Review Article

Fatty Liver Disease and Gut Microbiota: A Comprehensive Update

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is the accumulation of fat in the liver in the absence of secondary causes. NAFLD is a multifactorial disease that results from the interaction of genetic predisposition and metabolic, inflammatory and environmental factors. Among these factors, dysregulation of gut microbiome has been linked to the development of fatty liver disease. The microbiome composition can be modified by dietary habits leading to gut microbiome dysbiosis, especially when a diet is rich in saturated fats, animal products and fructose sugars. Different species of bacteria in the gut metabolize nutrients differently, triggering different pathways that contribute to the accumulation of fat within the liver and triggering inflammatory cascades that promote liver damage. In this review, we summarize the current understanding of the roles of gut microbiota in mediating NAFLD development and discuss possible gut microbiota-targeted therapies for NAFLD. We summarize experimental and clinical evidence, and draw conclusions on the therapeutic potential of manipulating gut microbiota to decrease the incidence and prevalence of fatty liver disease.
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Introduction

Nonalcoholic fatty liver disease (NAFLD) includes steatosis without inflammation, nonalcoholic steatohepatitis (NASH) or NASH-induced cirrhosis.[1] Linked to the worldwide rise in obesity and metabolic syndrome, NAFLD has become the most common types of chronic liver disease.[2] It is most commonly diagnosed by imaging and often confirmed with liver biopsy. Worldwide, NAFLD has a reported prevalence of 4–46%.[3] However, the prevalence of NAFLD differs by region.[3]

Initially, it was thought that liver injury from NAFLD resulted from inflammatory cytokines and adipokines that were stimulated by the gradual accumulation of triglycerides in the liver, leading to mitochondrial dysfunction and oxidative stress, resulting in steatohepatitis and ultimately fibrosis.[4,5] More recent studies have shown that gut microbial organisms play an important role in the metabolic regulation of glucose and lipids contributing to the development of metabolic syndrome and NAFLD.[4,6]

The liver and the gut have an intimate functional and anatomical relationship, as the liver receives approximately 70% (1000–1200 mL/min) of its blood supply from the intestines through the portal circulation.[7] The enteric lining of the intestines serves as an immunological barrier, preventing the movement of antigens in the gut from entering the portal circulation.[7] Antigens that do cross the barrier are mostly eliminated by the innate immune system, preventing them from reaching the liver and thereafter the systemic circulation.

The gut microbiome comprises all of the microorganisms in the digestive tract. Imbalance of the bacteria found in the gut is known as dysbiosis and can prevent the immunologic barrier in the gut from functioning properly. Dysbiosis has also been associated with the development of chronic metabolic conditions, such as insulin resistance, diabetes, cardiovascular disease, obesity and NAFLD[8] Bacteria dysbiosis has been linked to altered energy homeostasis, increased inflammation, and choline and bile acid metabolism, which have all been thought important to the development of NAFLD.[8]

Dysbiosis also leads to endotoxemia and inflammation of the gut wall and activation of Kupffer cells and hepatic stellate cells.[7] This causes the activation of proinflammatory cells via activation of the Toll-like receptors (TLRs) 9 and 4 and the tumor necrosis factor-alpha (TNF-α) receptors, leading to liver injury and inflammation (Fig. 1).[8,9] There are multiple animal and human studies that have demonstrated the relationship between the gut microbiome and development of NAFLD[3] (Table 1). Studies have also shown that lean individuals with a body mass index less than 25 have a different microbiome composition, when compared to obese individual with a body mass index greater than 30[10–12] (Table 1).

The gut microbiome

The gastrointestinal tract has approximately one hundred trillion commensal organisms and contains over 7000 different strains of bacteria.[8] The number of bacteria varies based on the location within the gut, increasing from the stomach to the colon. The bacterial composition also depends on age and diet.[13] Real-time PCR, microarray, and pyrosequencing have been used to improve the resolution of the microbial biodiversity and quantification of microbial species.[11] Most studies

