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REVIEW

Influence of gut microbiota on the development and progression of nonalcoholic steatohepatitis

Fabiana de Faria Ghetti[1,4] · Daiane Gonçalves Oliveira[1] · Juliano Machado de Oliveira[1] · Lincoln Eduardo Villela Vieira de Castro Ferreira[1] · Dionéia Evangelista Cesar[2] · Ana Paula Boroni Moreira[3]

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Abstract
Introduction Nonalcoholic steatohepatitis (NASH) is characterized by the presence of steatosis, inflammation, and ballooning degeneration of hepatocytes, with or without fibrosis. The prevalence of NASH has increased with the obesity epidemic, but its etiology is multifactorial. The current studies suggest the role of gut microbiota in the development and progression of NASH. The aim is to review the studies that investigate the relationship between gut microbiota and NASH. These review also discusses the pathophysiological mechanisms and the influence of diet on the gut–liver axis.
Result The available literature has proposed mechanisms for an association between gut microbiota and NASH, such as: modification energy homeostasis, lipopolysaccharides (LPS)–endotoxemia, increased endogenous production of ethanol, and alteration in the metabolism of bile acid and choline. There is evidence to suggest that NASH patients have a higher prevalence of bacterial overgrowth in the small intestine and changes in the composition of the gut microbiota. However, there is still a controversy regarding the microbiome profile in this population. The abundance of Bacteroidetes phylum may be increased, decreased, or unaltered in NASH patients. There is an increase in the *Escherichia* and *Bacteroides* genus. There is depletion of certain taxa, such as *Prevotella* and *Faecalibacterium*.
Conclusion Although few studies have evaluated the composition of the gut microbiota in patients with NASH, it is observed that these individuals have a distinct gut microbiota, compared to the control groups, which explains, at least in part, the genesis and progression of the disease through multiple mechanisms. Modulation of the gut microbiota through diet control offers new challenges for future studies.

Keywords Nonalcoholic fatty liver disease · Dysbiosis · Steatohepatitis · Gut microbiota · Microbiome

Introduction

Nonalcoholic steatohepatitis (NASH) is the most severe histological form of nonalcoholic fatty liver disease (NAFLD) [1], characterized by the presence of hepatic steatosis and inflammation, associated with ballooning degeneration, with or without fibrosis [2]. Even as the prevalence of NASH in the general population is approximately 2–5% [3, 4], around 70% of morbidly obese individuals are affected by this condition [5]. Although in most cases, NASH carriers present no symptoms, this condition may increase the risk of cirrhosis, liver failure, and hepatocellular carcinoma [6–9].

The exact cause of NASH is not yet clear, but studies have suggested the role of the gut microbiota in the pathogenesis of this disease [10–12]. In fact, it has been shown in animal models that gut microbiota increase intrahepatic fat through mechanisms associated with increased dietary energy extraction or change in lipogenesis and β-oxidation [10, 11]. Furthermore, hepatocellular inflammation may be secondary to increased intestinal permeability and translocation of

✉ Fabiana de Faria Ghetti
bia.ghetti@hotmail.com

[1] Universitary Hospital and School of Medicine, Federal University of Juiz de Fora, Juiz de Fora, Minas Gerais, Brazil

[2] Department of Biology, Federal University of Juiz de Fora, Juiz de Fora, Minas Gerais, Brazil

[3] Department of Nutrition, Federal University of Juiz de Fora, Juiz de Fora, Minas Gerais, Brazil

[4] Unidade de Nutrição Clínica, Hospital Universitário, Rua Catulo Breviglieri, s/n, Bairro Santa Catarina, Juiz de Fora, Minas Gerais CEP 36036-330, Brazil

microbial cell components to the circulation [12]. Finally, gut microbiota can contribute to fibrogenesis through activation of hepatic stellate cells [13].

Although animal experiments have associated gut microbiota with the histological components of NAFLD, there are few clinical studies with emphasis on the composition and functionality of the microbiome in NASH. There is evidence that NASH patients have a higher prevalence of small intestinal bacterial overgrowth (SIBO) [14, 15] and the lowest percentage of Bacteroidetes in their fecal content when compared to healthy subjects [16]. On the other hand, other studies have observed a higher abundance of Bacteroidetes in the gut microbiota of patients with NASH, compared to healthy controls [17, 18]. Thus, the results of the studies are still controversial regarding the microbiome profile in this population.

A detailed study of the composition of the gut microbiota and its metabolic functions can determine which microorganisms contribute to gut health maintenance and what changes can lead to the development of pathologies [19]. Therefore, the aim of this review is to highlight the studies that investigate the relationship between gut microbiota and NASH. Pathophysiological mechanisms and the influence of diet on the gut–liver axis are also discussed.

Development and progression of nonalcoholic steatohepatitis

Traditionally, the pathogenesis of NASH is explained by the hypothesis of "two hits" proposed by Day and James [20], in 1998. According to the authors, insulin resistance would be the first stimulus (the first "hit") that determines the accumulation of fat in hepatocytes, resulting in steatosis. The steatosis itself increases the sensitivity of the liver to the second "hit". The second "hit" would be the oxidative stress, which promotes liver injury, characterized by tissue lesions, inflammation, and fibrosis [20].

According to the hypothesis of the "two hits", insulin resistance promotes hepatic lipogenesis and lipolysis in the adipose tissue, increasing the amount of fatty acids released to the liver in the initial event. On a smaller scale, the availability of fatty acids in the liver may result from the transport mediated by lipoproteins after intestinal absorption of dietary fats [21]. Upon entering the hepatocytes, the free fatty acids are oxidized by mitochondria to generate energy or are esterified in triacylglycerols (TG), incorporated into very-low density lipoprotein (VLDL) particles, and exported from the liver to the peripheral tissues [22]. When free fatty acids in the hepatocytes exceed their metabolization and export capacity, they can cause hepatic steatosis [22]. The accumulation of fatty acids in the liver results in excessive increase in the production of reactive oxygen species (ROS) from the mitochondria [23]. In addition, peroxisomes and microsomal oxidation pathways are activated and generate more ROS, culminating in hepatic oxidative stress [24]. Oxidative stress appears to be responsible for initiating necroinflammation. The consequence of oxidative stress is hepatic lipid peroxidation in cell membranes and mitochondria, producing malondialdehyde and hydroxynonenal, resulting in mitochondrial dysfunction [25]. Malondialdehyde activates the regulatory transcription factor and the expression of proinflammatory cytokines and adhesion molecules (NF-kB), stimulating production of the tumor necrosis factor alpha (TNF-α), interleukin 8 (IL-8), and selectin E [26]. Hydroxynonenal activates hepatic stellate cells, promoting collagen deposition, and hence, the development of fibrosis [27]. Thus, ROS, lipid peroxidation products, and cytokines are involved in the second hit, which induces the progression of simple steatosis to NASH.

