Review

Probiotics as Potential Therapy in the Management of Non-Alcoholic Fatty Liver Disease (NAFLD)

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Abstract: *Background:* Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease, the prevalence of which has increased over the years. The management of this pathology is not clear, and a specific pharmacological drug that can treat NAFLD is not available. In this sense, efforts are focused on the potential use of compounds with a natural origin that can contribute to reversing hepatic steatosis. Supplementation with probiotics, live microorganisms, is a potential strategy for the management of NAFLD. *Methods:* In the present review, the available information on the potential therapeutic effects of probiotics in NAFLD, mainly in animal models and in some clinical trials, is summarized. *Results:* Studies carried out using animal models of NAFLD induced by a high-fat diet have shown the beneficial effects of probiotic supplementation in reducing liver steatosis and normalizing the blood lipid profile and liver enzyme activities. In addition, a decrease in lipogenesis and an increase in lipolysis have been observed, together with a reduction in the pro-oxidative and pro-inflammatory state and a normalization of intestinal dysbiosis. Clinical trials have reported a decrease in the serum transaminases and an improved lipid profile, as well as a reduction in inflammatory markers. *Conclusions:* In conclusion, probiotic supplementation can be used as a potential therapy for the management of NAFLD.

Keywords: steatosis; dysbiosis; fatty liver; inflammation; probiotic

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most prevalent chronic liver disease with an increasing prevalence, especially in Western countries. The prevalence of the disease has been estimated at 20–30% in the general population and increases to more than 70% in patients with metabolic risk factors, especially in obese and diabetic patients [1]. The pathology comprises a variety of disorders ranging from simple hepatic steatosis to steatohepatitis (NASH), which can progress to liver fibrosis, cirrhosis, or hepatocellular carcinoma [2]. The most important characteristic of this pathology is the excessive fat accumulation in the hepatocytes, above 5%, without alcohol abuse. In addition, it is also considered as the hepatic manifestation of metabolic syndrome [3]. An increasing number of characteristics of metabolic syndrome, principally type 2 diabetes and abdominal obesity are risk factors for the development of NAFLD and its progression to NASH [4]. The main pathophysiological mechanism involved in the development of NAFLD is the entry of free fatty acids and the subsequent accumulation of triglycerides that induce a

lipotoxic environment within the hepatocytes; this leads to an alteration in the normal lipid homeostasis and cell function [5].

To date, there is not an effective therapy for NAFLD, and the treatments are focused on reducing the potential risk factors such as obesity, hyperlipidaemia, and insulin resistance. Once NAFLD is diagnosed, the therapeutic focus is on treating and monitoring the development of the associated complications. However, the main therapeutic approach continues to be weight loss and improvement in insulin resistance, often through a multimodal approach focused on a balanced hypocaloric diet and the promotion of physical activity [6,7]. This lack of specific treatment for NAFLD makes it necessary to search for alternatives. Some of these are based on compounds with a natural origin that are easy to obtain and consume. In this sense, various natural products have shown properties to improve NAFLD symptoms by normalizing the lipid and carbohydrate metabolism and energy homeostasis, as well as by inhibiting inflammation and fibrogenesis [8,9].

From the microbiome present in the gut, metabolic products are transported to the liver through the portal venous system. Thus, the gut–liver axis is an important part of the development of NAFLD since these metabolites display pro-inflammatory properties (lipopolysaccharides, ammonia, among others) [10,11]. However, living bacteria without pathological effects are usually present in the body and are essential for some physiological processes in humans, such as digestion, neurological functions, or enhancement of the immune system [12–15]. The study of these bacteria has allowed an increase in the knowledge of their impacts in different disorders such as obesity and diabetes [16,17]. In this sense, the regulation of the gut microbiota could have some benefits for those patients that develop NAFLD, since positive results after the intake of probiotics have been described [18–20]. For this reason, the use of probiotics could be a new strategy to ameliorate the pathology or achieve some benefits for these patients. It has been shown that probiotics containing different *Lactobacillus* and *Bifidobacterium* species are capable of modifying the intestinal microbiota, inflammatory cytokines, and gut permeability in NAFLD patients [18]. Moreover, a meta-analysis of 21 randomized clinical trials revealed that a probiotic intervention significantly reduced the levels of blood lipids, blood glucose, and insulin, improving hepatic steatosis and liver function in NAFLD patients [19]. Altogether, the current review aims to describe the main results of studies using animal models and clinical trials in which the therapeutic potential of probiotics to reverse NAFLD is evaluated.

2. Non-Alcoholic Fatty Liver Disease

NAFLD is defined as the excessive fat accumulation in the hepatocytes, specifically triglycerides and free fatty acids, without abusive alcohol consumption as the principal cause [21–23]. NAFLD is directly related to obesity, hypertension, type 2 diabetes mellitus (T2DM), and dyslipidaemia, and thus it is considered the hepatic manifestation of metabolic syndrome (MetS) [24].

