminococcaceae)], ↑Peptostreptococcus</td>
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<td>Wigg et al., 2001 (23)</td>
<td>Healthy controls (n = 23)</td>
<td>C-13-xylose and lactulose breath test</td>
<td>Small intestinal bacterial overgrowth was present in 50% of patients with nonalcoholic steatosis and in 22% of control subjects (P = 0.048)</td>
</tr>
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</table>

1 NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; ref, reference; rRNA, ribosomal RNA; SS, simple steatosis; ↑, increased; ↓, decreased.

[242] Mokhtari et al.
<table>
<thead>
<tr>
<th>Study, year (ref)</th>
<th>Animal model</th>
<th>Treatment</th>
<th>Method(s)</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saha and Reimer, 2014 (53)</td>
<td>Wistar rats</td>
<td>HF or HP diet from 3 to 15 wk of age; a high-fat, high-sucrose diet from 15 to 21 wk; the respective HF or HP diets from 21 to 25 wk</td>
<td>qPCR</td>
<td>HF vs. control group: ↑Total bacteria, ↑Bifidobacteria, and ↑Bacteroides/Prevotella. HF vs. HP: ↓Firmicutes, ↓Firmicutes: Bacteroidetes, ↓hepatic cholesterol content. Negative correlation between liver weight and Bacteroides: Prevotella (r = −0.415, P = 0.044)</td>
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<tr>
<td>Bomhof et al., 2014 (54)</td>
<td>Sprague-Dawley rats</td>
<td>Initiate with a high-fat, high-sucrose diet for 8 wk and then prebiotic OFSs vs. the probiotic BB-12 for 8 wk</td>
<td>qPCR</td>
<td>Prebiotic oligofructose vs control: ↓Energy intake, ↓weight gain, ↓fat mass, ↑PYY, ↑Bifidobacteria, ↑Lactobacilli. Improved glycemia and ↓insulin concentrations, ↓liver TGs in OFSs and BB-12. ↑GLP-1 in OFSs. ↑GLP-2 in probiotic BB-12. No differences in plasma LPS, TNF-α, IL-6, IL-1β.</td>
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<tr>
<td>Ritze et al., 2014 (55)</td>
<td>C57BL/6 mice</td>
<td>High-fructose diet with LGG vs. high-fructose diet over 8 wk</td>
<td>qPCR</td>
<td>High-fructose diet with LGG vs. high-fructose diet: ↓ALT, ↓fat, ↓accumulation in liver, ↓ChREBP, ↓ACC1, ↓FAS, ↓TNF-α, ↓IL-1β, ↓occludin, ↓LPS, ↑total bacterial numbers</td>
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<tr>
<td>Zeng et al., 2013 (56)</td>
<td>C57BL/6 mice</td>
<td>HFD vs. LFD for 10 wk</td>
<td>Sequencing 16S rRNA</td>
<td>HFD vs. LFD: ↑Hepatic lipid accumulation, ↑inflammatory cell infiltration, ↑leptin, ↑TNF-α, ↑Lactobacillus gasseri and/or ↑Lactobacillus taiwanensis. Positive correlation of L. gasseri and/or L. taiwanensis DNA and lipid droplets in liver.</td>
</tr>
<tr>
<td>Park et al., 2013 (57)</td>
<td>C57BL/6J mice</td>
<td>HFD + probiotic (Lactobacillus curvatus HY7601 and Lactobacillus plantarum KY1032) vs. HFD + placebo for 10 wk</td>
<td>Sequencing 16S rRNA</td>