Keywords: Nonalcoholic fatty liver disease; Gut microbiome; Dysbiosis; Endotoxins; Choline metabolism.
Abbreviations: LPL, lipoprotein lipase; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; SIBO, small intestinal bacterial overgrowth; TLRs, toll-like receptors; TNF, tumor necrosis factor.
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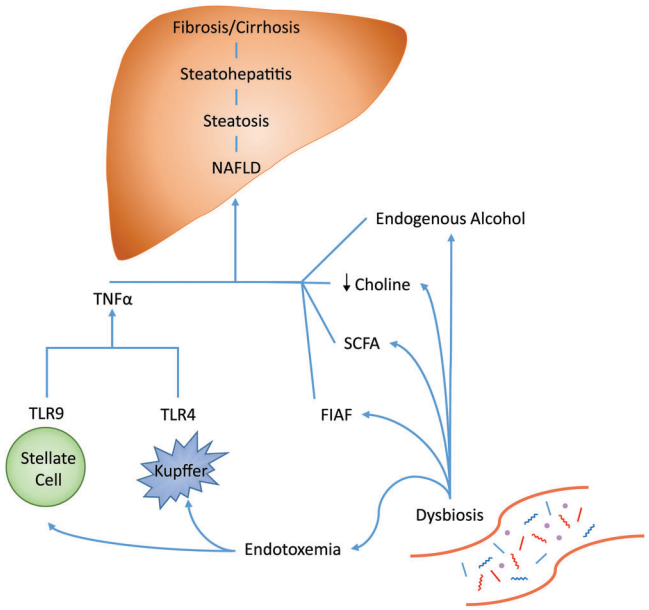

Fig. 1. Mechanisms of dysbiosis and NAFLD development. Dysbiosis in the gut results in increased levels of endogenous alcohol, SCFA, fasting-induced adipose factor (also known as Fiaf) and decreased production of the essential nutrient choline, all of which contribute to NAFLD, steatosis, steatohepatitis and ultimately fibrosis or cirrhosis as the process evolves. Dysbiosis also leads to endotoxemia by triggering Kupffer and stellate cells to active Toll-like receptors 9 and 4. This leads to the activation of TNF-α and ultimately NAFLD. Abbreviations: NAFLD, nonalcoholic fatty liver disease; SCFA, short chain fatty acids; TNF-α, tumor necrosis factor-alpha.

have shown a high level of bacteroidetes in the gut microbiota composition. Claesson *et al.*[14] analyzed the composition of intestinal microbiota by pyrosequencing and found that the phylum Bacteroidetes was the predominant species of intestinal microbiota, found in 68% of the individuals studied and accounting for 57% of bacteria in the microbiome. The phylum Firmicutes follows as a close second, accounting for up to 40% of bacteria in many individuals.[12] Other bacteria, including Proteobacteria, Actinobacteria, Faecalibacteria, Verrucomicrobia Cyanobacteria, and Lactobacillus are also found at lower levels in the human intestine.[12]

Microbial composition varies between cohabiting or related individuals. Studies in twins, that are born with a common gut microbiome revealed that the microbiota composition changes over time once a person is exposed to different environmental factors such as food, antibiotics, and infections.[15] Multiple studies have shown that obesity can change the composition of the common gut microbiome, leading to dysbiosis.[16] However, there is no consensus amongst studies about what the most common microbiota compositional changes are that occur in NALFD and obesity.

Studies have shown that bacteroides is seen at higher levels in lean individuals, whereas Actinobacteria and Firmicutes were found at higher levels in obese individuals.[11,17–20] This was supported in a study by Turnbaugh *et al.*,[18] that showed that the transplantation of intestinal bacteria from genetically obese mice to lean mice significantly enhanced the effectiveness of intestinal energy absorption and increased the body weight of the lean mice.[18] Similarly, analysis of the gut microbiome in NAFLD patients revealed a lower percentage of Bacteroides and higher levels of Prevotella and Porphyromonas species compared to lean controls.[11] Additionally, a change in the ratio between bacteroidetes to firmicutes is often noted in studies in both obese and NAFLD groups.[21]

A recent meta-analysis by Sze *et al.*[21] re-examined the relationship between gut microbiome and obesity and did not show a significant association between an increased ratio of bacteroides to firmicutes. They did not, however, comment on the relationship of gut microbial and NAFLD specifically.