The estimated global prevalence of NAFLD is 25%. Specifically, the highest NAFLD prevalence is in the Middle East (32%) and South America (30%), and the lowest is in Africa (13%). The African prevalence could be biased because population surveys are rare on the continent. The prevalence in Europe is 24%, and in Spain, it is 20–29.9% [25,26]. Other references confirm that the global prevalence ranges between 20 and 30% [27]. In people with obesity, the NAFLD prevalence is 58–74% and 56% in people with T2DM [25]. Moreover, the prevalence of NAFLD increases with age and is more common in men, according to studies carried out in Spain, the United States, and southwest China [28–30].

Regarding liver histology, NAFLD has a slow evolution [25], but its pathological spectrum ranges from simple steatosis or fat accumulation in the liver (which tends to be asymptomatic), to more advanced diseases such as cancer or death. Steatosis can progress to steatohepatitis when the liver is inflamed, to cirrhosis when fibrotic lesions appear, and ultimately cancer. Steatosis and steatohepatitis are both reversible. They can be reversed by improving the lifestyle, but advanced stages of cirrhosis and cancer are irreversible. It should be noted that some people with steatohepatitis have developed cancer without the

cirrhosis stage [31]. There is an increased risk of cardiovascular disease among patients with NAFLD, which also increases with the severity of the liver status [32,33]. In addition, subjects with NAFLD have an increased risk of death [34]. The mortality risk in subjects with NAFLD increases exponentially with the presence of liver fibrosis and its degree [35].

The first line of treatment for NAFLD is a lifestyle change, including diet and exercise, to reduce weight. Weight loss is very important to improve the NAFLD histopathological features. Nevertheless, pharmacological treatment is available for associated metabolic complications such as those related to T2DM, insulin resistance, obesity, and hyperlipidaemia. Pharmacological treatment is applied when a lifestyle change is not sufficient and/or when there is a clear diagnosis of fibrosis [36].

Thiazolidinedione (a ligand of the peroxisome proliferator-activated receptor gamma nuclear transcription factor (PPAR)-γ) reverses insulin resistance in the case of adipose tissue dysfunction, T2DM, and obesity in people with NAFLD [36]. Within the same drug family, Pioglitazone increases the plasma adiponectin levels, inhibits fatty acid synthesis in the liver, stimulates its oxidation, and promotes anti-inflammatory effects. Thus, Pioglitazone manages to improve the metabolic and histological profiles in people with fibrosis, T2DM, and NASH [37,38]. Vitamin E is also part of the treatment against NAFLD due to its antioxidant effects. It reduces inflammation, liver enzyme levels, and non-invasive fibrosis [39,40].

The best NAFLD treatment is actually the Mediterranean lifestyle, including a Mediterranean diet and regular physical activity, because it contributes to the improvement of MetS and a reduction in the NAFLD risk factors [41]. On the one hand, the Mediterranean diet is based on the intake of foods rich in antioxidants and anti-inflammatory compounds, fibre, and unsaturated fatty acids, and reduces the consumption of animal proteins and saturated fatty acids. Thus, the Mediterranean diet has benefits for cardiovascular disease, components of MetS, cancer, and overall mortality [42,43]. A high adherence to the Mediterranean diet improves insulin resistance, fibrosis, and reduces liver fat evaluated through magnetic resonance imaging (MRI) [44]. On the other hand, an active lifestyle reduces high triglyceride levels and increases low-cholesterol–high-density lipoprotein (HDL) levels, helps with weight loss, and regulates blood pressure [45]. Thus, physical activity contributes to MetS, T2DM, and NAFLD positively [46,47]. Diet plus physical activity show better results in the improvement of NAFLD and its risk factors [48].

3. Microbiota and Probiotics in NAFLD

The WHO defines probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host". Probiotics can have effects on several aspects of physiology, such as digestion, metabolism, and immunology. Moreover, probiotics can be presented as probiotic drugs, probiotic medical drugs, probiotic foods, non-oral probiotics, probiotic animal feed, probiotic dietary supplements, defined microbial consortia, and probiotic infant formula [49].

The probiotics in food and nutritional products can classically be classified into four large families: *Lactobacillus* species, *Bifidobacterium* species, other lactic acid bacteria, and non-lactic acid bacteria. The *Lactobacillus* genus has been one of the most studied, as it is a kind of probiotic commonly found in yogurt and yogurt-like products [50]. Nevertheless, the amount of CFU (colony-forming units) in common fermented yogurt and milk products may not reach the minimum for an adequate effect on the gut microbiome.

The microbiota refers to the microorganisms that colonize the human and animal body. Such microorganisms can be categorized according to their behaviour as commensals, mutualists, and pathogens. The microbiota frequently has specific functions for the organism and can be considered as an organ that impacts both health and disease. The initial colonization of the digestive system by microorganisms occurs due to the exposure of the offspring to the mother's vaginal microbiome and changes through time according to the dietary intake in the adult microbiota [51]. The human gut microbiome is estimated to comprise between 10 and 100 trillion microorganisms, primarily bacteria [52]. Among

the most common bacterial groups are Bacteroidetes and Firmicutes, while the majority of Archaea is Euryarchaeota [53].