<td>HFD + probiotic vs. HFD + placebo: ↓ALT, ↓FA oxidation–related genes, ↓proinflammatory genes (TNFα, IL6, IL1b), ↓gut microbiota diversity, ↑Bifidobacterium Pseudolongum.</td>
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<tr>
<td>Pachikian et al., 2013 (58)</td>
<td>C57BL/6J mice</td>
<td>n-3 PUFA–depleted diet for 3 mo supplemented with FOSs during the last 10 d of treatment</td>
<td>DGGE, qPCR</td>
<td>n-3 PUFA–depleted diet supplemented with FOSs vs. n-3 PUFA–depleted diet: ↑Bifidobacterium spp. ↓Roseburia spp., ↓SREBP2, ↑PPAR-α, ↓LDL, ↓HDL, ↑GLP-1.</td>
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<tr>
<td>Cano et al., 2013 (59)</td>
<td>C57BL/6 mice</td>
<td>HFD supplemented with Bifidobacterium pseudocatenulatum CECT 7765 vs. HFD for 7 wk</td>
<td>qPCR</td>
<td>HFD supplemented with B. pseudocatenulatum CECT 7765 vs. HFD: ↓Serum cholesterol, ↓serum TGs, ↓serum glucose, ↓insulin resistance, ↓hepatocytes with grade 3 steatosis, ↓fat absorption, ↓leptin, ↓IL-6, ↓MCP-1, ↑IL-4, ↑IL-10, ↑Bifidobacteria. ↓Enterobacteriaceae, ↓body weight gain, ↓food intake</td>
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(Continued)
With the use of germ-free animal models, it has been shown that the gut microbiota increases energy harvested from dietary polysaccharides and augments key enzymes in hepatic de novo FA biosynthesis, including acetyl-CoA carboxylase (ACC) and FA synthase (FAS), and consequently, hepatic TG accumulation. On the other hand, the gut microbiota enters to increase LPL activity and hepatic production of TGs by the inhibition of FIAF (an LPL inhibitor) in intestinal epithelium cells (35). In addition, germ-free mice showed increased AMP-activated protein kinase (AMPK) activity, which is an intracellular energy sensor in the liver and skeletal muscle, leading to increased FA oxidation and insulin sensitivity and reduced glycogen concentrations. This would protect germ-free mice against diet-induced obesity. In normal mice with normal gut microbiota, inactive AMPK is associated with decreased FA oxidation in skeletal muscle (36). In addition, gut satiety hormones are affected by the gut microbiome through the gut-brain axis (66). Therefore, the gut microbiome has a significant effect on energy intake and energy expenditure. Any changes in the gut microbiota can increase gastrointestinal satiety hormones, hence influencing energy homeostasis (66). Another key function of the intestinal bacteria is to hydrolyze polysaccharides and oligosaccharides that are not completely catabolized by the host enzymes. Although much is still to be learned about which microbes synthesize and/or metabolize the SCFAs and the roles of these potent metabolites, saccharolytic fermentations are major players.

