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Chapter

Intestinal Dysbiosis and Non-Alcoholic Fatty Liver Disease

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Abstract

Non-alcoholic fatty liver disease (NAFLD) affects 20–30% of the population, with an increased prevalence in industrialized regions. Some patients with NAFLD develop an inflammatory condition termed non-alcoholic steatohepatitis (NASH) that is characterized by hepatocellular injury, innate immune cell-mediated inflammation, and progressive liver fibrosis. In clinical practice, abdominal imaging, which reveals hepatic steatosis, is sufficient for NAFLD diagnosis if other diseases have been rejected. However, a liver biopsy is needed to differentiate NASH from simple steatosis. Therapeutic strategies used to treat obesity and metabolic syndrome improve NAFLD, but there is no specific treatment effective for NASH. The gut microbiota (GM) is composed of millions of microorganisms. Changes in the GM have a significant impact on host health. Intestinal dysbiosis is an imbalance in the GM that can induce increased permeability of the epithelial barrier, with migration of GM-derived mediators through portal vein to the liver. These mediators, such as lipopolysaccharides, short-chain fatty acids, bile acids (BAs), choline, and endogenous ethanol, seem to be involved in NAFLD pathogenesis. Given this evidence, it would be interesting to consider GM-derived mediator determination through omics techniques as a noninvasive diagnostic tool for NASH and to focus research on microbiota modulation as a possible treatment for NASH.

Keywords: non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, gut microbiota, intestinal dysbiosis, gut microbiota-derived mediators, noninvasive biomarker, therapeutic target

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is currently the most prevalent chronic liver disease worldwide [1]. A subset of NAFLD patients have the progressive form of NAFLD termed non-alcoholic steatohepatitis (NASH). NASH is typically characterized by a specific pattern on liver histology, including steatosis, lobular inflammation, and ballooning with or without perisinusoidal fibrosis [2]. It can progress to advanced fibrosis, cirrhosis, hepatocellular carcinoma, and liver-related morbidity and mortality. Liver disease is only the third leading cause of death in patients with NAFLD, following cardiovascular disease and malignancy [3].

Precise histological diagnosis of NAFLD is commonly based on liver biopsy [4]; however, biopsies present several potential problems [5]. Thus, there is a need

for reliable and cost-effective noninvasive biomarkers to avoid the invasiveness of biopsy [6].

Although there are some clinical strategies to ameliorate NAFLD progression, such as treatments for obesity or type 2 diabetes mellitus (T2DM), there is no medication proven to be effective as a treatment for NASH [7]. Therefore, it is necessary to improve the research on possible therapeutic targets for NASH due to the severity of this pathological condition.

Previous evidences have linked gut dysbiosis with obesity, insulin resistance (IR), metabolic syndrome (MS), and NAFLD [8, 9]. The impact of the GM on NAFLD/NASH has been attributed to increased gut permeability, intestinal endotoxemia, endogenous alcohol production, upregulation of hepatic de novo lipogenesis and triglyceride synthesis, reduction in choline metabolism, and aggravation of IR [10]. The increased permeability of the intestinal barrier results in the release of substances such as lipopolysaccharides (LPS), bacterial components, short-chain fatty acids (SCFAs), bile acids (BAs), choline metabolites, and endogenous ethanol that reach the liver and seem to contribute to the pathogenesis of NAFLD (**Figure 1**) [11, 12]. It is important to note that some of these substances could perhaps be employed as potential noninvasive biomarkers of NAFLD progression.

Manipulation of the microbiota through probiotics, prebiotics, and antibiotic treatment yields encouraging results for the treatment of obesity, T2DM, and NASH in animal models, but data in humans are scarce. In regard to NAFLD, this