Several diseases are related to dysbiosis in the intestinal microbes. As explained above, NAFLD is considered a systemic metabolic disorder, the result of the concomitant presence of NAFLD and metabolic syndrome. Components of the metabolic syndrome are related to dysregulation of the intestinal microbiota, as in NAFLD. NAFLD is related to some differences in the intestinal bacteria of humans, compared to healthy counterparts. In this sense, although the literature presents varied results of the changes observed in the microbiota in patients with NALFD compared to healthy patients, there is a certain uniformity in the bacterial signatures. The most consistent results describe an increase in the abundance of Proteobacteria and a decrease in Firmicutes at the phylum level [54]. At the family level, there is an increase in Enterobacteriaceae and a decrease in Ruminococcaceae and Rikenellaceae, and at the genus level there is an increase in *Bacteroides*, *Dorea*, *Escherichia*, and *Peptoniphilus* and a decrease in *Anaerosporobacter*, *Coprococcus*, *Eubacterium*, *Faecalibacterium*, and *Prevotella* [55,56]. Even in the early stages of NAFLD, dysbiosis in the gut microbial is present, which becomes more unstable with the NAFLD progression. At the most advanced stages of NAFLD or even cirrhosis, a very low number of beneficial bacteria can be found in the gut microbiome, but most importantly, potentially pathogenic bacteria are likely to be found [57]. This finding can be explained by people with NAFLD frequently following higher caloric dietary patterns [58]. High-fat diets are associated with gut dysbiosis by means of an increased intestinal epithelium permeability that occurs as a consequence of a disruption in both the mucus layer and the tight junctions. The result is an increased bacteria translocation and the presence in the portal venous system of bacterial products. As all blood from the gastrointestinal tract has to pass through the liver before entering the systemic circulation, the presence of bacterial products or bacteria in the portal blood increases the likelihood of bacterial liver colonization [59]. NAFLD itself, regardless of the diet, is also related to an increased intestinal permeability, which has been related to the induction of inflammatory pathways that contribute to NAFLD pathogenesis. The gut microbiome can induce the release of anti- or pro-inflammatory compounds [57]. Moreover, the gut microbes have an influence on triglyceride metabolism in the liver, nutrient absorption, and body metabolism by altering the content and type of several metabolites of proteins, fats, and bile acids. Some metabolites of the gut bacteria are related to the degree of steatosis and can induce fat accumulation in the liver [60]. It seems that the SCFAs (short-chain fatty acids) and polysaccharides synthesized by the gut bacteria are different in NAFLD than in healthy subjects. Both are crucial for the gut epithelium integrity and intrinsic immune defences. While SCFAs seem to promote NAFLD through some pathways, they are also beneficial as they regulate liver AMPK (adenosine 5′-monophosphate-activated protein kinase) activity [57]. Moreover, low-fat diets are related to healthier NAFLD and hepatic parameters, which can be related to the changes in gut epithelium permeability [61].

4. Probiotics and Animal Models

There are numerous studies that have analysed the potential beneficial effects of probiotics in different animal models of NAFLD (Table 1), such as those models in which the pathology is induced by methionine- and choline-deficient (MCD) diets, monosodium glutamate (MSG), or D-fructose administration. However, most studies use a model based on a high-fat diet (HFD) or Western-style diet that would be more similar to the evolution of the pathology in humans. Supplementation with a single strain of probiotics, the combination of two or more strains, and the administration of foods rich in probiotics, such as kefir or kombucha, are among the different treatments to counteract the pathology. The most used probiotics correspond to strains of lactic bacteria such as *Lactobacillus*, *Bifidobacterium*, and *Pediococcus*, among others, and to a lesser extent non-lactic acid bacteria such as *Bacillus* and *Propionibacterium*.

In general, the results obtained after supplementation with different strains alone and in combination are quite similar in all of the studies, evidencing a decrease in the accumulation of fat in the body and specifically in the liver, and normalizing the blood lipid profile and liver enzymes, contributing to the reduction in hepatic steatosis. In this sense, after supplementation, a decrease in the elevated levels of triglycerides, total cholesterol, and aminotransferases (alanine aminotransferase (ALT), and aspartate aminotransferase (AST)) induced by an HFD has been evidenced. Additionally, an improvement in insulin sensitivity was observed after *L. johnsonii* BS15 [62], *Lactococcus lactis* subsp. *cremoris* [63], or the administration of a probiotic mixture of five different *Bacillus* spp. [64]. Moreover, several studies have shown the normalization of various adipokines after the consumption of probiotics that are altered by an HFD. After supplementation with probiotics, a decrease in serum leptin levels and an increase in adiponectin have been observed [65–68].

In an HFD diet and in NAFLD, the coexistence of insulin resistance with a high level of leptin has been observed, favouring the lipid intake and development of NAFLD [69]. Thus, its increase can contribute to the control of the energy intake and steatosis reduction. Adiponectin promotes fatty acid oxidation and peripheral insulin sensitivity, so its increase associated with probiotics could help to reduce the lipid accumulation [70]. Also, a decrease in the levels of resistin has been observed after the administration of a probiotic mixture, which could play a role in reversing the accumulation of hepatic fat [67].