At present, it is believed that the process of "two hits" is insufficient to explain the pathogenesis of this heterogeneous disease, particularly in non-obese individuals [28]. Furthermore, simple hepatic steatosis, which is benign and nonprogressive in a majority of patients, and NASH, may reflect different pathogenesis [29]. In fact, the accumulation of free fatty acids in the liver occurs mainly in the form of TG [30]. There is evidence to indicate that TG by themselves is not hepatotoxic, at least in mice (BKS.Cg-m/Leprdb/J) with steatohepatitis [30]. Therefore, TG synthesis seems to be an adaptive, beneficial response in situations, where hepatocytes are exposed to potentially toxic TG metabolites [30]. On the contrary, several other lipids, such as free fatty acids, diacylglycerol, cholesterol, ceramide, and phospholipids, also accumulate in the liver, and they are considered as ''aggressive'' lipids [31], that induce endoplasmic reticulum (ER) stress, mitochondrial dysfunction, and oxidative stress, resulting in hepatic inflammation and fibrogenesis [32, 33].

In this sense, a new theory was proposed by Tilg et al. [31]: the hypothesis of "multiple parallel hits". This hypothesis proposes that several concurrent and not consecutive stimuli ("multiple parallel hits") induce oxidative stress, which results in both hepatic steatosis and steatohepatitis. Even as the two "hits" hypothesis suggests that steatosis always precedes inflammation, the "multiple parallel hits" hypothesis indicates that inflammation in NASH may precede steatosis in some cases [31]. In this new paradigm, NASH results not only from oxidative stress, but also from the interaction between different "hits", including altered lipid metabolism, mitochondrial dysfunction, ER stress, genetic predisposition, and gut microbiota alterations [31]. It is increasingly recognized that the gut microbiota is implicated in the pathogenesis and progression of NASH [34]. Thus, the gut microbiota has become a topic of interest in recent investigations and a potential target of intervention [35, 36].

Gut microbiota

The human gut contains a large number of microorganisms, mostly bacteria, collectively called gut microbiota [37]. The latest estimate for the total number of bacteria cells in the body is around 40 trillion (3.8×10^{13}), the same order as the number of human cells (3.0×10^{13}), and their total mass is about 0.2 kg [38]. More than 1000 cultured gastrointestinal species have been identified in the human microbiota [39]. Recent advances in molecular techniques, sequencing and bioinformatic programs, have allowed the identification of specific taxonomic groups, that is, phyla, classes, orders, families, genera, and bacterial species [40]. Currently, metagenomic techniques have been used to characterize the composition, diversity, and potential physiological effects of entire microbial communities, without cultivation and isolation of the members of the community [41].

The Firmicutes and Bacteroidetes are the most prevalent phyla in adults, followed by Actinobacteria, Proteobacteria, Fusobacteria, Spirochaetae, and Verrucomicrobia [42]. The stomach and small intestine are rich in Firmicutes (Lactobacillaceae) and Proteobacteria (Enterobacteriaceae), whereas the large intestine shows higher counts of Bacteroidetes (Bacteroidaceae, Prevotellaceae, and Rikenellaceae) and Firmicutes (Lachnospiraceae and Ruminococcaceae) [43]. The composition of adult microbiota remains relatively stable, although the microbial diversity is acquired within the first hours post birth, and is shaped over time as the diet becomes more complex and the immune-system matures [44]. A combination of multiple factors, including genetic and environmental characteristics (type of delivery, antibiotic therapy, diet composition, lifestyle, social interactions, and exposure to various xenobiotics) shapes the gut microbiota to make every individual microbially unique [45]. The gut microbiota can also be very dynamic and change rapidly, for example, in response to dietary changes. One study shows that an increase in caloric intake from 2400 to 3400 kcal/day (with a similar nutrient profile that includes 24% protein, 16% fat, and 60% carbohydrates) over 3 days increases Firmicutes ratio and decreases the ratio of Bacteroidetes [10]. Diet is a major factor driving the composition and metabolism of the gut microbiota [40] and the influence of the diet on gut microbiota will be described in more detail in the next sessions.

Nowadays, the gut microbiota is considered a metabolic organ, which performs a wide range of functions including an important role in the physiology of energy homeostasis [46]. For example, some members of the Firmicutes phylum are among the butyrate-producing bacteria that increase the energy extraction from the diet [47]. In contrast, members of the phylum Bacteroidetes participate in carbohydrate metabolism and accomplish this by expressing enzymes similar to glycosyl transferases, glycoside hydrolases, and polysaccharide lyases [48]. In this context, the knowledge of the composition and functions associated with the microbial community is fundamental, as alterations in the composition of the gut microbiota and/or its functions (called 'dysbiosis') are associated with metabolic diseases, such as NASH [17, 18, 49]. Although human studies are scarce in the literature (Table 1), animal experiments support the link between gut microbiota and the development of NASH.

Dysbiosis and nonalcoholic steatohepatitis

Experimental data

Animal experiments have demonstrated direct roles for gut microbiota in the development and progression of nonalcoholic steatohepatitis (NASH). Using germ-free C57BL/6J mice, Bäckhead et al. [50] have previously demonstrated that mice devoid of gut microbiota are resistant to diet-induced obesity, steatosis, and insulin resistance. Subsequently, Le Roy et al. [51], using the transplantation experiment, have shown that differences in microbiota composition can determine responses to a high-fat diet (HFD) in mice and contribute to the development of steatosis, independent of obesity. In this study, the conventional C57BL/6J mice, fed with an HFD, have generally displayed liver steatosis, hyperglycemia, and systemic inflammation (called the 'responders'), but some mice are nonresponders, normoglycemic, and have a lower level of systemic inflammation, with the same diet. Germ-free mice have been colonized with gut microbiota from either the responders or the nonresponders and then fed the same HFD. Despite a similar weight gain, responder–receiver mice have been found to develop a higher level of liver steatosis, glycemia, and insulin resistance than nonresponder–receivers. Pyrosequencing of the 16S ribosomal RNA genes has revealed that responder and nonresponder mice have distinct gut microbiota including differences at the phylum, genera, and species levels. Responder mice harbour a significantly increased number of sequences belonging to the Firmicutes phylum, *Barnesiella*, *Roseburia* genera, *Lachnospiraceae bacterium* 609, and *Barnesiella intestinihominis* species [51].

Barnesiella intestinihominis, that is part of the Porphyromonadaceae family, in particular, showed an increase in inflammasome-deficient mice (C57Bl/6) associated with the progression of NASH. It was revealed that the nucleotide-binding domain, leucine-rich repeat protein (NLRP) 6, NLRP3 inflammasomes, and the effector protein IL-18 negatively regulated the exacerbated hepatic steatosis and inflammation via modulation of the gut microbiota. Antibiotic treatment with ciprofloxacin and metronidazole reduced the severity of NASH in inflammasome-deficient mice and abolished transmission of the phenotype to wild-type

Table 1 Human studies that have evaluated the gut microbiota in nonalcoholic steatohepatitis