Lipid metabolism and dysbiosis

Saturated fat accumulation has been found to induce hepatic steatosis by creating changes in the gut microbiome.[22] De Wit *et al.*,[22] studied the effect of dietary fat types in the development of obesity, insulin sensitivity, and liver triglyceride levels. Their findings suggest that a high-fat diet including palm oil, which is high in saturated fat, increases steatosis incidence, lowers microbial heterogeneity, and raises the Firmicutes-to-Bacteroidetes ratio, which we noted may be a marker for obesity.[22]

Table 1. Studies on gut microbiome and NAFLD

Type of study	Subjects	Outcome	Ref
Randomized control trial	38 patients total; 16 of the patients were NASH patients and 7 were given probiotic versus 9 usual care group versus 22 controls.	NASH patients had lower concentration of Faecalibacterium and Anaerosporobacter. There were higher concentrations of Parabacteroides and Allisonella.	11
Cross sectional study	63 children total; 16 controls, 25 obese patients and 22 NASH patients.	Proteobacteria/Enterobacteriaceae/Escherichia was equally represented in both healthy and obese microbiomes but was higher in NASH.	24
Cross sectional study	60 patients total; 30 NAFLD patients versus 30 controls.	Lactobacillus were higher in NAFLD patients.	19
Observational study	15 female patients were placed on choline diets and the levels were modified.	In patients with low-choline diet there were increased levels of Gammaproteobacteria and Erysipelotrichi bacteria, and fatty liver.	35
Randomized controlled trial	48 children with NAFLD were treated with VSL#3 versus placebo.	VSL#3 supplementation for a total of 4 months improved NAFLD in children.	12

In another study, Backhead *et al.*[15] demonstrated that microbiome transplantation from conventional mice (mice that obtained a microbiota beginning at birth) to germ-free mice (mice raised in the absence of any microorganisms) produced a 57% increase in total body fat content and a 2.3-fold increase in the content of liver triglycerides in germ-free mice. Real-time polymerase chain reaction assays confirmed that the colonization elevated the liver mRNA levels of two key enzymes for the fatty acid biosynthetic pathway—(acetyl-CoA carboxylase (also known as Acc1) and fatty acid synthase (also known as Fas).[15]

These lipogenic enzymes increase and activate microbiota by liver carbohydrate response element binding protein (also known as ChREBP) and sterol regulatory element binding protein-1 (also known as SREBP-1), leading to uptake of dietary polysaccharides.[4,9,15] These complex carbohydrates are not able to be digested by the gut bacteria and instead undergo fermentation to form inulin, acetate, propionate and butylate.[4,7,15] Butylate is used as a substrate by the bacteria in the intestinal mucosa. Acetate and propionate are energy substrates in lipogenesis.[7] They can enhance the host energy production in the intestinal tract, thus contributing to the development of obesity and metabolic disease.[7]

Multiple studies have also shown that gut microbiota promote intestinal absorption of monosaccharides, accelerating *de novo* hepatic lipogenesis and suppressing fasting-induced adipocyte factor, which results in the accumulation of triglycerides in adipocytes[16] and thus contributes to fatty liver development. A study on NASH patients by Vrieze *et al.*[23] showed that insulin sensitivity improved significantly after allogenic/autologous fecal transplant, along with levels of butyrate-producing intestinal microbiota.

Lipoprotein lipase (LPL) is a key regulator of the release of fatty acid from the adipocytes, and heart and muscle cells.[13] It has been demonstrated that gut bacteria enhance the storage of triglycerides by suppressing the intestinal expression of LPL inhibitor.[13] This suppression occurs through a decrease of the LPL inhibitor Fiaf, the fasting-induced adipose factor, which leads to the subsequent increase in LPL activity in adipocytes, thereby promoting an increase in hepatic lipogenesis and storage of calories supplied by the diet.[4,9] This further fuels the development of fatty liver disease by predisposing towards obesity.

Ethanol and NAFLD

Ethanol is a normal by-product of metabolism in the human body. The liver removes ethanol through liver alcohol dehydrogenases, catalases and the microsomal oxidizing system.[4] The alcohol hypothesis postulates that increased levels of endogenous ethanol are associated with increased oxidative stress, directly leading to activation of the inflammatory cascade which ultimately causes fatty liver and cirrhosis.[4] Zhu *et al.*[24] studied ethanol blood alcohol levels in NASH patients who had not consumed any ethanol-containing beverages and compared the findings to non-NASH patients. The NASH patients were found to have significantly higher levels of blood ethanol levels when compared to obese and lean patients who did not have NASH.[22]

Interestingly, it is commonly noted that liver biopsies from patients with NASH have similar histology to liver biopsies from patients with alcoholic liver disease, perhaps pointing to a common promoter as the cause of the liver changes. Additionally, increased ethanol levels have been shown to increase gut permeability, which leads to increased lipopolysaccharide levels.[4] The mechanism by which increased ethanol levels occur in NAFLD individuals is not fully understood. Some studies have shown that intestinal microflora are the major source of endogenous alcohol.[25] However, a single bacterial culprit has not been identified. *Escherichia coli* is thought to be the primary producer of ethanol, but multiple studies have not found high levels of *E. coli* in the microbiomes of NAFLD patients.[17]