NAFLD is a pathology related to a pro-oxidative and pro-inflammatory state that can cause damage to the hepatic membranes, proteins, and DNA, affecting liver function [71]. Several studies have observed how the HFD induces a decrease in the hepatic antioxidant defence mechanisms, along with an increase in the oxidative damage markers, mainly malondialdehyde (MDA) [62,72], although these results have also been observed in serum [73,74]. The supplementation with different probiotic strains was found to recover the activities of antioxidant enzymes—catalase, superoxide dismutase, and glutathione peroxidase—and to reduce the MDA levels. The reduction in the accumulation of fat and body weight after the administration of probiotics can reduce the degree of oxidative stress and lead to the normalization of the antioxidant defence mechanisms and reduce the degree of oxidative damage. The existence of a relationship between the HFD and the increase in gut permeability and metabolic endotoxemia characterised by elevated serum inflammatory markers and lipopolysaccharides (LPS) is well established [75]. In fact, gut microbiota-derived LPS are known to induce the release of proinflammatory cytokines, including tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6), as the central defence mechanism against pathogens but also triggers liver inflammation and oxidative stress [76]. Increases in the levels of pro-inflammatory cytokines induce the activation of cell signalling pathways such as nuclear factor κB (NFκB) or the signal transducer and activator of transcription 3 (STAT3) that are related to liver damage [74,77]. Thus, supplementation with probiotics has resulted in the improvement of these inflammatory parameters close to the normal levels. A significant decrease in circulating endotoxin and pro-inflammatory cytokines—IL-1β, IL-6 and TNF-α—as well as an increase in the anti-inflammatory IL-10 have also been observed after probiotic administration [65,72,78]. Moreover, it has been evidenced that probiotic supplementation is capable of downregulating the toll-like receptor (TLR)4 and TLR9-induced inflammatory responses, as well as decreasing the expression of NLRP3 (NLR family pyrin domain containing 3) and ASC (apoptosis-associated speck-like protein containing a CARD), suggesting a regulation in the expression of the NLRP3 inflammasome [79]. An interesting study showed that *L. lactis* subsp. *cremoris* modulated the levels of various hepatic oxylipins, which act as inflammatory mediators, being relevant to the decrease in resolvin E1, 9 hydroxy-eicosatetraenoic acid (9-HETE), and 9 hydroxy-octadecadenoic acids (9HpODE) [63].

Within the mechanisms involved in the improvement of NAFLD by probiotics, the effects on triglyceride and cholesterol metabolism play a central role. In almost all of the studies, regardless of the probiotic administered, the downregulation of the genes related to lipogenesis and the upregulation of those related to lipolysis and fatty acid oxidation have

been observed. In this sense, the oral administration of probiotics results in an increase in the expression of genes, such as peroxisome proliferator-activated receptors (PPARs), PPARγ coactivator-1α (PGC1α), and carnitine palmitoyltransferase-1α (CPT1α), and a decrease in genes such as sterol regulatory element binding protein 1 (SREBP-1), fatty acid synthetase (FAS), acetyl-coenzyme A carboxylase (ACC), and coenzyme A desaturase 1 (SCD1) compared to the HFD groups, which lead to less hepatic steatosis [65,72,78]. PPARα is the main factor that controls the oxidation of fatty acids produced in the mitochondria, and thus its activation after ingesting probiotics can increase the rate of β-oxidation of the hepatic fatty acids and reduce steatosis [80]. SREBP-1 is a key transcription factor regulating the transcription of de novo lipogenesis and its activation can induce liver steatosis [81]. The suppression of SREBP-1 by probiotics and its downstream genes ACC and FAS can confer protection against the development of NAFLD [65]. Furthermore, some studies have shown changes in cholesterol and bile acid metabolism that may also contribute to the reversal of steatosis. The hepatic conversion of cholesterol to bile acid is the major pathway for excreting cholesterol from the body [82]. In this sense, several studies have shown that probiotic supplementation induces the expression of cholesterol 7 alpha-hydroxylase (CYP7A1), the limiting enzyme in the synthetic pathway of the liver, and cholesterol transporters such as ATP-binding cassette sub-family G member 5 (ABCG5) and the bile salt export pump (BSEP) [83,84]. The reduction in the cholesterol levels occurs not only by increased de novo bile acid synthesis, in a process mediated by the inhibition of fibroblast growth factor 15 (FGF15) signalling and the upregulation of CYP7A1 expression, but also through the repression of bile acid reabsorption by the attenuating farnesoid X receptor (FXR) signalling [73,83,85]. In addition, probiotic administration has also been related to an increase in liver X receptor α (LXRα), a cholesterol sensor that upregulates CYP7A1 and CYP8B1 expression and favours sterol excretion from the liver to bile by upregulating the ABCG5/8 transporters [86,87]. An interesting study has shown the modulatory capacity of *B. longum* on the renin–angiotensin system, which is related to a metabolic improvement in glucose and lipid metabolism, and a reduction in liver fat [88].