References	Study	Sample	Method	Results and *p* value
Boursier et al. [49]	Cross-sectional	Adult NASH ($n = 35$), obese no-NASH ($n = 22$)	16S rRNA	↑*Bacteroides* and ↓*Prevotella* in NASH vs. obese no-NASH ($p = 0.013$ e $p = 0.053$, respectively)
Del Chierico et al. [69]	Cross-sectional	Children NASH ($n = 26$), healthy controls ($n = 54$)	16S rRNA	↑*Ruminococcus*, *Blautia*, *Dorea* ↓*Oscillospira* in NASH vs. healthy controls ($p < 0.05$)
Zhu et al. [18]	Cross-sectional	Children NASH ($n = 22$), obesity ($n = 25$) and healthy controls ($n = 16$)	16S rRNA	↑Bacteroidetes and ↓Firmicutes in NASH vs. healthy controls ($p = 0.009$ e $p = 0.002$, respectively) ↑Proteobacteria, Enterobacteriaceae and *Escherichia* in NASH vs. healthy controls and obesity ($p = 0.0007$)
Wong et al. [17]	Longitudinal (6 months)	Adult NASH ($n = 16$) and healthy control ($n = 22$)	16S rRNA	↓*Faecalibacterium* and *Anaerosporobacter* in NASH vs. healthy controls ($p < 0.05$) ↑*Parabacteroides* and *Allisonella* in NASH vs. healthy control ($p < 0.05$) ↓Firmicutes in NASH vs. healthy controls ($p < 0.05$)
Mouzaki et al. [16]	Cross-sectional	Adult NASH ($n = 22$), SS ($n = 11$) and healthy controls ($n = 17$)	qRT-PCR	↓Bacteroidetes in NASH vs. SS and healthy controls ($p = 0.006$)
Shanab et al. [60]	Cross-sectional	Adult NASH ($n = 18$) and healthy controls ($n = 16$)	Lactulose breath hydrogen test	SIBO in NASH (77.78%) vs. SIBO in healthy controls (31.25%) ($p < 0.0001$)
Wigg et al. [14]	Cross-sectional	Adult NASH ($n = 22$) and healthy controls ($n = 23$)	Combined xylose and lactulose breath test	SIBO in NASH (50%) vs. SIBO in healthy controls (22%) ($p = 0.048$)

↑, increase; ↓, decrease; 16S rRNA, 16S ribosomal RNA sequencing; NASH, nonalcoholic steatohepatitis; qRT-PCR, quantitative real-time polymerase chain reaction; SS, simple steatosis

animals, showing that gut microbiota drove NASH progression in this model [52].

Progression of NASH was also strictly related to reduced microbial diversity and an increased ratio of Firmicutes to Bacteroidetes in model C57BL/6J mice [53]. The abundance of *Bacteroides* spp., *Bacteroides vulgatus*, *Desulfovibrio* spp., *Atopobium* spp., *Clostridium cocleatum*, and *Clostridium xylanolyticumin* was increased in these animals and positively correlated with the increased levels of lipopolysaccharides (LPS) [53], an endotoxin present on the cell surface of Gram-negative bacteria, which induced inflammation [54]. The authors also observed a reduction in the abundance of gut barrier-protecting bacteria, such as the *Lactobacillus* spp. [53]. Another study with rats also showed that during the progression of NASH, the levels of LPS were highly increased. In addition, an increase was found in *Escherichia coli* and *Enterococcus* as well as a decrease was seen in *Lactobacillus*, *Bifidobacteria,* and *Bacteroide* [36]. Based on the connection between the intestine and liver, also termed 'gut–liver axis', the gut microbiota and their metabolic by-products may influence liver pathology [36].

Dysbiosis could also promote liver fibrogenesis. Indeed, C57BL/6 mice fed an HFD developed more severe liver fibrosis than control mice that were fed a standard chow diet, by changes in gut microbiota, activating an inflammasome cascade [55]. HFD-related increases in liver fibrosis were associated with an increase in the percentage of Gram-negative (mainly Proteobacteria) versus Gram-positive bacteria (mainly reduction in Erysipelotrichaceae and a complete disappearance of Bifidobacteriaceae) and a reduced ratio between Bacteroidetes and Firmicutes [55]. Bifidobacteriaceae (Firmicutes) was known to exert a protective role during liver injury [56, 57], whereas Proteobacteria was considered the main pathogen bacteria, expressing endotoxins [58]. Thus, the outcome suggested that dietary habits, by increasing the percentage of intestinal Gram-negative

endotoxin producers, might accelerate liver fibrogenesis, introducing dysbiosis as a cofactor that contributed to chronic liver injury in NASH [55].

Human data

The first report, on humans, of the relationship between gut microbiota and pathogenesis of NASH was published by Drenick et al. [59]. In this study, patients undergoing intestinal bypass developed parallel NASH and SIBO. After being treated with antibiotics, the patients showed regression of hepatic steatosis, suggesting that microbiota were the possible cause of NASH [59]. Subsequently, other studies investigated gut microbiota in patients with NASH (Table 1). Wigg et al. [14] observed small intestinal bacterial overgrowth (SIBO) in 50% of the patients with NASH and in 22% of the control subjects ($p = 0.048$). Shanab et al. [60] also observed a higher prevalence of SIBO in the NASH group, compared to the control group (77 vs. 31%).

SIBO may be characterized by an increase in the number of bacteria in the proximal small intestine ($\geq 10^5$ colony-forming units/mL of intestinal content) or a change in microbial composition, with a profile that is typical of the microorganism species that colonize the large intestine [61]. Although the "gold standard" for the diagnosis of SIBO is still thought to be jejunal aspiration and culture, this technique requires intestinal intubation that may not be well tolerated and may not detect the un-culturable species [62]. To investigate the possible presence of SIBO, all the studies in patients with NASH used the breath test (Table 1), because it provides the simplest noninvasive and widely available diagnostic modality for suspected SIBO, by determination of hydrogen and/or methane concentration produced by intestinal bacterial metabolism in the exhaled air [63]. However, there is a lack of consensus on the breath test interpretation [64]. Studies seeking to validate breath testing have calculated sensitivities and specificities ranging from 31 to 77 and 44 to 100%, respectively [65, 66], leading to high false-positive rates [67]. In addition, a variety of test methods and diagnostic criteria are used in studies and they are not standardized to define a positive test for SIBO. These factors have led to a controversy regarding the diagnostic utility of breath testing in SIBO [64].

Many microbial studies have focused on the fecal microbiota. It is important to highlight the major drawback of the use of stool analyses. It is the fact that a fecal sample does not reflect the microbiota composition from the small intestine, because it represents mainly fecal samples from the end of the colon [68]. Therefore, studies with NASH patients show changes in the composition of the fecal microbiota, but there is controversy regarding the profile of resident bacteria in the gut. For example, Mouzaki et al. [16] show a low percentage of Bacteroidetes (Bacteroidetes to total bacteria counts) and no differences in Firmicutes, in the stool samples of NASH patients. Instead, two studies [17, 18] have observed an increase in Bacteroidetes and decrease in Firmicutes in NASH patients, compared to the healthy controls. There is recent evidence that shows that the abundance of Bacteroidetes and Firmicutes is similar between NASH and no-NASH patients [49].

Other changes in the gut microbiota are related to NASH (Table 1). Recent evidence shows that the percentage of *Bacteroides* genus, one of the most important genera within the Bacteroidetes phylum, is significantly increased in NASH, whereas the percentage of the *Prevotella* genus is decreased [49]. In addition, pediatric NASH patients have a lower fecal abundance of *Faecalibacterium* and *Anaerosporobacter*, but higher abundance of *Parabacteroides* and *Allisonella*. A significant difference is observed at the phylum, family, and genera level in the fecal samples of children with NASH. Proteobacteria/Enterobacteriaceae/*Escherichia* are higher in NASH compared to healthy controls and obese patients [18]. Another study with pediatric patients shows a decrease of *Oscillospira* and increases of *Ruminococcus*, *Blautia*, and *Dorea* in NASH compared to the controls [69].