Progression of NAFLD

NAFLD progression is presumed to result in part from interactions between the innate immune system and the gut bacteria. This may be due to a mechanism mediated by the inflammasome.[26] The inflammasome is a cytoplasmic, multiprotein complex that senses pathogenic bacteria and leads to the activation of an inflammatory response and cellular pyroptosis. Studies have shown that inflammasome deficiency is associated with negative changes in the composition of gut microbiota, and increased fat accumulation in the liver.[24,27]

Imajo *et al.*[20] used a rat model, with rats fed a high-fat diet to show that obesity induces increased leptin levels, which induces increased levels of multiple profibrogenic factors, such as leading to increased signaling in Kupffer cells. This ultimately results in enhanced response to low-dose lipopolysaccharides, a bacterial endotoxin, leading to the liver inflammation and fibrosis as seen in NASH.[20] It is therefore possible that a bacteria-mediated mechanism underlies the progression from simple fatty liver to NASH in humans as well.

Small intestinal bacterial overgrowth (SIBO) and the microbiome

Sebate *et al.*[28] showed a relationship between SIBO, obesity and hepatic steatosis. They measured SIBO by hydrogen breath test concentrations, as a surrogate marker for bacterial concentration, and compared the levels in obese and lean individuals. It was determined that the prevalence of bacterial overgrowth is higher in obese patients than in lean controls and is associated with hepatic steatosis.[26] In other studies, patients with SIBO had increased expression of TLR4 and increased release of interleukin-8, which is associated with NAFLD.[29] Bacterial overgrowth has also been shown to increase intestinal permeability and promoted bacterial translocation contribute to the progression of liver diseases.[30,31]

Fructose and the gut microbiome

Dietary fructose intake has also been associated with NAFLD.[4] Several studies in mice have shown that dietary fructose induces fatty liver and leads to activation of TLR4 and TLR8, which promote hepatic steatosis.[32] Animals fed with a high-fructose diet were found to have an increase in the number of macrophages and increased expression of Toll-like receptors. These factors increase the production of endotoxins by Gram-negative bacteria and lipopolysaccharides, thereby promoting inflammation and hepatic fibrosis.[4] This was mainly exhibited in patients with intestinal inflammation or portal hypertension.[4]

Choline deficiency

The intestinal microbiome also plays a role in the regulation of dietary choline and the anaerobic degradation of choline.[33]

Low-choline diets have been shown to be associated with NAFLD development.[30] Choline levels appear to affect the gut microbiota composition. Choline deficiency leads to an increase in the levels of the Gram-negative bacteria Gammaproteobacteria and Erysipelotrichi. The presence of these species has been associated with increases in fat content in the liver.[32] Furthermore, as we noted before, Gram-negative bacteria contain lipopolysaccharides which promote chronic inflammation, metabolic dysfunction, insulin resistance, and diabetes.[13,34]

In one study, mice that were bred to have a genetic predisposition to insulin resistance and NAFLD were fed a high-fat diet. This led to a change in the gut microbiota and in lower levels of choline.[32] Spencer *et al.*[35] demonstrated that a similar relationship between fatty liver and choline exists in humans. They observed 15 women in the hospital who were fed a low-choline diet for 42 days. Sequencing of their gut microbiota during the diet identified two classes of bacteria, Firmicutes/Erysipelotrichi and Proteobacteria/Gammaproteobacteri, both of which were associated with decreased choline production. Importantly, the study subjects were found to have increased hepatic steatosis at the end of the study.[35]

NAFLD prevention

The current gold standard for the management of NAFLD is weight loss and minimizing metabolic risk factors.[19] Data suggest that diet is the most important intervention to target NAFLD and NASH.[19] A reduction in body weight of only 5% can improve steatosis and a reduction of 10% can improve steatohepatitis.[36] However, many patients struggle to adhere to the recommended lifestyle modifications.[1] Many interventions have been studied which could be beneficial for the management of NAFLD[37] Weight loss medications, such as ursodeoxycholic acid, thiazolidinedione, polyunsaturated fatty acids, metformin, lipid-lowering drugs and the antioxidant vitamins E and C treatments, have been assessed as treatment options for the management of NAFLD.[1] However, the majority of the trials have been of short duration and did not include histological endpoints.[1]