### TABLE 2 (Continued)

<table>
<thead>
<tr>
<th>Study, year</th>
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<th>Treatment</th>
<th>Method(s)</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axling et al., 2012 (60)</td>
<td>C57BL/6j mice</td>
<td>HFD with or without a supplement GT and supplemented with Lp or the combination of both (Lp + GT) for 22 wk</td>
<td>Sequencing 16S rRNA, qPCR</td>
<td>Lp + GT vs. control: ( \uparrow \text{Lactobacillus}, \uparrow \text{diversity of bacteria,} \downarrow \text{liver weights,} \downarrow \text{liver size,} \downarrow \text{TGs,} \downarrow \text{ALT} ) Akkermansia correlated negatively with body fat content ( r = -0.43, P = 0.04 ), plasma insulin ( r = -0.47, P = 0.03 ), and liver TGs ( r = -0.44, P = 0.03 ).</td>
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<tr>
<td>Neyrinck et al., 2011 (61)</td>
<td>C57BL/6j mice</td>
<td>HFD supplemented with arabinoxylans from wheat vs. HFD for 4 wk</td>
<td>DGGE, qPCR</td>
<td>HFD supplemented with arabinoxylans from wheat vs. HFD: ( \uparrow \text{Bifidobacteria,} \downarrow \text{adipocyte size,} \downarrow \text{serum and hepatic cholesterol accumulation and insulin resistance,} \uparrow \text{tight junction proteins (ZO-1 and occludin),} \downarrow \text{inflammatory markers (IL-6 and MCP-1).} )</td>
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<tr>
<td>Cani et al., 2009 (62)</td>
<td>C57BL/6j mice</td>
<td>Prebiotic (oligofructose) vs. nonprebiotic carbohydrates (microcrystalline cellulose) for 4 wk</td>
<td>DGGE, qPCR analysis</td>
<td>Prebiotic vs. nonprebiotic: ( \uparrow \text{total bacteria count,} \uparrow \text{Lactobacillus spp.,} \uparrow \text{Bifidobacterium spp.,} \uparrow \text{Clostridium cocooides–Eubacterium rectale cluster,} \downarrow \text{LPS,} \downarrow \text{ZO-1 and occludin,} \downarrow \text{PAI-1,} \downarrow \text{NADPHox,} \downarrow \text{iNOS,} \downarrow \text{TLR4,} \downarrow \text{TNF-( \alpha ),} \downarrow \text{GLP-2,} \downarrow \text{hepatic expression of inflammatory and oxidative stress markers.} )</td>
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<tr>
<td>Cani et al., 2007 (33)</td>
<td>C57BL/6j mice</td>
<td>High-fat and carbohydrate-free diet vs. normal-diet group for 4 wk</td>
<td>FISH</td>
<td>High-fat and carbohydrate-free diet vs. normal-diet group: ( \uparrow \text{Liver weight,} \uparrow \text{Liver TGs,} \uparrow \text{TNF-( \alpha ),} \downarrow \text{IL-6,} \downarrow \text{LPS,} \downarrow \text{Bifidobacterium spp.,} \downarrow \text{E. rectale/C. cocooides} )</td>
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1. ACC1, acetyl-CoA carboxylase 1; ALT, alanin transferase; BB-12, Bifidobacterium animalis subsp. lactis BB-12; ChREBP, transcription factor carbohydrate-responsive element-binding protein; DGGE, denaturing gradient gel electrophoresis; FAS, fatty acid synthase; FISH, fluorescent in situ hybridization; FOS, fructo-oligosaccharide; GLP, glucagon-like peptide; GT, green tea powder; HFD, high-fat diet; HP, high protein; iNOS, inducible nitric oxide synthase; LFD, low-fat diet; LGG, Lactobacillus rhamnosus GG; Lp, Lactobacillus Lp + GT vs. control: \( \uparrow \text{Lactobacillus}, \uparrow \text{diversity of bacteria,} \downarrow \text{liver weights,} \downarrow \text{liver size,} \downarrow \text{TGs,} \downarrow \text{ALT} \) Akkermansia correlated negatively with body fat content \( r = -0.43, P = 0.04 \), plasma insulin \( r = -0.47, P = 0.03 \), and liver TGs \( r = -0.44, P = 0.03 \). |
in metabolic disorders (69). A wide range of bacterial groups are able to produce acetate, whereas the production of propionate and butyrate seems to be more specific. Akkermansia muciniphila has been known as a major propionate-producing bacterium (70) and Eubacterium hallii, Eubacterium rectale, Roseburia inulinivorans, Fecalibacterium prausnitzii, Clostridium gracile, Bacteroides uniformis, and Ruminococcus bromii appear to be responsible for most of butyrate production (70–72). Butyrate and propionate at low amounts exert multiple advantageous effects on the host, including the prevention of colonic carcinogenesis, inflammation, and oxidative stress; improvement in intestinal barrier function; and stimulation of satiety and lipid oxidation in hepatocytes (73, 74). Acetate may be more important for modulating insulin sensitivity and metabolic disease (75) and the development of diabetes (76). However, more studies are required to understand which microbes produce each SCFA and the relation this has on liver health and disease.

SCFAs are ligands for G-protein–coupled receptor (GPR) 43 expressed on intestinal epithelial cells. The stimulation of GPR43 by SCFAs is necessary during immune responses (77). GPR41 is another SCFAs receptor. Binding to GPR41 is associated with upregulation of peptide YY (PYY) and glucagon-like peptide 1 (GLP1), anorectic hormones] that have effects on appetite control; therefore, SCFAs are linked to food intake by activating these receptors (78, 79). Current evidence indicates that SCFAs have paradoxical effects on hepatic health. SCFAs have been identified as rich sources of energy for the host and act as intestinotropic agents to promote intestinal absorption of nutrients. On the other hand, through their receptor GPR43 and other pathways, they reduce gut permeability and invasion of bacterial products through the portal vein and liver, and thus protect the liver from the ensuing damaging inflammation, which then results in downregulation of insulin signaling in adipose tissue, thereby decreasing fat accumulation (80). Thus, it seems that SCFAs are beneficial for hepatic health if the body is receiving a balanced diet, whereas they exaggerate steatosis when extra calories are consumed. However, more mechanistic studies are required to understand the role of each of the SCFAs on NAFLD.