The variability of methods (qPCR vs. pyrosequencing), exclusion of all taxa with an abundance below 1% and profile of subjects (adults vs. children) may explain, in part, why there is still no consensus in the literature about which bacterial groups are increased or reduced in the gut of NASH patients, compared to no-NASH patients. In these studies, all NASH patients have a high body mass index (BMI) (>29 kg/m^2), and the BMI of NASH patients is significantly higher than that of healthy controls in two studies [16, 18]. As obesity itself is linked to gut microbiota composition changes [47, 70], BMI can be a major confounder [28]. Thus far, no study has directly assessed the gut microbiota composition in non-obese patients with NASH, but recent evidence has shown that non-obese patients with nonalcoholic fatty liver disease (NAFLD) have 20% more Bacteroidetes phylum and 24% fewer Firmicutes phylum, compared to healthy controls [28]. Future studies should include non-obese NASH patients in their analyses, to exclude the impact of obesity.

Although few studies have evaluated the composition of the gut microbiota in NASH patients, it was observed that these individuals have a distinct gut microbiota, compared to the healthy control groups, which explains, at least in part, the genesis and progression of the disease through multiple mechanisms.

Mechanistic pathways in the development and progression of NASH

The mechanisms involved in the relationship between gut microbiota and NASH are not yet fully known, but the

proposed mechanisms in the literature are described below (Fig. 1). The proposed mechanisms may potentiate each other through shared molecular pathways of fat accumulation, activation of inflammation, and fibrogenesis in the liver [71].

Modification of energy homeostasis

Energy harvest from the diet

The gut microbiota has the ability to extract energy from food via glycoside hydrolases and polysaccharide lyases, which are not encoded by the human genome (Fig. 1). Such enzymes in the colon metabolize undigested polysaccharides into monosaccharides and short chain fatty acids (SCFA) [50]. The monosaccharides produced by microbial fermentation are absorbed and transferred to the liver through portal circulation, where they activate factors like the carbohydrate-responsive element-binding protein (ChREBP), which increases the transcription of proteins involved in hepatic lipogenesis [72]. The SCFA (acetate, propionate, and butyrate) can be used for lipid or gluconeogenesis [73]. Thus, bacterial SCFA provide an additional source of energy for the body, promoting fatty liver accumulation [74].

The first investigation in this line of evidence has been conducted by Bäckhed et al. [11]. Colonization of C57BL/6 mice, germ-free (raised in absence of microorganisms), with cecal content from mice that were colonized with a normal microbiota at birth (termed 'conventionally raised') resulted in 60% of increased total body fat and consequently hepatic TG accumulation, without any increase in food consumption or energy expenditure [11]. Subsequently, Turnbaugh et al. [47] showed that the C57BL/6J obese mice had a higher concentration of SCFA and fewer calories in their stool, suggesting that in these animals, the microbiota contribute to the extraction of additional calories from their diet. These animals showed higher levels of Firmicutes than Bacteroidetes compared to their lean counterparts. The changes observed in obese mice microbiota could increase energy delivery to the liver and reduce fecal energy loss [47].

Although the experimental data indicate that the gut microbiota influence the energy balance, it remains uncertain as to what extent gut microbiota are an important regulator of nutrient absorption in humans. One clinical study showed that the total amount of SCFA and propionate were higher in the obese group than in the lean group [73]. However, another study found no difference in energy excretion in the stools and no difference in bacterial abundance between the obese and lean groups [10].

There is evidence that SCFA produced in the colon contribute to approximately 5–10% of the energy requirements [75]. It may be possible that the additional calories provided to the host by the microbiota, due to the fermentation of undigested dietary molecules, are not sufficient to induce significant changes in weight [76]. One of the arguments to support this hypothesis is that consumption of a high-fiber diet could increase SCFA production, which usually helps to reduce weight and adipose tissue [77, 78]. Studies with

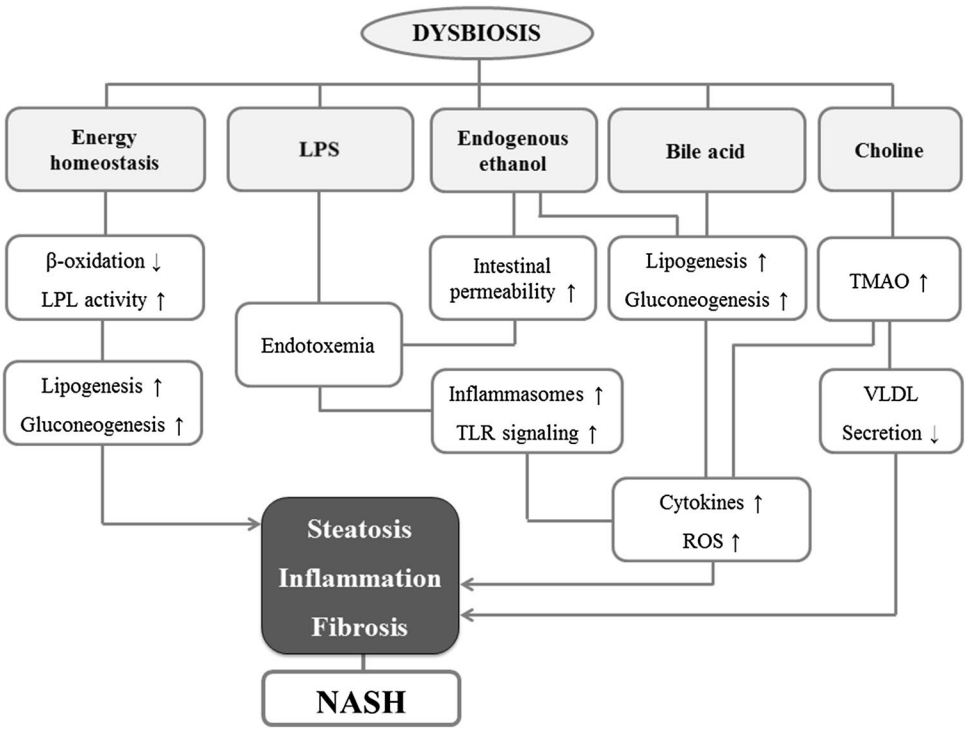

Fig. 1 Mechanisms proposed in the relationship between gut microbiota and nonalcoholic steatohepatitis. *LPL* lipoprotein lipase, *LPS* lipopolysaccharide, *NASH* nonalcoholic steatohepatitis, *ROS* reactive oxygen species, *TMAO* trimethylamine-*N*-oxide, *TLR* toll-like receptor, *VLDL* very-low density lipoprotein

prebiotics also have indicated that a higher intestinal production of SCFA is associated with an increase in satiety and a consequent reduction in dietary intake. These effects are in part related to the increase of glucagon-like peptides (GLP-1 and GLP-2) and peptide YY (PYY), which lead to hypothalamic effects related to the reward mechanism [79, 80]. Thus, more mechanistic studies are required to understand the role of each of the SCFA on NASH.