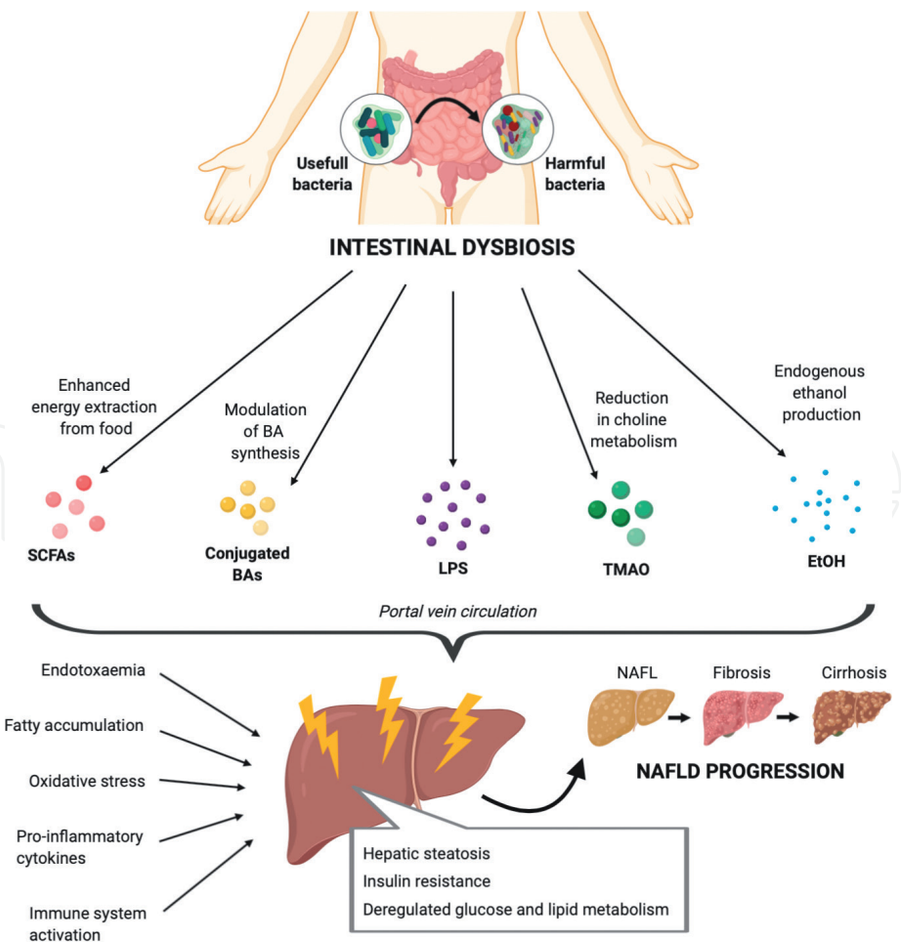

Figure 1.
Implication of intestinal dysbiosis in NAFLD pathogenesis. Short-chain fatty acids (SCFAs), bile acids (BAs), lipopolysaccharides (LPS), trimethylamine-N-oxide (TMAO), ethanol (EtOH), non-alcoholic fatty liver (NAFL), and non-alcoholic fatty liver disease (NAFLD).

therapeutic strategy seeks to prevent the endotoxicity produced by the microbiota-derived metabolites that reach the liver and promote the progression of the disease [13]. Thus, there is a need to focus research on the GM as a therapeutic target to ameliorate NASH.

To provide a broad overview of the relationship between intestinal dysbiosis and NAFLD, we have elaborated on this subject in this book chapter. In this sense, this narrative chapter will explain (a) non-alcoholic fatty liver disease, (b) the gut microbiota, (c) gut microbiota-derived mediators involved in NAFLD, and (d) the gut microbiota as a therapeutic target in NAFLD.

2. Non-alcoholic fatty liver disease

NAFLD has emerged as the most common form of chronic liver disease worldwide. The incidence of NAFLD has drastically increased in parallel with obesity in recent years. Currently, the global prevalence of NAFLD is approximately 25% [1], but it can increase to 58% in individuals who are overweight or as high as 98% in individuals with nondiabetic morbid obesity [14].

NAFLD comprises a spectrum of disorders extending from simple steatosis (SS) to NASH, fibrosis, and cirrhosis [2, 15]. This pathology has potentially serious sequelae [16]. Although SS tends to develop into a favorable clinical course [3], NASH can develop into liver cirrhosis and hepatocellular carcinoma [15]. Thus, liver-related mortality increases exponentially with an advance in the fibrosis stage [17]. In this regard, NASH is a very common cause of liver transplant worldwide [1]. Although the most common cause of death in patients with NAFLD is cardiovascular disease, independent of other metabolic comorbidities, NAFLD is becoming a major cause of liver disease-related morbidity (e.g., cirrhosis, end-stage liver disease, hepatocellular carcinoma, and liver transplantation).

NAFLD is characterized by significant lipid deposition in the hepatocytes of the liver parenchyma [18]. Obesity, T2DM, dyslipidemia, MS, and IR are the main risk factors for NAFLD [19]. Most NAFLD patients are asymptomatic, and the evidence of hepatic steatosis should be detected via a routine blood test, showing a deregulation in liver enzymes. Currently, it is not possible to diagnose NAFLD with only a blood test, but the aspartate aminotransferase (AST)-alanine aminotransferase ratio (ALT) can be used as a first step [20–22]. However, the ALT level correlation with histological findings has poor sensitivity and specificity for the diagnosis of NASH [23]. Then, it is necessary to rule out other causes of liver damage, such as alcoholic fatty liver disease, drug-induced liver injury, viral hepatitis, autoimmune liver disease, hemochromatosis, celiac disease, and Wilson's disease [1]. Finally, ultrasonography is the most common noninvasive tool used to detect NAFLD. There are also other imaging techniques used to detect liver steatosis, such as computer tomography or magnetic resonance imaging, but ultrasound is the technique that provides the most information without irradiation [24, 25].

One-third of the NAFLD-affected subjects progress to NASH. This condition is characterized by the presence of hepatocellular ballooning and inflammation and has a prevalence of 2–3% worldwide [2]. Key issues in NAFLD patients are the differentiation of NASH from SS and the identification of advanced hepatic fibrosis. To date, liver biopsy has been the *gold standard* for identifying these two critical end points but has well-known limitations, including invasiveness; rare but potentially life-threatening complications; poor tolerance; sampling variability; and cost. Furthermore, due to the epidemic proportion of individuals with NAFLD worldwide, liver biopsy evaluation is impractical, and noninvasive assessment for the diagnosis of NASH and fibrosis is needed [5]. NASH is confirmed when the hepatic

tissue shows the presence of perilobular inflammation, hepatocellular ballooning, Mallory's hyaline, and acidophil bodies with or without fibrosis. Although there are other noninvasive tests, such as the fatty liver index, NAFLD fibrosis score, and FibroMeter, and elastographic techniques, such as FibroScan, that can suggest the presence of NASH and detect fibrosis [15], a precise histological diagnosis of NASH is commonly based on liver biopsy [26]. The development of alternative noninvasive strategies has been an area of intensive research over the past decade and currently.