Macronutrient intakes and the gut microbiome in NAFLD. Several studies have shown that high-fat diets (HFDs) participate in the development of dysbiosis through diet-induced reductions in Bacteroidetes, Lactobacilli, and Bifidobacteria and increases in Firmicutes (81, 82). Thus, an HFD may contribute to the development of NAFLD at least partially through dysbiosis, which may increase intestinal permeability. Furthermore, HFD exposure impairs choline metabolism via dysbiosis. Choline is converted to methanobactin (dimethylamine, trimethylamine, and trimethylamine–N-oxide) by intestinal microbes with consumption of an HFD. It is associated with a reduction in plasma phosphatidylcholine and increases in urinary methylamines. Indeed, changes in the gut microbiota mimic the effect of a choline-deficient diet, which leads to accumulation of TGs in hepatocytes and reduced VLDL secretion (83). Maintenance of mice fed an HFD for 4 wk resulted in a significant increase in Bifidobacterium spp. and the E. rectale/Clostridium cocoides group, while decreasing the C. cocoides group and Bifidobacteria E. rectale. Moreover, fat consumption can produce a wide amount of chylomicrons, which translocate LPS toward other organs, especially the liver (84), which can bind to TLR4 on hepatic immune cells and initiate the inflammatory process. It has been shown that plasma inflammatory cytokines are positively correlated with plasma LPS concentrations and negatively correlated with intestinal Bifidobacteria count (85). An HFD not only changes the gut microbiota but also plays a role in the development of steatohepatitis in mice (56). It has been shown that Lactobacillus gasseri and Lactobacillus tairwanensis increase after consumption of an HFD. These bacteria are bile acid–resistant and probably contribute to bile acid deconjugation and reduction in fat absorption. There was a positive significant association between L. gasseri and/or L. tairwanensis DNA and lipid droplets in the liver ($R^2 = 0.3$, $P = 0.02$). Increased fat intake simultaneously increases bile acid secretion and bile acid–resistant bacteria. Increased Lactobacillus in HFD may affect lipid metabolism through bile acids (86, 87).

A high-protein diet also affects the gut microbiome, favoring a potentially pathogenic and proinflammatory microbiota profile with increased ammonia, phenols, and hydrogen sulfide concentrations, which induce mucosal inflammation and alter the enteric nervous system and intestinal motility (88). In addition, the dietary source of proteins plays a critical role in shaping the gut microbiota and their ability to produce secondary metabolites, so that meat protein–fed rats showed a higher relative abundance of the beneficial genus Lactobacillus but lower amounts of SCFAs and SCFA-producing bacteria, including Fusobacterium, Bacteroides, and Prevotella spp. compared with the soy protein–fed group (89). Overall, studies evaluating the effects of different dietary patterns on the human gut microbiome are limited; further studies are needed to elucidate more details in this area.

Probiotics and the gut microbiome in NAFLD. Current studies hypothesize the use of probiotics for modulating dysbiosis in many diseases, including NAFLD. Probiotics are beneficial microorganisms when ingested in specified dosages. Several studies showed that gut microbiota manipulation by probiotic supplements is associated with reduced liver damage, decreased concentrations of LPS, as well as improved aminotransferase concentrations (90–93). A recent review summarized the studies that show that probiotic supplementation in animal models and human studies improve inflammatory status and clinical manifestations in NAFLD (94). Manipulating the gut microbiota with probiotics may improve dysbiosis associated with an HFD. In support of this, consumption of an HFD upregulates the expression of genes involved in hepatic lipid biosynthesis, and supplementation with Lactobacillus curvatus HY7601 and...
Lactobacillus plantarum KY1032 upregulates the expression of genes involved in hepatic FA oxidation (57). Another study showed that increasing tissue n-3 PUFAs in the gut alters the gut bacterial composition, which is associated with a decrease in LPS and gut permeability and eventually results in metabolic endotoxemia and inflammation (95). Overall, although there are some exciting possibilities with regard to the use of probiotics as a treatment for NAFLD, experimental studies are still required to test the mechanisms of action and clinical trials are still required to test their efficacy.