Activation of G protein-coupled receptors

The SCFA act on the G protein-coupled receptors, such as Gpr41 and Gpr43, expressed in intestine and adipose tissues [81]. GPR41 and GPR43 have been renamed free fatty acid receptors FFA3 and FFA2, respectively [82], based on their responsiveness to SCFA. There is a power order of SCFA in activating human FFA2 and FFA3 receptors, where FFA2 is activated more potently by acetate = propionate > butyrate, whereas for FFA3, it is propionate = butyrate > acetate [83].

The FFA3 activation stimulates enteroendocrine cells to increase production of the PYY, a hormone that reduces intestinal motility and provides greater absorption of nutrients, particularly of SCFA [84]. The FFA2 activation contributes to inhibition of lipolysis and adipocyte differentiation, leading to increased adipose tissue [85]. FFA2 is also present on intestinal neutrophils and might, therefore, contribute to NASH pathogenesis by increasing intestinal inflammation and permeability [86, 87].

Effects of adenosine 5′-monophosphate protein kinase and fasting-induced adipose factor

Experimental studies suggest that the presence of microbiota inhibits the enzyme adenosine 5′-monophosphate protein kinase (AMPK) pathway and suppresses intestinal expression of the protein fasting-induced adipose factor (FIAF) [11, 50]. AMPK is a key enzyme that controls the cellular energy status, which in turn activates the key enzymes of mitochondrial fatty acid oxidation, including acetyl-CoA carboxylase (ACC) and carnitine-palmitoyltransferase I (CTP1). When inhibited, the AMPK suppresses muscle oxidation of fatty acids, favoring adiposity [50]. FIAF is a circulating lipoprotein lipase (Lpl) inhibitor produced by the intestine, liver, and adipose tissue [88]. Inhibition of FIAF increases the activity of the lipoprotein lipase, leading to fat accumulation in the adipose tissue and increases hepatic uptake of free fatty acids [11]. Inhibition of FIAF further decreases expression of the peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC1-α) and enzymes involved in mitochondrial fatty acid oxidation [50]. Together, these effects may increase insulin resistance, resulting in obesity and hepatic steatosis (Fig. 1) [11, 50].

Lipopolysaccharide–endotoxemia

It has been proposed that changes in the gut microbiota favor an increase in circulatory lipopolysaccharides (LPS), particularly when the diets are rich in fat and energy [89, 90]. Increased circulatory LPS may contribute to metabolic endotoxemia (low-grade inflammation) [91], which plays a pivotal role in the development and progression of NASH (Fig. 1) [90]. LPS is recognized by pattern recognition receptors. These receptors include membranous toll-like receptors (TLRs) (especially TLR-4) and intracellular NOD-like receptors (NLRP3 and NLRP6 inflammasomes). Stimulation of TLR-4 results in the activation of several different intracellular signaling cascades, inducing the synthesis of a variety of inflammatory cytokines (especially TNF-α), which induce inflammation, oxidative stress, and insulin resistance [92]. Kupffer cells, which express the highest levels of TLR-4 liver, are cells that respond to LPS to produce cytokines and ROS [93]. The interaction between LPS and TLR-4 also activates receptors on stellate cells, resulting in hepatic fibrogenesis [15]. Increase in the levels of LPS also leads to liver injury through a mechanism mediated by the inflammasome, which includes NLRPs, a group of cytoplasmatic and multiprotein complexes [94]. NLRPs manipulate the cleavage of proinflammatory interleukins (ILs), such as pro-IL-1β and pro-IL-18 [94]. Henao-Mejia et al. [52] experimentally revealed that the NLRP6 and NLRP3 inflammasome alterations or IL-18 deficiency cause intestinal microbial changes by enhancing portal influx of TLR-4 and TLR-9 ligands, which in turn increase hepatic TNF-α production in C57Bl/6 mice. Apart from LPS, TNF-α, ILs, and plasminogen activator inhibitor-1 (PAI-1) may represent a good marker of NASH. The increased portal endotoxemia could induce the expression of PAI-1, a fibrinolysis inhibitor [95]. Elevated PAI-1 has been correlated with enhanced LPS-induced liver damage and induction of liver inflammatory response [96, 97].

Several studies conducted on animals and humans support the link between LPS–endotoxemia and the development of NASH [54, 98–100]. An animal study demonstrated that chronic infusion of LPS caused endotoxemia, hepatic steatosis, and changed gut microbiota in HFD C57bl6/J mice [54]. Genetically obese mice exhibit increased sensitivity to endotoxin hepatotoxicity, quickly developing steatohepatitis after exposure to low doses of LPS [99]. In addition, intraperitoneal administration of LPS-augmented hepatic inflammation, apoptosis, and reactive substances in the methionine choline-deficient nutritional model of NASH/C57/BL6 mice [100]. In human studies, increased levels of endotoxin were found in NASH patients, as compared to healthy individuals [101, 102]. Similarly, it was reported that morbidly obese subjects have increased levels of LPS and

LPS-binding proteins (LBP), which correlate with a major liver expression of TNF-α and the presence of NASH [98].

Possible mechanisms for endotoxemia in patients with NASH include SIBO and disruption of the intestinal mucosa barrier integrity, which may lead to an increased intestinal permeability and excessive absorption of LPS, resulting in low-grade inflammation and hepatic fibrosis [15, 103]. This association is supported by a growing body of experimental and human data. Genetically obese C57BL/6J mice display enhanced intestinal permeability, leading to portal endotoxemia. Moreover, murine hepatic stellate cells isolated from the livers of the animals were more sensitive to LPS, developing a stronger inflammatory and fibrogenic phenotype [103]. In human studies, Miele et al. [15] have shown that patients with NAFLD have increased intestinal permeability, and this abnormality is related to the increased prevalence of SIBO and disrupt tight junctions compared to healthy adults. Another study has associated SIBO with the expression of TLR-4 and IL-8 in NASH patients [60]. Wigg et al. [14] have found increased prevalence of SIBO and elevated TNF-α levels in patients with NASH, but have found no difference in the intestinal permeability or serum endotoxin levels. Despite the negative result of this study, the authors have suggested that endotoxin may still be an important factor in the pathogenesis of NASH. Some possible explanations for the paradox in this study are underestimated endotoxin levels, due to a retrospective collection of endotoxins. Endotoxins bound to plasma proteins are not measured and systemic levels may not reflect portal endotoxins [14]. In addition, NASH patients may have significant susceptibility to gut leakiness, and gut leakiness may still be an important pathogenic factor in patients with NASH and 'normal' intestinal permeability [104].

Increased endogenous ethanol production

The hypothesis that endogenous ethanol contributes to the pathogenesis of NASH dates back from the Cope et al. study [105]. The authors have reported elevated alcohol concentration in the breath of obese mice and have demonstrated that breath alcohol concentration can be reduced by gut microbial intervention with antibiotics [105]. Human studies have reported increased endogenous ethanol in NASH. Nair et al. [106] have demonstrated that obese women with NASH have higher breath ethanol concentrations than healthy controls detected by gas chromatography. Another study has shown that pediatric NASH patients have higher plasma concentrations of ethanol when compared to healthy or obese individuals [18]. In addition, an increased expression of ethanol-metabolizing enzymes, alcohol dehydrogenase, catalase, and aldehyde dehydrogenase is seen in NASH liver [107]. In summary, these outcomes suggest that the microbiota of patients with NASH produce more ethanol, which induces the expression of ethanol-metabolizing enzymes in the liver [107].