Regarding NAFLD therapeutics, all forms of treatment of metabolic disorders are able to modify liver damage. Diet and lifestyle modification and insulin-sensitizing agents appear to be promisingly effective against NAFLD progression. However, these approaches may not be effective in some patients. Many other drugs are currently being studied to establish treatments for NAFLD. At present, no accepted drug treatment for NASH has been stated [24]. In this sense, it is very important to improve the knowledge of NAFLD physiopathology. Actually, the underlying precise mechanisms of NAFLD pathogenesis have just begun to be understood. The classic "multiple hit" theory states that lipid accumulation initiates hepatic steatosis and subsequently triggers multiple insults acting together (hormones/adipokines from adipose tissue, inflammation, deregulated fat metabolism, lipotoxicity, oxidative stress, mitochondrial dysfunction, and genetic and epigenetic factors), ultimately inducing NASH and cirrhosis [27]. Progression to NASH is linked to systemic inflammation, and it is associated with other pathological processes, such as innate immunity alterations, endoplasmic-reticulum stress, toll-like receptor (TLR) signaling, mitochondrial dysfunction, and intestinal dysbiosis [6, 28–32]. Regarding this last process, approximately 70–75% of blood that reaches the liver comes from the portal vein circulation that communicates the liver with the intestine [33]. The liver is continually exposed to GM-derived mediators, including bacteria and bacterial components, such as LPS, promoting an inflammatory response that contributes to liver injury [13].

3. The gut microbiota

Millions of symbiotic microorganisms live on and within human beings and play an important role in human health and disease. Initial colonization occurs at the time of birth, and humans progressively acquire ~10^{14} bacterial cells at equilibrium, which remain for life [13].

The human microbiota, especially the GM, has even been considered to be an "essential organ," carrying approximately 150 times more genes than the human genome [34]. The GM is composed of an immense number of microorganisms (bacteria, viruses, and fungi) with several functions, such as host nutrition, bone mineralization, immune system regulation, xenobiotic metabolism, proliferation of intestinal cells, and protection against pathogens [35, 36]. This bacterial community is dominated by anaerobic bacteria and includes 500–1000 species [37]. *Firmicutes* and *Bacteroidetes* are the most important phyla among the intestinal bacteria, with a proportion of over 90% of the total community [38].

The duodenum and proximal jejunum normally contain small numbers of bacteria, usually lactobacilli and enterococci, which are facultative anaerobes. The distal ileum is a transition zone between sparse populations of aerobic bacteria of the proximal small intestine and very dense populations of anaerobic microorganisms in the large bowel. Occasional groups of bacteria can be found in low concentrations within the lumen of the small intestine. Bacteria do not form clusters, and the luminal contents are separated from the mucosa by a mucus layer [13].

The GM is specific to an individual and highly resilient to changes. However, it can be affected by several factors, intrinsic and extrinsic to the host, such as the subject's genetic makeup, dietary habits, antibiotic use, and environmental changes [13, 39, 40]. A disruption in the composition of the normal GM is known as intestinal dysbiosis [41, 42]. Generally, this process includes an unfavorable change in the bacterial composition, with a reduction in autochthonous bacteria and growth of others that prejudice host health [43].

3.1 Intestinal dysbiosis

Intestinal dysbiosis is a process that may adversely impact metabolism and produce immune responses, favoring NAFLD progression. Important studies on the relationship of the GM with obesity have identified profound changes in the composition and metabolic function of the GM in subjects with obesity. Moreover, these studies demonstrated that the GM interacts with host epithelial cells to indirectly control energy expenditure and storage and activate inflammatory responses in NASH pathogenesis [44]. Qualitative or quantitative imbalances in the GM might have serious health consequences for the host, including small intestinal bacterial overgrowth (SIBO) syndrome [13]. Due to gut dysbiosis, there is an elevated production of toxic bacterial components and metabolic mediators, which consequently accumulate in the intestine. In addition, an increase in intestinal permeability and further disruption of the epithelial barrier lead to the release of these GM-derived mediators [42], which could reach the liver through portal circulation, favoring hepatic inflammation and the development of NAFLD [45, 46]. After disruption of the gut epithelial barrier, the liver is exposed to microbial products and metabolites resulting from bacterial metabolism [47, 48]. In this sense, it has been demonstrated that patients with NAFLD have gut dysbiosis, gut epithelial barrier dysfunction, and increased translocation of bacterial components to the liver [49]. For this reason, mediators derived from gut dysbiosis might also be related to the pathogenesis of the disease. Several previous studies in clinical settings have associated intestinal dysbiosis with the occurrence of NAFLD [50–52] and with the progression to NASH [10, 53].

Among the various factors, dietary habits are considered to be most influential on the gut microbiome in subjects with obesity and NAFLD patients. It is well-known that a high-fat diet causes gut dysbiosis characterized by lowered species richness and changes in microbial composition, such as decreased *Bacteroidetes* and increased *Firmicutes* and *Proteobacteria* abundances [43]. On the other hand, *Prevotella*, a member of the phylum *Bacteroidetes*, is associated with plant-rich diets. *Prevotella*-dominated microbiotas have higher fiber utilizing capacity than *Bacteroides*-dominated microbiotas, producing higher amounts of SCFAs [54]. There are some studies that consider *Prevotella* to be a beneficial commensal bacterium [10, 55], but there are others that noted enriched fecal *Prevotella* in NASH or cirrhotic patients [56–58]. These contradictory results may be partly explained by the differences in populations, age, or NAFLD stages between the studies. In this sense, further studies on *Prevotella* should be directed to characterize properties at the species level and to evaluate these species in different stages of NAFLD.