Prebiotics and intestinal microbiota in NAFLD. In addition to probiotics being potentially protective in NAFLD, accumulating data also suggest that there are beneficial effects of prebiotics in NAFLD. A prebiotic is defined as “a selectively fermented ingredient that results in specific changes, in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health” (96). Prebiotics are naturally found in foods such as onions, asparagus, garlic, and leeks and resistant starch (RS) sources such as legumes, potatoes, and cereals. Prebiotics could modulate the intestinal microbiota, because they would increase beneficial bacteria for the host, in particular the indigenous Bifidobacteria and Lactobacilli, and inhibit bacterial activities harmful to host health (97). Furthermore, in the large intestine, inulin-type fructans and RS are completely catabolized by the microbiota into SCFAs, bacterial biomass, and organic acids such as lactic acid and gases (carbon dioxide, hydrogen, methane).

Prebiotics have beneficial effects on the gut microbiota, serum lipids, and the prevention of hepatic steatosis (61, 62, 98, 99). In fact, the addition of RS to rodent feed pellets increases Bacterioidetes, Actinobacteria (Bifidobacteria spp.), and Verrucomicrobia (A. muciniphila) while decreasing Firmicutes (100–102). Also, in humans, the administration of RS has been shown to induce phylum-level changes, selectively increasing numbers of Actinobacteria and Bacterioidetes and reducing Firmicutes; at the species level, an increase in Bifidobacterium adolescentis, E. rectale, Roseburia spp., and R. bromii have been reported (103–107). These various bacteria differ significantly in the production of metabolic byproducts and potential interactions with the host. It has been well established that Bifidobacteria spp., a source of lactate and acetate and low amounts of ethanol and formate, are broadly used as probiotics and have health-promoting properties because of their potential modulating effects on the immune system, metabolism, and elimination of pathogenic bacteria of the host (108). Members of E. rectale, Roseburia, and R. bromii are main producers of butyrate in the colon (71, 107, 109). It has been shown that increasing butyrate-producing bacteria and butyrate production is closely linked with prevention of the progression from steatosis to hepatocarcinogenesis and improving hepatic inflammatory and oxidative stress indexes and gut integrity (74, 110, 111). This would appear to indicate that there is a mutualistic relation between the gut microbiota and the host; dietary ingredients such as RS could induce special species of bacteria that produce metabolic products such as butyrate. Butyrate could act as a signaling molecule in the host that participates in various signaling pathways (110); thus, products of the gut microbiota could explain part of the role of nutritional factors in the gut liver axis and their relation with NAFLD.

It has been reported that prebiotic supplementation can modulate the effects of HFDs. Prebiotic consumption leads to increased Bifidobacteria and total bacteria Bacteroides: Prevotella spp. and decreased Firmicutes (53). Cano et al. (59) reported that Bifidobacterium pseudocatenulatum consumption improved glucose tolerance and inflammatory status while decreasing serum LPS and hepatic steatosis in HFD-fed mice. Another study showed that prebiotic oligofructose in combination with probiotic Bifidobacterium animalis subsp. lactis BB-12 decreased the ratio of Firmicutes to Bacteroidetes and improved glycemia. This decrease in the Firmicutes-to-Bacteroidetes ratio is hypothesized to decrease obesity-inducing effects of the microbiome (54). In addition, oligofructose decreases energy intake, weight gain, and fat mass (54). In this study, prebiotics and probiotics had no effect on increased LPS and inflammatory factors induced by high-fat and high-sucrose diets (54). A significant increase in the abundance of Bifidobacterium spp. and Lactobacillus spp. by the administration of inulin-type fructo-oligosaccharides (FOSs) has been documented (112). Increasing total bacteria counts of Lactobacillus spp. and Bifidobacterium spp. by prebiotics improved intestinal barrier function and hepatic inflammation and oxidation. Furthermore, stimulating the proliferation of these bacteria was associated with a reduction in intestinal endotoxin and modulated gut mucosal function (113, 114). Lactobacillus spp. inhibit proinflammatory responses that result from endotoxins by suppressing NF-κB (115).