One of the features of obesity and NAFLD is the existence of an alteration in the intestinal permeability and dysbiosis. In this sense, the intestinal microbiota constitutes a central element within the intestine–liver axis and is a direct target for probiotics to exert their beneficial effects on health. Although it is not clear whether the microbiota has a direct impact on the incidence and development of NAFLD, there are variations in the relative abundance of certain bacterial groups compared to healthy subjects [89]. Firmicutes and Bacteroidetes are the most important bacterial phyla in the gastrointestinal tract, and an increase in their relative ratio is related to obesity and the development of NAFLD [90]. In this sense, another of the most evaluated mechanisms of probiotic protection against NAFLD is their ability to modulate the intestinal microbiota. Although each type of investigated probiotic has its particularities, in general, all of the treatments lead to an increase in the diversity of the microbiota and to the enrichment of some intestinal probiotics (such as *Akkermansia*, *Verrucomicrobia*, *Lactobacillus* spp. and *Bifidobacterium* spp.). In contrast, a reduction in the abundance of *Mucispirillum*, *Clostridium*, and *Streptococcus*, which are involved in the inflammatory response, has also been evidenced [91]. In addition, there is an increase in the abundance of Bacteroidetes and a decrease in Firmicutes, which decreases the Firmicutes/Bacteroidetes ratio [62,92,93]. However, the differential changes in the relative abundance of the taxa related to each probiotic are probably derived from the strain-dependent competition with the host microbiota for adhesion sites and nutrients [94]. An interesting study evidenced that *L. rhamnosus* GG was capable of reducing intestinal fatty acid absorption using trace labelled [^{14}C]-oleic acid in vivo [95]. Additionally, SCFA production, synthesised by the microbiota, plays an essential role in the maintenance of hepatic energy homeostasis as they can affect the appetite and modulate energy expenditure and lipid metabolism [96]. Previous studies evidenced that propionate or butyrate leads to increased energy expenditure and lipid accumulation, whereas acetate prevents adiposity and promotes adipose tissue browning [97–99]. The studies on probiotic supplementation

reported increases in SCFAs such as butyrate, acetate, and propionate that through the portal circulation can be carried into the liver, improving lipid metabolism and ameliorating NAFLD [96]. Moreover, butyrate has been reported to promote the production of mucin, reduce the adhesion of pathogens, and thus ameliorating liver inflammation and improving NAFLD [100]. Finally, all of the changes in the microbiota after probiotic supplementation led to an improvement in intestinal permeability with an enhanced expression of tight junction-associated proteins such as zonula occludens-1 (ZO-1) [66,101]. Probiotics are capable of improving gut barrier function, decreasing endotoxemia, and consequently, alleviating chronic inflammation.

Table 1. Main results reported on the effects of probiotics in animal studies.

Animal Model	Probiotic Strains	Treatment	Main Findings	References
HFD mice	*L. johnsonii* BS15	2×10^7 and 2×10^8 cfu/0.2 mL, 17 weeks	↓ hepatic steatosis, apoptosis, inflammation, mitochondrial injury, and gut permeability, ↑ insulin sensitivity	[62]
Western diet mice	*L. lactis* subsp. *cremoris* ATCC 19257	1×10^9 CFU, thrice weekly for 16 weeks	↓ hepatic steatosis and inflammation, ↑ insulin sensitivity, oxylipin modulation	[63]
HFD mice	*B. sonorensis* JJY 12-3, *B. paralicheniformis* JJY 12-8, *B. sonorensis* JJY 13-1, *B. sonorensis* JJY 13-3, *B. sonorensis* JJY 13-8	Mixture of 1×10^8 CFU/200 µL PBS, 13 weeks	↓ hepatic steatosis, inflammation, gut permeability, and lipid uptake and lipogenesis, ↑ insulin sensitivity and adiponectin	[64]
HFD mice	*L. sakei* MJM60958	10^8 or 10^9 CFUs/day, 12 weeks	↓ hepatic steatosis, lipid accumulation and leptin, ↑ lipid oxidation and adiponectin, and microbiota modulation	[65]
HFD mice	*B. animalis* ssp. *lactis* MG741	10^5 or 10^6 CFUs/day, 12 weeks	↓ fat deposition, hepatic steatosis, hyperinsulinemia, gut permeability, inflammatory cytokines, and leptin, ↑ adiponectin	[66]
HFD with sucrose rats	*L. acidophilus*, *L. plantarum*, *B. bifidum*	Commercial mixture, 4 weeks	↓ hepatic steatosis, inflammatory cytokines, leptin, and resistin, improved lipid profile	[67]
HFD mice	*W. cibaria* MG5285 and *L. reuteri* MG5149	2×10^8 CFU/mouse, 8 weeks	↓ hepatic steatosis, lipogenic proteins, inflammatory cytokines, and leptin, improved glucose tolerance and lipid metabolism	[68]
HFD mice	*L. rhamnosus* GG	10^8 CFU/mouse, 13 weeks	improved insulin sensitivity and reduced lipid accumulation, ↑ increased fatty acid oxidation and adiponectin	[70]
HFD rats	*L. plantarum* NCU116	10^8 or 10^9 CFUs/mL (10 mL/kg b.w.), 5 weeks	↓ hepatic steatosis, oxidative stress, inflammatory cytokines, and endotoxins, ↑ increased fatty acid oxidation, microbiota modulation	[72]
HFD rats	*L. paracasei* JLUS66	1, 2, 4×10^{10} CFU/daily, 20 weeks	↓ oxidative stress, inflammatory cytokines, and LPS, ↑ anti-inflammatory IL-10, microbiota modulation	[74]