GM-derived mediators resulting from intestinal dysbiosis could play a key role in NAFLD progression through several mechanisms: (1) enhanced energy extraction from food nutrients by formation of SCFAs; (2) modulation of BA synthesis, which is crucial for fat absorption and affects metabolism of glucose via farnesoid X receptor (FXR); (3) innate immune system activation by bacterial component translocation; (4) endogenous ethanol production; and (5) reduction in choline metabolism, which reduces efflux of very-low-density lipoprotein (VLDL) from hepatocytes, promoting inflammation. These mechanisms involve translocation

of these mediators, such as SCFAs, BAs, endogenous ethanol, and choline metabolites, which may be potentially evaluated as noninvasive blood markers of NAFLD progression [59].

4. Gut microbiota-derived mediators involved in NAFLD

4.1 Short-chain fatty acids

SCFAs are molecules with seven carbon atoms or less, for example, acetic, propionic, and butyric acids, that are produced by the gut bacterial fermentation of cellulose, xylans, resistant starch, or inulin since humans lack enzymes that digest fibers. These substances can strongly regulate host metabolism [60]. In general, these SCFAs have several effects on energy metabolism, the immune response, and adipose tissue expansion and act as signaling molecules between the GM and the host. SCFAs provide not only important sources of nutrients and energy for the intestinal epithelium but also serve as precursors for lipogenesis and gluconeogenesis [61, 62]. SCFAs can directly act as lipid precursors in the liver and mediate other effects as ligands for G protein-coupled receptors, specifically the subtypes GPR41 and GPR43 [59]. Experimental studies have demonstrated that these SCFAs can modulate regulatory T-cell expansion and enhance neutrophil chemotaxis, promoting inflammation in mouse models [63–66]. Furthermore, SCFAs modulate the production of several inflammatory cytokines, including tumor necrosis factor (TNF)-α, interleukin 2 (IL-2), interleukin 6 (IL-6), and interleukin 10 (IL-10) [67]. Recently, some studies found that high concentrations of intestinal SCFAs as a result of dysbiosis and their G-protein coupled receptors play an important role in NAFLD progression [68, 69]. Activation of GPR41 and GPR43 stimulates secretion of peptide-YY, inhibits gut motility, and slows intestinal transit. Therefore, nutrient absorption and energy capture from the diet increase and may promote hepatic lipogenesis [56, 70]. Additionally, activation of GPR41 and GPR43 induces secretion of glucagon-like peptide-1 (GLP-1), which activates genes in hepatocytes that regulate fatty acid β-oxidation and insulin sensitivity [56, 71], promoting NAFLD occurrence and progression. Furthermore, clinical studies have demonstrated SCFA enrichment in fecal samples of children and adults with NAFLD [72, 73].

However, other previously published studies have reported that SCFAs could be beneficial in the progression of NAFLD. In this regard, butyrate activates AMP-activated protein kinase (AMPK) in the liver and accelerates the assembly of tight junction proteins [74, 75], improving intestinal barrier dysfunction and reducing metabolic endotoxemia. In addition, butyrate is able to modulate regulatory T-cell activity, suppressing the immune response and reducing liver inflammation [76].

The close relationship between intestinal dysbiosis and SCFA production, according to the results of previous experimental and clinical studies, provides evidence of their potential use as markers of NAFLD progression. In this sense, in a recent study, we studied this possibility, but we failed to demonstrate any relationship between circulating SCFA levels and histological degrees of NAFLD in a cohort of patients with morbid obesity [6]. However, additional studies are necessary to accurately determine the specific role of SCFAs in NAFLD.

4.2 Bile acids

As previously mentioned, the gut-liver axis, which involves gut hormone release and the immune response, is essential to regulate systemic metabolism.

BAs participate in communication along this axis. They are steroid-derivative components of bile synthesized after cholesterol oxidation by enzymes present in hepatocytes, and they are involved in the absorption of lipids and vitamins in bile salt-dependent flow regulation. BAs participate in the digestion and solubilization of lipids and regulate hepatic glucose and inflammation [59, 60]. Moreover, they are capable of controlling their own synthesis through the activation of FXR [77, 78]. In addition, BAs act as signaling molecules that modulate several physiological processes, and GM dysbiosis can change BA pool characteristics through its effects on BA metabolism [78, 79].

The GM is a critical modulator of BA pool size and composition, and the process of dysbiosis could substantially alter concentrations of conjugated and/or secondary bile acids, as well as increase their synthesis.

Unmodified BAs, also called primary BAs (cholic acid (CA) and chenodeoxycholic acid (CDCA)), undergo a deconjugation process by GM components after reaching the colon and become secondary BAs, such as deoxycholic acid (DCA) and lithocholic acid (LCA); they can be transported again to the liver via the portal vein in a mechanism called "enterohepatic circulation." BAs prevent the overgrowth of bacteria in the gut to maintain gut homeostasis. This protective effect is mediated by their detergent properties and the activation of FXR, which protects the distal small intestine from bacterial proliferation. It is recognized that these circulating BAs, in addition to the abovementioned functions, can coordinate a wide number of pathways mediated by specific nuclear receptors (NRs) [60].