Inulin-type fructans or FOSs are also able to modify groups of bacteria other than Bifidobacteria or Lactobacilli. Inulin-containing diets result in an increase in bacteria belonging to C. coccoides and E. rectale cluster in animal models (116). In addition, inulin can stimulate the metabolic activity of Roseburia spp. and E. rectale genera of the Firmicutes phylum (117, 118). Roseburia spp. and E. rectale belong to the butyrate-producing bacteria; therefore, they may mediate some of the advantageous properties of prebiotics. Furthermore, the consumption of inulin, galacto-oligosaccharides, and raffinose stimulates F. prausnitzii (119–121). F. prausnitzii, which belong to the butyrate-producing bacteria, could be crucial to gut barrier function and could modulate systemic inflammation (common to NAFLD) (79, 122). All of these proposed actions may play a role in the beneficial effects of prebiotics in NAFLD prevention and/or treatment. However, well-designed clinical trials are required to support the efficacy of prebiotics during NAFLD.

Choline and other nutrients and the gut microbiome in NAFLD. Choline deficiency is related to NAFLD pathogenesis. Although choline is synthesized in the body, it is considered to be an essential nutrient and dietary intake is
required to fulfill its roles in the body (123). Choline plays a role in brain function, acetylcholine and neurotransmitter synthesis, cell signaling, lipid transport, and lipid metabolism (124, 125). Several studies have shown that choline is critical for the development of hepatic steatosis due to its contribution to lipid metabolism (125–127). When hepatic de novo lipogenesis or TG synthesis from diacylglycerol is greater than the lipid secretion from the liver and/or fat oxidation, hepatic steatosis occurs. Choline is one of the methyl group donors in the body that contributes to hepatic synthesis of phosphatidylcholine (125). Phosphatidylcholine is required for VLDL synthesis and secretion. Reduced phosphatidylcholine contributes to TG accumulation in the liver and NAFLD (128). Choline-deficient diets disturb the mitochondrial function, resulting in increased hepatic lipid accumulation that contributes to NAFLD pathogenesis (129). Gut bacteria can convert choline to trimethylamine and reduce choline bioavailability, mimicking choline deficiency side effects (130). A reduction in choline bioavailability is associated with increased reactive oxygen species resulting from FFA oxidation, more hepatic lipid accumulation, and reduced hepatic VLDL excretion, all of which contribute to the development of hepatic steatosis (125, 126). Choline metabolism is mainly disrupted by 3 main bacterial phyla in the gut microbiota: Proteobacteria, Firmicutes, and Actinobacteria (130). Spencer et al. (131), in a longitudinal experimental study in healthy women who consumed a rigorously controlled diet, showed that choline deficiency led to hepatic lipid accumulation. In addition, decreased amounts of Gammaproteobacteria and increased Erysipelotrichi were directly associated with elevated accumulation of hepatic fat. This study was the only study, to our knowledge, that examined gut microbiota composition in relation to diet and development of NAFLD in humans.

In addition to the evidence of gut dysbiosis in NAFLD, patients with NASH also have dysbiosis, including small intestinal bacterial overgrowth (23). Bacterial overgrowth enhances the number of bacteria metabolizing choline and influences hepatic lipid accumulation (125). Pyruvate produced by the gut microbiota from carbohydrate increases metabolites such as acetaldehyde and ethanol. These metabolites are toxic and associated with gut permeability (132, 133). Overall, although better designed studies are needed, there are sufficient data to support that choline and the gut microbiome together play a role in liver disease. High-fructose dietary intake is also considered to be a significant risk factor in the development of NAFLD (134, 135). Some studies have indicated that antibiotics, prebiotics, and probiotics are protective factors in fructose-induced liver damage (136–139). These studies suggested that gut microbe manipulation may protect from the side effects of high-fructose diets on the liver. The chronic intake of fructose is associated with bacterial overgrowth, which causes increases in endotoxins in the portal vein, development of inflammatory responses in the liver, and insulin resistance in hepatocytes, resulting in increased hepatic lipid accumulation (140, 141). Ritz et al. (55) reported that the use of Lactobacillus rhamnosus GG protects against NAFLD in animals fed a high-fructose diet through increasing the proteins involved in gut integrity and decreasing inflammatory factors and endotoxins. The authors suggested that L. rhamnosus GG may increase occludin and claudin-1 (effective proteins in gut integrity), which lead to reductions in LPS and other toxins translocated to the liver.