The normal microbiota in the human large intestine is capable of producing and metabolizing ethanol [108]. It has been shown that under anaerobic conditions, the bacterial metabolism of pyruvate, produced during the breakdown of carbohydrates, generates acetaldehyde, which can then be further reduced to form ethanol [109]. This metabolic fate of carbohydrates is favored when there is intestinal overgrowth of bacteria or yeast [110] or if carbohydrates, particularly sugar (e.g., glucose, sucrose, and fructose), are consumed excessively [111].

Despite the lack of consistent NASH-related gut microbiota changes, the possible overgrowth of ethanol-producing bacteria may underlie an increase in the circulation of ethanol levels in NASH. Zhu et al. [18] have shown a higher *E. coli* rate in the NASH group, compared to groups without NASH. *E. coli* is a member of the Family of Enterobacteriaceae, which typically aerobically degrade carbohydrates by mixed acid fermentation [112]. Ethanol is one of the common-end products of this pathway [112]. Furthermore, it is possible to suggest that the intestinal overgrowth of other bacteria-producing ethanol or yeast (e.g., *Lactobacillus fermentum, Weissella confusa,* and *Saccharomyces cerevisiae*) explain the higher plasma concentration of ethanol in some NASH patients [113], mainly in those with rich-in-carbohydrate diets [114]. *L. fermentum* and *W. confusa* are both heterolactic organisms [115, 116]. Ethanol is one of the dominating metabolites of heterolactic intestinal microbes by mixed acid fermentation [115]. Finally, *S. cerevisiae*, typically for yeasts, metabolizes hexoses via ethanol fermentation, yielding just ethanol and carbon dioxide [117].

The intestinally derived ethanol may contribute to the pathogenesis of NASH (Fig. 1), because gut-derived ethanol can induce hepatic steatosis [118]. In addition, increases in the production of ethanol by gut microbiota may injure the intestinal barrier and promote increasing permeability and endotoxemia [105, 119, 120]. Consequently, tissues, including the liver, that are exposed to this blood flow are stimulated to produce cytokines, such as TNF-α [105] and ROS, causing liver injury [119].

Altered bile acid metabolism

Primary bile acid (BA) species (cholic and chenodeoxycholic acids) are synthesized and conjugated with glycine or taurine in the liver, stored in the gallbladder, and released into the duodenum until ingestion of a fat meal (Fig. 2) [121]. In the intestine, BA are metabolized by bacteria to more hydrophobic BA species, through 7α-dehydroxylation and/or deconjugation of hydrophilic groups, resulting in secondary BA species (deoxycholic

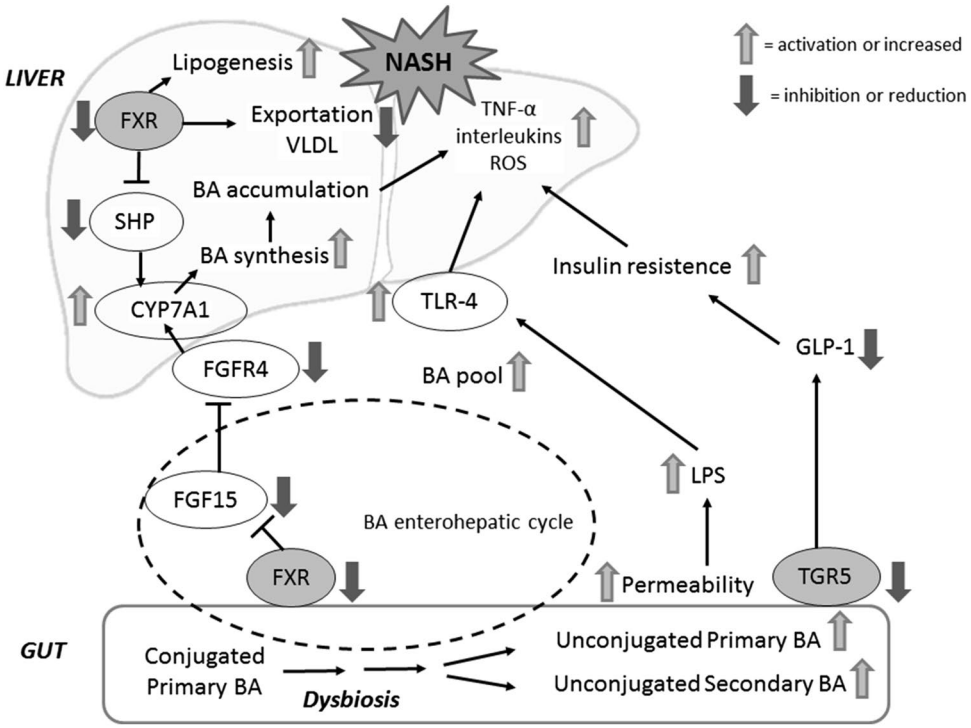

Fig. 2 Influence of gut microbiota in the development of nonalcoholic steatohepatitis by altering bile acid metabolism and its regulated signaling pathways. *BA* bile acid, *CYP7A1* the first enzyme of bile acid synthesis, *FGF15* fibroblast growth factor 15, *FGFR4* FGF receptor 4, *FXR* farsenoide X receptor; *GLP-1* glucagon-like peptide-1, *LPS* lipopolysaccharide, *NASH* nonalcoholic steatohepatitis, *ROS* reactive oxygen species, *TNF-α* tumor necrosis factor alpha, *TRG-5* G protein-coupled receptor, *TLR-4* Toll-like receptor 4, *SHP* short heterodimer partner, *VLDL* very-low density lipoprotein

and lithocholic acid) [122]. Over 95% of BA are reabsorbed in the distal ileum and then recycled via the portal vein into the liver [123].

In addition to promoting the absorption of fat, cholesterol, and fat-soluble vitamins in the intestinal tract, BA also act as signaling molecules that modulate a variety of physiological processes [124]. Regulatory actions of BA are mediated through specific BA-activated receptors, including the farnesoid X receptor (FXR), and members of the G protein-coupled receptor, mainly the TGR5 [125]. FXR, which is highly expressed in hepatocytes and enterocytes, is activated by free and conjugated primary BA [124]. FXR induces the expression of a short heterodimer partner (SHP), which inhibits CYP7A1 activation, the first enzyme of BA synthesis [121]. In the small intestine, FXR induces the fibroblast growth factor (human FGF19 and mouse FGF15), an intestinal hormone, to repress hepatic BA synthesis through FGF receptor 4 (FGFR4) expressed in the liver [125]. Activation of the FXR pathways not only regulates the synthesis and enterohepatic cycle of BA, but also acts on the control of hepatic de novo lipogenesis in the liver, exportation of TG by VLDL, and gluconeogenesis [126]. TGR5 is widely distributed and expressed in various tissues, including the intestines, enteroendocrine cells, and liver [121]. Activation of TGR5 by secondary BA induces intestinal glucagon-like peptide-1 (GLP-1) release from the intestinal enteroendocrine L cells and GLP-1-associated improvements in glucose tolerance and liver function [127]. Therefore, BA plays a crucial role in lipid and glucose homeostasis [126].