The PIVENS trial was a large randomized controlled trial of vitamin E versus pioglitazone (a diabetic medication) for 96 weeks in nondiabetic NASH patients.[38] A significant improvement in hepatic steatosis and lobular inflammation was demonstrated by both treatments, but neither had a significant effect on hepatic fibrosis scores.[38] Guidelines suggest that in nondiabetic patients with biopsy proven NASH, the use of vitamin E is reasonable at a dose of 800 IU/day despite the reported inability to observe an effect on hepatic fibrosis scores.[1,38] However, until further data supports its effectiveness, vitamin E should not be considered a standard treatment of NASH in diabetic patients, NAFLD without liver biopsy, NASH cirrhosis, or cryptogenic cirrhosis.[1,38] Larger randomized controlled trials focusing on histological endpoints are necessary to precisely assess the efficacy of current treatment options for NASH.[1]

The integrity of the gut microbiota depends in part on diet.[39] "Plant-based diets" rich in vegetables, grains, fruits, and legumes, compared to "animal-based diets" composed of cheese, egg and meats, have demonstrated significant effects on gut bacteria composition.[39] An animal-based diet promotes gut microbiota to increase the production of deoxycholic acid, a precursor to bile acids, and branched-chain fatty acids. Also, the expression of genes for the degradation of polycyclic aromatic hydrocarbons (which are carcinogenic compounds), vitamin biosynthesis and b-lactamase are increased in individuals on an animal-based diet.[39] Fructose and saturated fat are more likely to stimulate hepatic lipid accumulation and progression to NASH.[39] In mice, high-fructose diet stimulates gut-derived portal endotoxemia, leading to liver steatosis through the activation of TLR4 and TNF-α.[39,40] Therefore, recommendations that include promoting diets low in saturated fats and fructose may protect against the development of NAFLD.

Supplements that are sold over the counter, such as probiotics (commensal bacteria in pill form) and prebiotics (chemicals that induce growth or activity of "good bacteria"), have been proposed as possible treatments for gut microbiome dysbiosis.[27] Several studies have evaluated probiotics and prebiotics in animal and human models, but due to the diversity of product manufacturing worldwide, there is no standardized dose nor a clear understanding of the treatment duration.[40] Additionally, there has not been sufficient studies specifically looking at prebiotics and probiotics and the development or treatment of NAFLD to make firm recommendations for or against their use.[41,42] Of the available products on the market, lactobacillus has been the best studied supplement in animal models.[43] One animal study found that after 8 weeks of oral administration of *Lactobacillus rhamnosus* there was a reduction in liver steatosis in diet-induced obese mice, confirmed by liver biopsies.[33] In a different study, mice fed with a high-fructose diet and then treated with *L. acidophilus* and *L. casei* showed a delayed onset of glucose intolerance, reduced insulin levels, and improved steatosis.[33] Therefore, further studies of Lactobacillus in human subjects is warranted.

Conclusions

NAFLD is a multifactorial disease that results from the interaction of genetic predisposition, and metabolic, inflammatory and environmental factors which influence the composition of the gut microbiome.[4,7] The microbiome composition can be modified by dietary intake, leading to gut microbiome dysbiosis, especially when these diets are rich in saturated fats, fructose, and animal-based foods. Studies have shown that lean and obese individuals have different types of bacteria in their microbiome.[4,7,8] This change seems to trigger the activation of inflammatory pathways that lead to progression of NAFLD.

A recent meta-analysis was published by Sze *et al.*,[36] which examined the relationship between gut microbiome and obesity, did not find an association between obesity and the presence or absence of bacteroidetes and firmicutes in the microbiome. This lack of an association underscores the necessity for further investigation into the relationship between the microbiome and NAFLD independent of obesity. There are many mechanisms that link the gut microbiome with the development of NAFLD which may offer potential targets for further investigation and novel therapeutic interventions, such as targeting the pathways involved with choline metabolism and inflammation.

Conflict of interest

Nikolaos Pyrsopoulos declares separate research grants from Gilead Sciences, Intercept, Shire, Genfit, Prometheus, Conatus. The other authors have no conflict of interests related to this publication.

Author contributions

All authors contributed equally to this paper in conception and design of the literature review and analysis as well as in

drafting, critical revision, editing, and final approval of the final version (LC, SE, TA, NP).

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