Several recent studies reported that polyphenol compounds attenuate inflammatory markers and hepatic steatosis grade in patients with NAFLD (142–145). Some of these act through the amelioration of insulin resistance, whereas some act through the modulation of the gut microbiome (146, 147). These compounds can reduce pathogen bacteria and increase lactic acid bacteria, such as some strains of Bifidobacteria (148). The manipulation of the gut microbiota may be 1 of the mechanisms of action involved in the beneficial effects of polyphenols on NAFLD. Axling et al. (60) reported that green tea in combination with Lactobacillus plantarum (which metabolizes phenolic acids) in HFD-fed mice was able to promote growth of Lactobacillus in the intestine and to attenuate HFD-induced inflammation and hepatic TG and cholesterol accumulation. Furthermore, there was a significant inverse association between the amount of Akkermansia and hepatic TG accumulation. A. muciniphila is a mucus-degrading bacterium that contributes to the regulation of immune responses through upregulation of the expression of some genes involved in intestinal mucus integrity (149).

**n–3 FAs and the intestinal microbiota in NAFLD.** A low intake of n–3 PUFA is associated with hepatic steatosis (150). n–3 PUFA plays an important role in lipid metabolism because they can induce FA oxidation by activating PPAR-α and inhibition of lipogenesis by suppressing sterol regulatory element binding protein 1 (SREBP-1) (151). It has been shown that n–3 PUFA supplementation, and not total calories from fat, alters the gut microbial composition by enhancing numbers of Lactobacillus and Bifidobacteria spp. in adult mice (152) as well as in aged mice, which modulated both gut immunity and oxidative stress (153). In support of these data, other studies have shown that changes in gut microbiota composition by n–3 PUFA deficiency can be modulated by prebiotics such as FOS. FOS consumption was associated with increased cecal Bifidobacterium spp. and reduced Roseburia spp. in n–3 PUFA–depleted mice (58). Moreover, it has been shown that an increase in lactic acid bacteria, such as Bifidobacterium spp., is related to a reduction in cholesterol absorption (154) and an increase in bile acid deconjugation, which leads to more excretion of bile acids and elimination of hepatic cholesterol (155).

**Conclusions**

In summary, intestinal pathogenic bacteria contribute to NAFLD pathogenesis through the following ways: 1) by increasing LPS, which binds to TLR4 in hepatic immune cells, resulting in the activation of the NF-κB pathway and its related inflammatory pathways, leading to hepatic injury, necrosis, and finally fibrosis; 2) inhibition of FIAF, which...
increases the activity of LPL and lipogenesis; 3) enhancement in polysaccharide digestion and absorption, which increases the production of SCFAs and hepatic lipogenesis; and 4) conversion of choline into methyllamines, which reduces choline availability and induces fat accumulation and reactive oxygen species production in the liver.

Nutritional factors and the gut microbiota have bilateral interactions that contribute to the development and/or protection from NAFLD. High-fat, high-protein, and high-fructose diets and diets that are low in n-3 FAs (Western dietary pattern) may contribute to the development of NAFLD by dysbiosis in the intestinal microbiota. On the other hand, gut microbiota manipulation by nutritional factors such as prebiotics, probiotics, and polyphenols can improve characteristics of NAFLD. Few studies have investigated the effects of nutritional factors on the gut microbiota in patients with NAFLD, and in particular, human studies are scarce. Further studies are needed to elucidate the exact mechanism(s) of action and probable therapeutic options.

Acknowledgments

ZM and AH contributed to the conception of the article, the literature search and interpretation, writing the article, and the critical revisions related to important intellectual content of the manuscript and approved the final manuscript; and DLG contributed to the final revisions of the manuscript. All authors read and approved the final manuscript.

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