The increased intestinal permeability associated with BA modifications has been linked to metabolic endotoxemia, IR, and inflammatory cytokine release with enhanced proinflammatory signaling cascades, which are common findings in patients with NAFLD [59]. An increased level of BAs causes activation of the cell death pathway mediated by inflammatory and oxidative stress cascades in liver tissue [80, 81].

Regarding hepatic lipid metabolism, Watanabe et al. demonstrated that hepatic FXR activation mediated by BAs could induce the expression of the atypical NR small heterodimer partner (SHP), which promotes the inhibition of sterol-regulatory element-binding protein-1c (SREBP-1c), thus reducing hepatic synthesis of triglycerides. In addition, FXR can limit lipid accumulation in the liver by promoting fatty acid oxidation after the activation of peroxisome proliferator-activated receptor alpha (PPARα) and by the induction of plasma VLDL-triglyceride clearance [82–85]. FXR activation in the liver was also demonstrated to coordinate glucose homeostasis via the inhibition of gluconeogenesis and glycolysis. Interestingly, the activation of FXR in the intestine can generate crucial endocrine feedback regulation [86]. Experimental studies have demonstrated that intestinal dysbiosis can modulate the activity of FXR in the intestine, affecting lipid metabolism in the liver [4]. Specifically, FXR not only plays an important role in maintaining BA levels but also regulates glucose and lipid metabolism via different mechanisms, such as increasing insulin sensitivity, repressing hepatic gluconeogenic genes, and increasing hepatic glycogen synthesis [87, 88].

Previous investigations have demonstrated a BA level increase in the biological fluids of patients with NASH compared to that in the biological fluids of subjects with healthy livers and an evident association with intestinal dysbiosis [89–91]. Additionally, the levels of BAs have been correlated with histopathological features, such as the degree of hepatic steatosis, the presence of cellular ballooning, and the severity of fibrosis in patients with NASH [92]. These studies confirmed the disruption in BA homeostasis in NASH physiopathology [65] and the correlation of BAs with NASH severity parameters (portal inflammation, lobular inflammation, and hepatocyte ballooning) [93]. In children with NAFLD, changes in the

circulating BA profile have also been reported. Troisi et al. demonstrated that serum BA levels decrease in early NAFLD and increase during progression to fibrosis in obese children. These authors postulated that BAs may have value as a noninvasive biomarker in pediatric NAFLD progression [83, 94]. In a previous study by our research group, we found that FXR jejunal expression was lower in NASH patients than in normal liver (NL) subjects; in regard to BAs, we also found that levels of glycolic acid (GCA), a primary BA, and DCA, a secondary BA, were significantly higher in NAFLD patients than in NL subjects [6].

Considering the numerous published experimental and clinical studies associating gut dysbiosis, BAs and NAFLD, it is expected that BAs could be proposed as potential noninvasive markers of the disease. For example, Svegliati-Baroni et al. specifically proposed DCA and LCA, which can only be produced by bacterial fermentation [95].

4.3 Bacterial components

The liver is exposed to potentially harmful substances derived from the gut, considered pathogen-associated molecular patterns (PAMPs), that include translocated bacteria, LPS, bacterial DNA, bacterial RNA, and endotoxins, which are potent inducers of tissue inflammation [41, 96]. These PAMPs might contribute to the pathogenesis of NAFLD by activating the innate immune system via TLRs, which recognize these gut-derived bacterial components. The healthy liver expresses low mRNA levels of TLRs (TLR1, TLR2, TLR4, TLR6, TLR7, TLR8, TLR9, and TLR10), implying a high tolerance of the liver to TLR ligands from the microbiota. The translocation of these bacterial components from the gut into the portal system is facilitated by intestinal barrier disruption due to GM dysbiosis [13, 96]. In this sense, there is evidence that dysbiosis causes permeability changes that increase portal levels of gut-derived TLR ligands (LPS or endotoxin), which further activate TLR4 on hepatic Kupffer and stellate cells [97]. LPS is the major structural component of gram-negative bacteria and the major component of endotoxin. LPS may be recognized by LPS-binding protein (LBP) in serum and is the major activator of the innate immune response [98]. Ruiz et al. indicated that the serum levels of LBP were increased in patients with obesity and NASH compared to those in patients with obesity and SS and the increased serum LBP level was correlated to an upregulated expression of TNF-α in liver tissue [99].

During TLR4 activation, the adaptor molecule myeloid differentiation factor 88 (MyD88) is activated, and the downstream signaling MyD88-dependent pathway results in the activation of necrosis factor kappa beta (NF-κB), leading to the expression of proinflammatory cytokines (TNF-α, IL-6, IL-8 and IL-12) and chemokines (interferon γ (IFN-γ) and monocyte chemotactic protein-1 (MCP-1)), promoting inflammation [68, 97]. There are several intracellular cascades involved in this process, generating oxidative stress, low-grade systemic inflammation, and hepatic injury [100]. In addition, TLR signaling can also lead to the production of inflammasomes in peripheral and parenchymal cells, which activate a variety of processes, including activation of caspase-1, resulting in cell death [101].

The inflammasome, which is a multimeric signaling platform that leads to the production of IL-18 and IL-1β through the NOD-like receptors pyrin domain-containing (NLRP3 and NLRP6), is activated by LPS derived from intestinal dysbiosis via TLR4 and TLR9 responses. Reports have associated inflammasome activation with the development of liver steatosis, inflammation, and fibrosis in NAFLD patients [102, 103].