By altering BA metabolism and its regulated signaling pathways, gut microbiota could contribute to the pathogenesis of NASH (Figs. 1, 2) [128]. Although the precise mechanism is unknown, altered BA concentrations have been reported in patients with NASH [34, 129, 130]. Patients with NASH have higher fasting and postprandial total serum BA concentrations, including secondary BA, which tend to be a more hydrophobic and cytotoxic species [129]. In a similar study [130], total and secondary BA were increased in the liver tissues of NASH patients. This increase in BA concentration could be the consequence of a higher rate of BA synthesis or possibly be an adaptive response to the accumulation of TG in the liver [131]. A healthy liver is very efficient in removing BAs from the enterohepatic cycle. When the liver function is compromised, more BA appears in the circulation, because the liver is not adequately removing them [131]. Higher levels of serum 7α-hydroxy-4-cholesten-3-one (C4), a BA synthesis intermediate and a reliable marker of de novo BA synthesis, were also observed in NASH patients. C4 may represent the hepatic response to the increased fecal BA losses [35]. Indeed, higher fecal BA levels have been demonstrated in patients with NASH. In this study, higher levels of unconjugated primary BA in the stool correlated with dysbiosis [35].

Dysbiosis could substantially alter BA homeostasis [35], especially in the colon, where some bacteria, including *Bacteroides*, *Clostridium*, and *Escherichia,* are able to deconjugate and/or dehydroxylate BA, which may lead to an increase in the circulation of unconjugated secondary BA

species [129]. At high levels, BA are able to activate inflammatory and oxidative stress, resulting in apoptosis or necrosis, and eventually fibrosis and cirrhosis [132]. In contrast, the relative abundance of *Clostridium leptum* (*C. leptum* to total bacteria counts) is decreased in patients with NASH compared to the controls and correlates with higher cholic and chenodeoxycholic acids in the stool [35]. Higher levels of unconjugated primary BA in the stool are positively correlated with steatosis, ballooning, and fibrosis. These findings may represent the hepatotoxic impact of hydrophobic BA. BA can also contribute to the development of NASH through its effects on intestinal permeability [35]. BA has bactericidal activity and reduces the intestinal permeability to endotoxin [133]. However, increased deconjugation of BA reduces the bactericidal properties of the bile, causing growth of bacteria that promotes more deconjugation of BA, and ultimately, translocation and endotoxemia in homeostasis conditions [134]. The interplay between BA and gut microbiota in human NASH needs to be investigated further.

Altered choline metabolism

Choline is an essential nutrient and phospholipid component of cell membranes required for the formation of VLDL and exportation of liver lipids [135]. The metabolism of dietary choline by microbiota reduces the bioavailability of choline free to the secretion of VLDL, favoring the accumulation of fat in the liver (Fig. 1) [135, 136]. It is also known that enzymes produced by the gut microbiota catalyze the conversion of dietary choline into toxic methylamines, such as trimethylamine (TMA) [1]. TMA is subsequently oxidized in the liver, forming trimethylamine-*N*-oxide (TMAO), which induces liver inflammation. Moreover, TMAO may affect glucose and lipid metabolism, promoting the development of fatty liver [136]. TMAO increases fasting insulin levels and homeostasis model assessment-estimated insulin resistance (HOMA-IR) and also exacerbates the impaired glucose tolerance in HFD mice (C57BL/6). These effects are associated with the expression of genes related to the insulin signal pathway, glycogen synthesis, gluconeogenesis, and glucose transport in the liver [137]. The effects of TMAO on lipid metabolism involve the overexpression of flavin containing monooxygenase (FMO3) in the human hepatoma cell line, resulting in increased lipogenesis. These effects may be mediated through the peroxisome proliferator-activated receptor alpha (PPARα) and Kruppel-like factor 15 (KLF15) pathways [138].

A few studies have examined the association of choline and its metabolites with fatty liver disease. A choline-deficient diet greatly exacerbates a fatty liver induced by HFD consumption in C57Bl/6 mice [139]. Moreover, Pfp/Rag2 mice, submitted to a choline-deficient diet, develop a fatty liver featuring fibrosis and elevation of the proinflammatory markers serum amyloid A (SAA) and TNFα. Hepatic TG is significantly increased as well as alanine aminotransferase, demonstrating inflammation-linked hepatocyte damage [140]. Human studies have shown that the consumption of a low-choline diet promotes accumulation of TG in the liver and worsens fibrosis [141]. In a recent study, Chen et al. [136] have shown adverse associations between the circulating TMAO level and the presence and severity of NAFLD in Chinese adults, but no significant choline–NAFLD association has been observed. The composition of the gut microbiota can change with a low-choline diet. Healthy women during choline depletion show rate variations of *Erysipelotrichi* (Firmicutes) and *Gammaproteobacteria* (Proteobacteria) in their fecal contents, which are directly associated with changes in the liver fat [142]. The *Gammaproteobacteria* genera in particular, identified in their study, including *Klebsiella* spp., *Enterobacter* spp., and *Escherichia* spp., are gram-negative bacteria with LPS-containing membranes [142]. Therefore, the increase of circulating LPS can be one of the possible mechanisms involved in NASH development in choline-deficient patients.

On the basis of all these mechanistic pathways, it is possible to suggest that modulation of the gut microbiota, through strategies that can include diet, probiotics, antibiotics, or fecal microbiota transplantation, is a possibility for the treatment of NASH [143]. However, diet appears to be the simplest, most physiological, and most effective method to improve intestinal health [144].

Diet and gut microbiota

The diet is among the most easily controlled factors that can potentially manipulate the gut microbiota [145]. As discussed above, a high-energy diet, HFD, high-carbohydrate diet (mainly high-fructose diet), and decreased choline intake can alter the gut microbiota, which has been shown to be associated with NASH. Correcting dietary habits is typically part of the standard recommendations for NASH treatment [146]. However, we have not found studies in the literature that have investigated the effects of dietary habit modifications on the gut microbiota of NASH patients, without the use of probiotics and/or prebiotics. There is some evidence in animal models and no-NASH subjects that suggests that the amount of dietary calories and the balance between the three dietary macronutrients (fats, carbohydrates, and proteins) have the potential to improve the gut microbiota [147].

The impact of calorie restriction on gut microbiota has been demonstrated in C57BL/6J mice [148]. Calorie restriction enriches phylotypes correlated with probiotic effects, such as *Lactobacillus* and *Bifidobacterium*, and reduces phylotypes correlated with inflammation and obesity, such

as *Streptococcaceae* and *Desulfovibrionaceae*. These calorie restriction-induced changes in the gut microbiota are concomitant with significantly reduced serum levels of the LPS-binding protein, suggesting that animals under calorie restriction can establish a structurally balanced architecture of the gut microbiota that may exert a health benefit to the host via reduction of antigen load from the gut. In obese subjects, the reduction in food energy content decreases the phylum Firmicutes and increases Bacteroidetes [10, 149, 150]. Moreover, these calorie-restricted diets increase microbial gene richness in subjects with obesity [151] and normalize the circulating LPS levels [152].