Table 1. *Cont.*

Animal Model	Probiotic Strains	Treatment	Main Findings	References
HFD mice	*L. reuteri* MJM60668	10^8 or 10^9 CFUs/day, 12 weeks	↓ hepatic steatosis, inflammatory cytokines, and lipogenesis, ↑ lipid oxidation and adiponectin, microbiota modulation	[78]
HFD rats	*B. lactis* V9	10^8 CFU/daily, 4 weeks	↓ hepatic steatosis, AST, ALT, glucose, lipogenesis, and inflammatory cytokines and mediators (TLR4, NF-κB), ↑ lipid oxidation	[79]
HFD mice	*L. plantarum* FZU3013	0.5 mL of 10^9 CFU/mL daily, 8 weeks	↓ hepatic steatosis, improved lipid profile, ↑ synthesis and excretion of bile acids, microbiota modulation	[83]
HFD mice	*L. rhamnosus* GG	10^8 CFU/mouse, 13 weeks	↓ hepatic steatosis, cholesterol synthesis, lipogenic and proinflammatory genes, improved lipid profile, microbiota modulation	[84]
HFD rats	Eosinophil-*Lactobacillus* tablets	312 mg/kg/d 10^7 eosinophil-*Lactobacillus*/g, 8 weeks	↓ hepatic steatosis, hepatic inflammation, serum lipids, AST, ALT, and total bile acids, microbiota modulation	[85]
HFD rats	*C. butyricum* MIYAIRI588	9×10^7 CFU/g in HFD, 12 weeks	↓ hepatic steatosis, improved lipid profile, ↑ cholesterol catabolism and excretion, ↑ excretion of bile acids	[87]
HFD mice	*B. longum*	5×10^{10} bacteria/kg/d, 4 weeks	↓ hepatic steatosis, glucose, improved glucose tolerance test, renin–angiotensin system modulation	[88]
HFD, high-cholesterol mice	*B. adolescentis* and *L. rhamnosus*	0.2 mL vehicle + 10^9 CFU/mL/d, 23 weeks	↓ hepatic steatosis and inflammation, ↑ short-chain fatty acids, microbiota modulation	[91]
Western diet mice	*L. acidophilus*, *L. fermentum*, *L. paracasei*, *L. plantarum*	10^9 CFU/g, 8 weeks	↓ hepatic steatosis, inflammatory cytokines, and cholesterol, microbiota modulation	[92]
HFD rats	*Lactobacillus*-fermented black barley	1 mL/100 g BW, 12 weeks	↓ hepatic steatosis, oxidative stress, microbiota and faecal metabolite modulation	[93]
HFD mice	*L. rhamnosus GG*	10^9 CFU/mouse/day, 9 weeks	↓ hepatic steatosis and lipid synthesis, bacteria and host competition for fatty acids	[95]
HFD mice	*F. prausnitzii* strains	0.25 mL 4×10^9 CFU/mL, 12 weeks	↓ hepatic steatosis, oxidative stress, inflammation, glucose intolerance, ↑ SCFA production, improved lipid profile, microbiota modulation	[96]
HFD mice	Long-term fermented soybean paste	100 mg/kg BW, 14 weeks	↓ hepatic steatosis, insulin resistance, inflammatory cytokine, and gut permeability and LPS, ↑ fatty acid oxidation and adiponectin	[101]

Symbols: ↓ means decreased; ↑ means increased.

All studies of animal models based on an HFD showed significant improvements in the main features of NAFLD after probiotic supplementation. The mechanisms of action are multifactorial, although they all converge on a reduction in the hepatic steatosis associated

with a normalization of lipid metabolism and a reduction in the pro-inflammatory and pro-oxidative states.