It has been shown that TLR2, TLR4, and TLR9 play an important role in the development of NASH [104]. In addition, other studies have established that the increase in endotoxin levels is related to IL-1α and TNF-α production [105, 106]. In

patients with NAFLD, gut permeability and SIBO due to intestinal dysbiosis have been associated with the severity of steatosis [107]. In biopsy-proven human NASH, plasma levels of IgG against endotoxin were found to be increased with NASH grade severity, suggesting the deleterious effect of chronic endotoxin exposure [108]. In our previous GM-derived metabolite study, we found overexpression of TLR9 jejunal expression in NAFLD subjects, which suggested the activation of the immune system during NAFLD progression [6]. Additionally, enhanced expression of TLR4, the release of IL-8, and high levels of LPS have been demonstrated in NAFLD patients [109, 110]. However, other reports did not reveal an association between endotoxemia and NAFLD progression, suggesting that endotoxemia may not be the only driver of disease development in all patients [111].

Multiple experimental studies have demonstrated that a high-fat diet can increase the proportion of LPS derived from the GM, and administration of endotoxin has been shown to induce IR and weight gain [99, 112]. On the other hand, some authors have recently proposed that the small intestine shields the liver from otherwise toxic fructose exposure via the GM [113].

There is a clear relation between gut dysbiosis, bacterial-derived components, the inflammatory response, and NAFLD; therefore, these bacterial mediators, especially circulating TLRs, might be used as potential noninvasive markers of disease progression.

4.4 Endogenous ethanol production

Intestinal dysbiosis increases endogenous ethanol production [111], which also affects gut permeability, disrupting intestinal tight junctions. This process allows endotoxins and ethanol to reach the liver and trigger the TLR response and inflammasome activation, contributing to liver damage [114]. In addition to the proinflammatory response, ethanol promotes oxidative stress and hepatocyte necrosis because of the formation of reactive oxygen and nitrogen species [94]. Endogenous ethanol inhibits the tricarboxylic acid cycle, thus increasing levels of acetate and thereby promoting triglyceride accumulation in hepatocytes [64]. Ethanol can also increase the activity of the enzyme cytochrome P450 2E1 (CYP2E1), which catalyzes the oxidation of ethanol but produces free radicals favoring oxidative damage, mitochondrial dysfunction, and liver inflammation [94, 115, 116].

Several studies have detected increased levels of non-dietary ethanol derived from bacteria in patients with obesity [111, 117] and in patients with NASH [111, 118, 119]. In this sense, Zhu et al. proposed that microbiomes rich in ethanol-producing *Escherichia* may be a risk factor for NAFLD progression [56]. *Escherichia*, *Bacteroides*, *Bifidobacterium*, and *Clostridium* can produce endogenous alcohol and generate significant ethanol-mediated liver damage [111]. Therefore, the production of endogenous ethanol by the GM may act as a hepatotoxin, contributing to the development of NAFLD and its progression to NASH [120]. In addition, children with NAFLD/NASH showed high levels of endogenous ethanol and LPS derived from the GM [111, 117, 121], confirming that endogenous ethanol might contribute to the pathogenesis of NAFLD and NASH.

Furthermore, Zhu et al. showed an increased abundance of alcohol-producing bacteria in NASH microbiomes, elevated blood-ethanol concentration in NASH patients, and the well-established role of alcohol metabolism in oxidative stress and liver inflammation [56]. In our previous GM-derived metabolite study, we found an interesting result about the higher circulating endogenous ethanol levels in NASH patients than in patients with SS. This fact suggested that circulating ethanol levels could distinguish between different degrees of liver damage. Moreover, in the same study, we evaluated the diagnostic efficacy of a biomarker panel including circulating ethanol, betaine,

GCA, and DCA levels as markers of NASH in a group of patients with liver histology indicative of NASH. A cutoff point and area under the curve were determined so that NASH could be diagnosed. The accuracy with which this panel discriminates NASH subjects from non-NASH subjects showed an area under the ROC curve (AUROC) of approximately 0.776 (0.632–0.921). Therefore, we concluded that the levels of certain circulating microbiota-related metabolites are associated with NAFLD severity and could be used as a "liquid biopsy" in the noninvasive diagnosis of NASH [6].

In summary, proinflammatory and prooxidative damage has been demonstrated as a result of endogenous ethanol in the liver, which might contribute to the pathogenesis of NAFLD, and previous reports may support its use as a noninvasive biomarker of disease progression.

4.5 Reduction of choline metabolism

Choline is an essential nutrient obtained through both dietary intake and endogenous synthesis and is an important constituent of the phospholipid membrane. The human GM actively metabolizes dietary components, including choline. Alterations in choline and phosphatidylcholine metabolism due to intestinal dysbiosis may have an impact on several physiological pathways, which could induce NAFLD. Choline deficiency prevents the synthesis and excretion of VLDL, leading to hepatic triglyceride accumulation and liver steatosis [122, 123]. In fact, the link between choline deficiency and the accumulation of hepatic lipids has been recognized for more than 50 years [124], leading to the establishment of choline-deficient diets to induce models of NAFLD in animals.

In addition, choline can be metabolized to its derivative trimethylamine (TMA) by the GM. TMA reaches the liver via portal circulation and is subsequently oxidized by hepatic flavin-containing monooxygenases in the liver, forming trimethylamine-N-oxide (TMAO), which is then released into blood circulation [125, 126]. Previous studies have revealed that TMAO may affect lipid absorption and cholesterol homeostasis and modulate glucose and lipid metabolism by decreasing the total BA pool size [122]. TMAO modulates glucose metabolism and increases IR in mice fed a high-fat diet [127]. TMAO also affects lipid absorption and cholesterol homeostasis by reducing the conversion of cholesterol into BAs [122].