The effects of dietary macronutrients on the human microbiota are still poorly understood. A number of studies focus on the impact of a "Western" diet (high in animal fat and protein and low in fiber), compared to a "non-Western" diet (low in animal fat and protein and high in fiber) [43, 153, 154]. Western and non-Western human diets are consistently associated with distinct gut microbial communities [43, 153, 154]. Amato et al. [154] observed elevated microbial richness and a relatively higher abundance of *Prevotella* in non-Western humans and a relatively elevated abundance of *Bacteroides* in Western humans. These results are concordant with a study of the human gut microbiota that associates diets high in protein and animal fat with high levels of *Bacteroides* and diets high in plant carbohydrates with high levels of *Prevotella* [155, 156]. Similarly, all published studies of Western and non-Western humans to date report higher microbial richness and a higher abundance of *Prevotella* in non-Western populations [43, 153, 154]. One study also shows a higher abundance of *Xylanibacter* [156]. The genera *Prevotella* and *Xylanibacter* are known to contain a set of bacterial genes for cellulose and xylan hydrolysis, completely lacking in the non-Western population. In addition, Enterobacteriaceae (*Shigella* and *Escherichia*) were significantly under-represented in non-Western populations compared to Western populations [156]. Moreover, a clinical trial has shown that healthy subjects with a prudent-style diet (20% of fat) for 1 month reduced plasma LPS levels by 38%, whereas a Western-style diet induced a 71% increase in plasma levels of endotoxin [157].

The composition of gut microbiota could also be influenced by the quality of dietary lipids. One experiment evaluated the effect of a fat-type diet, varying in polyunsaturated-to-saturated fatty acid ratios in the gut microbiota composition and hepatic TG accumulation [158]. C57Bl/6J mice were fed purified HFDs (45E% fat) containing palm oil (saturated lipids), olive oil (monounsaturated lipids), or safflower oil (polyunsaturated lipids) for 8 weeks. According to the authors, HFD containing palm oil induced a higher liver TG content, reduced microbial diversity, and increased the Firmicutes:Bacteroidetes ratio, whereas HFDs containing olive oil or safflower did not change the gut microbiota [157]. Similarly, Caesar et al. [159] fed mice isocaloric diets that differed only in fat composition (either lard or fish oil, which are rich in saturated and polyunsaturated lipids, respectively) to assess how the dietary fat sources affected the microbiota. This study showed that the genera *Bacteroides*, *Turicibacter*, and *Bilophila* had increased in lard-fed mice, while *Actinobacteria* (*Bifidobacterium* and *Adlercreutzia*), lactic acid bacteria (*Lactobacillus* and *Streptococcus*), *Verrucomicrobia* (*Akkermansia muciniphila*), *Alphaproteobacteria*, and *Deltaproteobacteria* had increased in fish-oil-fed mice. Moreover, TLR-4 was activated by serum from mice fed with lard, suggesting that a lard diet promotes an increase in the influx of microbial factors into the systemic circulation [159].

Regarding carbohydrate intake, there is evidence that low-carbohydrate diets can impair the gut microbiota, since they are also restricted in source foods, such as fruits, vegetables, and grains, which are rich in fiber (non-digestible carbohydrates) [160, 161]. In general, the consumption of fiber-rich diets is associated with greater richness and diversity of the gut microbiota, being positively associated with the presence of *Bacteroidetes* and *Actinobacteria* and with reduction in the Firmicutes:Bacteroides ratio [150]. Fiber-rich diets also promote a higher concentration of SCFA in the fecal contents, especially of butyrate, which has a beneficial effect on inflammation [162]. Thus, the replacement of fructose, and other simple carbohydrates, with non-digestible carbohydrates can avoid the consequences of dysbiosis in the gut–liver axis, especially in NASH.

Finally, the relationship between protein intake and gut microbiota, specifically in NASH patients, is lacking. The excess protein has been linked with potentially damaging effects on the gut microbiota and health. Hoodia et al. [163] have verified that a high-protein diet reducts *Faecalibacterium prausnitzii* and increases colon permeability and secretion of cytokines. Other evidence has reported high levels of *Clostridium* spp. and *Bacteroides* spp., with concurrent reductions in *Bifidobacterium* spp., *Roseburia* spp., and *Eubacterium* spp., in subjects who consumed a high-protein diet [161, 164, 165]. Reductions in *Bifidobacterium* spp., *Roseburia* spp., and *Eubacterium* may increase the risk of NASH, as these bacterial species are usually associated with butyrate production and control of endotoxemia [166]. It is important to mention that, generally, the highest proportion of dietary protein is accompanied by a reduction in the amount of carbohydrates. Therefore, it is possible that the impact of the consumption of high-protein diets on the gut microbiota is related not only to the production of toxic substances derived from protein fermentation, but also to the reduction of dietary carbohydrate consumption, especially of non-digestible carbohydrates [162]. Besides that, the source of protein varies between studies. For example, populations that consume meat-rich diets have higher fecal *Bacteroides*,

Bifidobacterium, *Peptococcus*, and anaerobic *Lactobacillus* species [167]. A vegetarian diet is associated with higher rates of *Bacteroides thetaiotaomicron*, *Clostridium clostridioforme*, and *F. prausnitzii* compared to an omnivorous diet [168].

Although the studies use different methods and varying manipulations of diets, which make comparability and generalization of the outcomes difficult, it seems clear that the various diets are important environmental factors that regulate and modify the gut microbiota.

Conclusions

The development and progression of NASH is a complex and multifactorial process, which cannot be completely explained by the "two hits" hypothesis. It is increasingly recognized that the gut microbiota is implicated in the pathogenesis and progression of NASH. There are evidences suggesting that NASH patients have a higher prevalence of bacterial overgrowth of the small intestine and changes in the composition of gut microbiota, but there is controversy regarding the profile of resident bacteria in the gut. An abundance of the Bacteroidetes phylum may be increased, decreased, or unaltered in NASH patients. There is an increase in the Enterobacteriaceae family (phylum Proteobacteria), especially *Escherichia*. Moreover, the *Bacteroides* genus (phylum Bacteroidetes) is also increased. There is depletion of certain taxa, such as *Prevotella*, *Fecalibacterium*, *Anaerosporobacter, Oscillospira, Ruminococcus*, *Blautia*, and *Dorea.* Although few studies have evaluated the gut microbiota in NASH patients, it was observed that these subjects have a distinct gut microbiota compared to the control groups, which explains, at least in part, the genesis and progression of the disease through multiple mechanisms.

Changes in the gut microbiota have consequences on energy homeostasis, resulting in hepatic steatosis. Dysbiosis is also responsible for increased intestinal permeability and metabolic endotoxemia, which correlate with inflammation and liver fibrosis. In addition, it is observed that the metabolism of other related pathways is affected by the gut microbiome in NASH, such as choline and bile acid metabolism and the endogenous production of ethanol. The role of LPS and bile acids in NASH pathogenesis has been discussed in several studies and they appear to be the most relevant factors in humans.

It is essential to identify strategies to modulate the gut microbiota and probably minimize the development and progression of NASH. Modulation of gut microbiota by diet control offers new challenges for future studies.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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