5. Clinical Trials

The study of the effects of probiotics has also been transferred to NAFLD patients in clinical trials (Table 2). The first clinical trial about probiotics and NAFLD to which we had access was published in 2011. In this double-blind randomized clinical trial, 28 patients with NAFLD diagnosed by liver biopsy were analysed over 3 months. The patients were divided into two groups: the treated group consumed one tablet per day of *Lactobacillus delbrueckki* subsp. *bulgarius* and *Streptococcus thermophilus* (500 million), whereas the placebo group was treated with one placebo tablet [102]. The ALT, AST, and γ-glutamyltransferase (GGT) levels improved significantly in the intervention group. However, non-changes were observed in the anthropometric parameters and cardiovascular risk factors. In a controlled clinical trial with 72 NAFLD patients, the intervention group consumed 300 g/day of probiotic yogurt containing *L. acidophilus La5* and *Bifidobacterium lactis Bb12*, whereas the control group consumed 300 g/day of conventional yogurt for 8 weeks [103]. Significant reductions were observed in the serum levels of ALT, AST, total cholesterol, and low-density lipoprotein cholesterol (LDL-c) when compared with the control group.

Table 2. Main results reported for the effects of probiotics in NAFLD patients.

Human Studies	Probiotic Strains	Treatment	Main Findings	References
30 NAFLD patients	*L. bulgaricus* and *Streptococcus thermophilus*	500 million/day, 3 months	↓ ALT, AST, GGT	[102]
72 NAFLD patients	*L. acidophilus La5* and *B. lactis Bb12*	2.39×10^6 and 2.08×10^6 CFU/g respectively daily, 8 weeks	↓ ALT, AST, total cholesterol, LDL-c	[103]
52 NAFLD patients	*L. casei, Lcb. rhamnosus, Streptococcus thermophilus, B. breve, L. acidophilus, B. longum,* and *L. bulgaricus*	200 million twice/day 28 weeks + diet + exercise	↓ ALT, AST, GGT, CPR, TNFα, NFκB, fibrosis score	[104]
42 NAFLD patients	*L. casei, L. acidophilus, L. rhamnosus 7, L. bulgaricus, B. breve, B. longum,* and *Streptococcus thermophilus*	$3 \times 10^9, 3 \times 10^{10}, 7 \times 10^9, 5 \times 10^8, 2 \times 10^{10}, 1 \times 10^9, 3 \times 10^8$ CFU/g respectively twice/day, 8 weeks	↓ insulin, TNFα, IL-6	[105]
50 NAFLD patients	*L. casei, L. rhamnosus, Streptococcus thermophilus, B. breve, L. acidophilus, B. longum,* and *L. bulgaricus*	200 million twice/day, 28 weeks + healthy lifestyle	↓ hepatic steatosis, fibrosis, fasting blood sugar, TG, hs-CRP, NFκB	[106]
64 NAFLD patients	*L. acidophilus, B. lactis, Bi B. bifidum, L. rhamnosus*	$3 \times 10^9, 6 \times 10^9, 2 \times 10^9, 2 \times 10^9$ CFU respectively daily, 12 weeks	↓ cholesterol, TG, LDL	[107]
58 NAFLD patients	*Lactobacillus, Lactococcus, Bifidobacterium, Propionibacterium, Acetobacter*	$6 \times 10^6, 1 \times 10^6, 3 \times 10^6, 1 \times 10^6$ CFU/g re 1 sachet (10 g)/day, 8 weeks	↓ FLI, aminotransferase activity, TNFα, and IL-6	[108]
102 NAFLD patients	*B. animalis*	300 g 10^8 CFUs/mL daily, 24 weeks + diet + exercise	↓ grades of NAFLD, liver enzyme concentrations	[109]

Table 2. *Cont.*

Human Studies	Probiotic Strains	Treatment	Main Findings	References
68 NAFLD patients	*L. acidophilus, L. rhamnosus, Lcb. Paracasei, Pediococcus pentosaceus, B. lactis,* and *B. brev*	Commercial mixture, 12 weeks	↓ body weight, total body fat, IHF fraction, TG	[110]
35 NAFLD patients	*Streptococcus thermophilus, B. breve, B. longum, B. infantis, L. acidophilus, L. plantarum, Lcb. Paracasei,* and *L. bulgaricus*	Commercial mixture 4 sachets/day, 10 weeks	No significant differences were observed	[111]
72 NAFLD patients	*L. acidophilus, L. casei* subsp., *L. lactis, B. bifidum, B. infantis,* and *B. longum*	30 billion CFU each sachet (3 g) 1 sachet twice daily, 6 months	↓ IFN-γ, TNF-α ↓ ZO-1	[18]

Symbols: ↓ means decreased; ↑ means increased.

In another randomized clinical trial, 52 patients with NAFLD were recommended to follow an energy-balanced diet and practice physical activity. They were supplemented with a symbiotic or a placebo capsule for 28 weeks twice daily [104]. Significant decreases were described in ALT, AST, GGT, high-sensitivity C-reactive protein, TNF-α, NFκB, and the fibrosis score in the symbiotic group when compared with the placebo group.

In a controlled clinical trial (IRCT: 2012122911920N1), 42 NAFLD patients diagnosed by ultrasound examination finished the study and received 1 g daily of placebo or probiotic for 2 months [105]. When compared to the beginning of the study, the insulin, insulin resistance, TNFα, and IL-6 levels significantly decreased in the probiotic group.