A small number of human studies have shown that the consumption of a low-choline diet promotes fatty liver and liver damage [123, 128]. Other studies have pointed out that plasma-free choline levels are positively related to the severity of NAFLD, fibrosis, and NASH [129, 130].

On the other hand, in our previous research, we analyzed circulating levels of these choline metabolites according to hepatic histology and observed that levels of TMAO were significantly higher in NAFLD patients than in NL subjects [6], which correlates with the previous statement that serum TMAO levels are significantly higher in patients with NAFLD than in healthy people and correlates with the development and severity of NAFLD through different mechanisms: modulating glucose metabolism, promoting inflammation in adipose tissue, and influencing lipid absorption and cholesterol homeostasis [125, 129, 131].

In summary, the evidence has demonstrated that choline and TMAO are associated with the progression of NAFLD, indicating the potential use of these GM-derived mediators as markers of disease progression.

5. Gut microbiota as therapeutic target in NAFLD

Although there are no treatments to directly reverse steatosis, fibrosis, or liver damage, lifestyle changes and therapeutic strategies to treat other MS-related

diseases, such as obesity, T2DM, or IR, could ameliorate NAFLD, avoiding its progression to NASH. Lifestyle intervention (diet and exercise), bariatric surgery, antidiabetic drugs, lipid-altering agents, and antihypertensive drugs can improve all of the features of NASH by ameliorating MS-related diseases [4]. Nevertheless, there is currently no specific treatment proven to be effective in treating NASH. Clarifying NAFLD risk factors could lead to more accurate prediction of disease progression and more effective treatments based on individualized drivers of disease [132]. The search for a possible therapy for NASH is focused on different pathways: metabolic targets, cell stress and apoptosis, immune targets, fibrosis, and GM modulation.

Currently, there are different mechanisms to manage NAFLD/NASH with metabolic targets focused on ameliorating other related diseases but also involved in NAFLD progression. Moreover, vitamin E acts as an antioxidant and hepatoprotective agent used to treat NASH (**Figure 2**).

On the other hand, there are many active studies and clinical trials focused on new therapeutic strategies with different pharmacological targets to avoid NAFLD and NASH progression. In this regard, PPAR agonists, antidiabetic drugs, FXR ligands, and anti-inflammatory and antiapoptotic agents can act as insulin sensitizers and improve the proinflammatory chronic state characteristic of NASH; antifibrotic agents can avoid NASH progression to fibrosis; and GM modulation can prevent the intestinal dysbiosis involved in NAFLD pathogenesis (**Figure 2**) [4, 7, 24, 133].

The key role that the GM plays in the progression of the disease opens the door to new ways of thinking about NASH prevention and treatment. The possibility of modulating the GM to treat NAFLD and NASH has gained interest in the potential use of probiotics, prebiotics, and antibiotics as effective treatments.

Probiotics are defined as viable microorganisms that when administered in adequate amounts, confer a health benefit to the host [134]. There are many mechanisms by which probiotics improve the GM and consequently ensure liver health (inhibition of intestinal bacterial enzymes, stimulation of host immunity, competition for limited nutrients, inhibition of bacterial mucosal adherence and epithelial

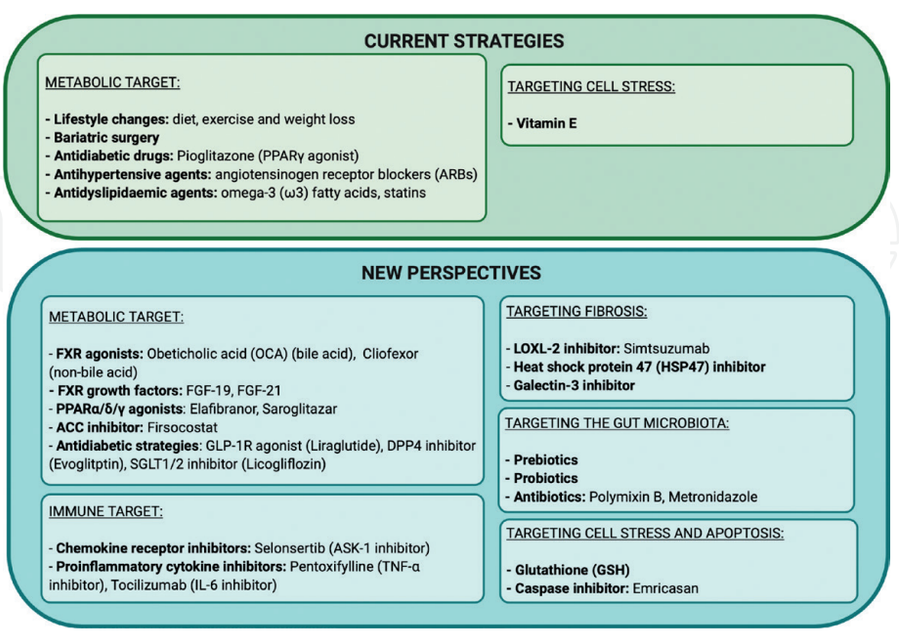

Figure 2.
Current and future treatment strategies to manage and treat NAFLD and NASH. Peroxisome proliferator-activated receptor (PPAR), farnesoid X receptor (FXR), farnesoid growth factor-19 (FGF-19), farnesoid growth factor-21 (FGF-21), acetyl-CoA carboxylase (ACC), glucagon-like peptide-1 receptor (GLP-1R), dipeptidyl peptidase-4 (DPP-4), sodium-glucose cotransporter 1/2 (SGLT-1/2), apoptosis signal-regulating kinase-1 (ASK-1), tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and lysyl oxidase-like 2 (LXL-2).

invasion, protection against intestinal permeability, and control of bacterial translocation from the gut to the portal vein circulation). The biological activity of probiotics depends on delivering anti-inflammatory mediators that downregulate proinflammatory cytokines [104]. Therefore, probiotic therapy offers an interesting approach to control hepatic injury and a low-grade proinflammatory state.

Another alternative is the use of prebiotic fiber, which is defined as an amount of nondigestible food ingredients that beneficially affect the host, by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon [135]. The health effects of prebiotic fiber are related to improved glucoregulation and modified lipid metabolism as well as selective modulation of the GM. Some mechanisms have been proposed to explain the beneficial effects of prebiotics on the accumulation of triglycerides in the liver observed in animals, including reduced de novo fatty acid synthesis and SCFA production, body weight and fat loss, and improved glycemic control, GM modulation, and anti-inflammatory effects [13, 104]. These promising preliminary results strongly indicate the potential use of probiotics and prebiotics for the prevention or treatment of NASH.

Prophylactic use of antibiotics in patients with chronic liver diseases is an established method of preventing infections or innate immune dysfunction in acute liver failure (ALF) [13]. In addition, it has been demonstrated in animal and human models that the positive effect of polymyxin B and metronidazole in reducing the severity of NAFLD during total parenteral nutrition or after intestinal bypass could be interesting for their use to treat NAFLD [136, 137]. However, direct evidence is currently lacking, and thus, antibiotics cannot be routinely recommended to treat NASH, although further research is needed.

Overall, to date, there have been only a few studies concerning the use of probiotics, prebiotics, and antibiotics in humans; therefore, large-scale randomized controlled trials with histological endpoints are indicated.

6. Conclusions

Intestinal dysbiosis can trigger gut inflammation and increase the permeability of the intestinal epithelial barrier, exposing the gut-liver axis to GM-derived mediators of dysbiosis, such as bacterial components or metabolites, which may induce hepatotoxicity, inflammation, and consequently NAFLD progression. Gut-derived mediators of dysbiosis contribute to NAFLD progression by activating the immune system, inducing oxidative stress, enhancing inflammation, and finally promoting fibrogenesis.

Despite the evident association between GM dysbiosis, obesity, and NAFLD derived from several experimental studies, few studies have been conducted in patients with NAFLD to explore the role of GM-derived mediators of dysbiosis in the occurrence and progression of the disease. Additionally, few studies have focused on gut-derived mediators of dysbiosis as noninvasive markers of disease progression. The study of these mediators may provide an opportunity to develop a specific diagnostic and prognostic biomarker for NAFLD and NASH. In this sense, we propose the metabolomic study of these mediators and other metabolites involved to achieve a metabolomic profile that could be used as biomarkers for evaluating the status of NAFLD. On the other hand, some previous evidence has focused on GM modulation using probiotics, prebiotics, and antibiotics as therapeutic strategies to prevent or treat NAFLD and NASH, which is more uncertain and requires future research. In this sense, it remains important to promote study of GM targeting to find an effective treatment for NAFLD and overall for NASH.

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Conflict of interest

The authors declare no conflict of interest.

Abbreviations

NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
GM	gut microbiota
T2DM	type 2 diabetes mellitus
IR	insulin resistance
MS	metabolic syndrome
LPS	lipopolysaccharides
SCFAs	short-chain fatty acids
BAs	bile acids
TMAO	trimethylamine-N-oxide
EtOH	ethanol
NAFL	non-alcoholic fatty liver
SS	simple steatosis
AST	aspartate aminotransferase
ALT	alanine aminotransferase
TLRs	toll-like receptors
SIBO	small intestinal bacterial overgrowth syndrome
FXR	farnesoid X receptor
VLDL	very-low density lipoprotein
GPR	G-protein coupled receptors
TNF-α	tumor necrosis factor alpha
IL	interleukin
GLP-1	glucagon-like peptide-1
AMPK	AMP-activated protein kinase
CA	cholic acid
CDCA	chenodeoxycholic acid
DCA	deoxycholic acid
LCA	lithocholic acid
NRs	nuclear receptors
SHP	small heterodimer partner
SREBP-1c	sterol-regulatory element-binding protein-1c
PPARα	proliferator-activated receptor alpha
NL	normal liver
GCA	glycolic acid
PAMPs	pathogen-associated molecular patterns
LPB	LPS-binding protein
NF-κB	necrosis factor-kappa beta

INF-γ	interferon gamma
MCP-1	monocyte chemotactic protein-1
NLRP	NOD-like receptors pyrin domain
CYP2E1	enzyme cytochrome P450 2E1
AUROC	area under the ROC curve
TMA	trimethylamine
FGF	farnesoid growth factor
ACC	acetyl-CoA carboxylase
DPP-4	dipeptidyl peptidase-4
SGLT-1/2	sodium-glucose cotransporter-1/2
ASK-1	apoptosis signal-regulating kinase-1
LXL-2	lysyl oxidase-like-2
ALF	acute liver failure

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