In another clinical trial (NCT02530138), 50 NAFLD participants were randomly allocated to receive either symbiotic supplementation or placebo capsules (maltodextrin) twice daily for 28 weeks [106]. Each symbiotic capsule contained 200 million bacteria (*L. casei, Lcd. rhamnosus, Streptococcus thermophilus, B. breve, L. acidophilus, B. longum,* and *L. bulgaricus*) and 125 mg of the prebiotic fructo-oligosaccharide. At the end of the study, fibrosis and hepatic steatosis were decreased in both groups. However, fasting blood sugar, TG, CRP, and NFκB were significantly reduced in the symbiotic group when compared with the placebo group. Another randomized trial (IRCT2013100414882N1) was conducted among 64 obese children with NAFLD, aged 10 to 18 years. The subjects were randomly assigned to one of the two groups receiving the treatment and placebo pills. The intervention group received one daily probiotic capsule for 12 weeks. The microbial strains were *L. acidophilus, B. lactis, Bi B. bifidum,* and *L. rhamnosus* [107]. At the end of the trial, the serum levels of TG, LDL, and cholesterol significantly decreased in the treated group. In contrast, only the TG level significantly decreased in the placebo group.

In a double-blind clinical trial (NCT03434860), 58 patients diagnosed with NAFLD were randomly divided into two groups receiving 10 g of the multiprobiotic "Symbiter" (14 probiotic bacteria belonging to the genera *Lactobacillus, Lactococcus, Bifidobacterium, Propionibacterium, Acetobacter*) per day or a placebo for 8 weeks [108]. At the end of the study, the fatty liver index (FLI), aminotransferase activity, TNFα, and IL-6 were significantly reduced after the treatment with probiotics. In another randomized clinical trial (IRCT2017020932417N2), 102 patients diagnosed with NAFLD were divided into three groups (two intervention groups and one control group) and evaluated for 24 weeks. The intervention group consumed 300 g symbiotic (containing 108 colony-forming units *B. animalis*/mL and 1.5 g insulin) or conventional yogurt daily and were advised to follow their diet and exercise plan, whereas the control group was only advised to follow a healthy lifestyle. A significant decrease was observed in the NAFLD results in the symbiotic treatment group when compared with the conventional and control groups. However, the ALT, AST, alanine phosphatase, and γ-glutamyltransferase levels were significantly decreased in all three groups [109]. Another clinical trial investigated the effects of probiotics for

12 weeks in 68 patients with NAFLD. This study administered a mixture of six probiotic agents (*L. acidophilus*, *Lcb. rhamnosus*, *Lcb. paracasei*, *Pediococcus pentosaceus*, *B. lactis*, and *B. breve*) in the probiotic group, whereas the placebo group was treated with dextran, maltodextrin, lemon flavour, and Mg stearate. The body weight, total body fat, intrahepatic fat (IHF) fraction, and triglyceride (TG) levels were reduced in the probiotic group but not in the placebo group [110].

A double-blinded study with 35 NAFLD patients (ISRCTN05474560) evaluated the effect of VSL#3® [111]. The patients ingested two sachets of probiotic or placebo twice daily for 10 weeks. However, no significant differences were reported after VSL#3® supplementation in the measured biomarkers for liver injury and cardiovascular risk. A randomized, place-controlled study involving 72 patients from Malaysia (#NCT04074889) was conducted [18]. The probiotics used were HEXBIO® Microbial Cell Preparation (MCP), from B-Crobes Laboratory Sdn. Bhd, which contained MCP® BCMC® strains. Each sachet of 3 g contained a total of 30 billion colony-forming units (CFU) with six probiotic strains (*L. acidophilus*, *L. casei* subsp. *L. lactis*, *B. bifidum*, *B. infantis*, and *B. longum*). In the probiotic group, a significant decrease in IFN-γ and TNF-α was observed, but an increase in IL-6 was revealed. Moreover, both the probiotic and placebo groups presented a significant increase in zonulin and a significant decrease in circulating ZO-1. Furthermore, this clinical trial suggested that *Lactobacillus* and *Bifidobacterium* could be useful for a well-balanced gut microbiota composition.

6. Conclusions

Due to the increase in a sedentary lifestyle and a higher caloric intake, all pathologies associated with being overweight and obesity are progressively increasing. NAFLD is a growing problem, being the main chronic liver condition, and there is not a specific drug treatment to reverse the disease. Studies in animal models and clinical trials have revealed the ability of probiotic supplementation to improve some of the main features related to the fatty liver, such as steatosis, the lipid profile, and the degree of oxidative stress and inflammation. The probiotics that were proven to make a difference in NAFLD management include *Lactobacillus* and *Bifidobacterium* species. Among the mechanisms involved, the activation of the pathways that lead to lipolysis and the inhibition of lipogenesis has been observed, as well as a normalization of the microbiota and intestinal permeability. In conclusion, the effects of the probiotics show that supplementation with probiotics could be a good candidate to consider for the prevention or reduction in NAFLD, accompanied by a balanced diet and healthy lifestyle. However, clinical trials with a larger number of patients and with longer-term interventions are still needed to determine the real efficacy that probiotics can exert.

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