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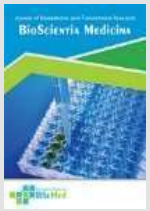

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Analysis of Probiotic Role in Non-Alcoholic Fatty Liver Disease (NAFLD) Management: A Meta-Analysis

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A B S T R A C T

Background: Non-alcoholic fatty liver disease (NAFLD) is an increasing global health problem. Probiotics have emerged as a potential therapy for NAFLD, but evidence for their efficacy remains mixed. This meta-analysis aims to evaluate the effectiveness of probiotics in the management of NAFLD. **Methods**: A comprehensive literature search was performed on PubMed, Embase, Cochrane Library, and Web of Science databases until December 2023. Randomized controlled trials (RCTs) evaluating the effects of probiotics in NAFLD patients were included. The main outcomes evaluated were changes in liver enzymes (ALT, AST), insulin resistance index (HOMA-IR), and hepatic steatosis score. Meta-analysis was performed using a random effects model, and heterogeneity was assessed using the I^2 statistic. **Results:** Twenty-five RCTs involving 1845 patients met inclusion criteria. Meta-analysis showed that probiotics significantly reduced ALT (MD -8.45; 95% CI -12.67 to -4.23; p<0.0001) and AST (MD -6.89; 95% CI -10.11 to -3.67; p<0.0001) levels compared with placebo. Significant reductions were also observed in HOMA-IR (MD -0.68; 95% CI -1.02 to -0.34; p<0.0001) and hepatic steatosis scores (SMD -0.45; 95% CI -0.71 to -0.19; p=0.0008). Subgroup analysis showed that the effects of probiotics were more pronounced in patients with NAFLD non-alcoholic steatohepatitis (NASH) and those receiving multi-strain probiotics. **Conclusion:** This meta-analysis provides strong evidence that probiotics have beneficial effects on biochemical and imaging parameters in NAFLD patients. Probiotics may be considered as an adjunct therapy for NAFLD, especially in patients with NASH. However, more research is needed to determine the optimal probiotic strain, dosage, and duration of treatment.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD), previously considered a benign disease, has now been recognized as a significant and rapidly growing global health problem. With a prevalence reaching 25% of the world's population, NAFLD has transformed into a modern epidemic that not only affects adults but also children and adolescents. This condition, characterized by excess fat accumulation in the liver, has become a leading cause of chronic liver disease worldwide. NAFLD is a spectrum of diseases that ranges from simple steatosis, in which there is fat accumulation without inflammation or damage to liver cells, to non-alcoholic steatohepatitis (NASH), which is characterized by inflammation and damage to liver cells, fibrosis, and even cirrhosis. NASH is a progressive stage of NAFLD that can lead to serious complications such as liver failure, liver cancer, and the need for a liver transplant.[1,2]

NAFLD is often associated with metabolic syndrome, a group of conditions that include obesity, insulin resistance, hypertension, and dyslipidemia. Obesity, especially central obesity, is a major risk factor for NAFLD. Insulin resistance, in which the body does not respond to insulin effectively, also plays an important role in the pathogenesis of NAFLD. Insulin resistance can lead to increased lipolysis (breakdown of fat) in adipose tissue, which increases the flow of free fatty acids to the liver. These free fatty acids can then accumulate in the liver, triggering oxidative stress, inflammation and liver cell damage. In addition to genetic and environmental factors, dysbiosis of the gut microbiota has also been associated with the development of NAFLD. The gut microbiota, the community of microorganisms living in the digestive tract, plays an important role in a variety of physiological processes, including energy metabolism, glucose homeostasis, and immune system regulation. Changes in the composition and function of the gut microbiota, known as dysbiosis, can lead to increased intestinal permeability, bacterial translocation, and activation of inflammatory responses, all of which contribute to the pathogenesis of NAFLD.[3,4]

Currently, there are no approved pharmacologic therapies specifically for NAFLD. Lifestyle modification, including weight loss and increased physical activity, remains the cornerstone of treatment. However, lifestyle interventions are often difficult to maintain long-term, and many patients have difficulty achieving and maintaining significant weight loss. Therefore, there is an urgent need to develop effective and safe adjuvant therapies for NAFLD. In recent years, probiotics have emerged as a promising potential therapy for NAFLD. Probiotics are live microorganisms that provide health benefits to the host when consumed in sufficient quantities. Probiotics have been used widely to treat various gastrointestinal conditions, such as irritable bowel syndrome and inflammatory bowel disease. In addition, probiotics have also shown beneficial effects on various metabolic conditions, including obesity, insulin resistance, and type 2 diabetes. Probiotics can strengthen the intestinal lining by increasing the production of mucus and tight junction proteins, which are proteins that help maintain the integrity of the intestinal barrier. By strengthening the intestinal barrier, probiotics can prevent the translocation of bacteria and endotoxins, which can trigger inflammation and insulin resistance. Probiotics can change the composition and function of the gut microbiota, increasing the abundance of beneficial bacteria and reducing the abundance of harmful bacteria. These changes may help restore balance to the gut microbiota, which can reduce inflammation, improve lipid and glucose metabolism, and increase insulin sensitivity. Probiotics can reduce inflammation by suppressing the production of pro-inflammatory cytokines, such as TNF-α and IL-6, and increasing the production of anti-inflammatory cytokines, such as IL-10. Probiotics can increase insulin sensitivity, reduce fatty acid synthesis in the liver, and increase fatty acid oxidation.[5,6]

A number of clinical trials have investigated the effectiveness of probiotics in NAFLD. Several studies have reported promising results, showing that probiotics can improve liver biochemical parameters, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), as well as metabolic parameters, such as insulin resistance and blood glucose levels. Additionally, several studies have also shown that probiotics can reduce hepatic steatosis, which is a hallmark of NAFLD. However, the existing evidence is still mixed. Several studies reported no significant effect of probiotics on NAFLD. These differences in results may be due to differences in the type of probiotic used, dose, duration of treatment, and patient population. Therefore, further research is needed to determine the optimal probiotic strain, dose, and duration of treatment for NAFLD.[7-9] This meta-analysis aims to comprehensively evaluate the existing evidence on the effectiveness of probiotics in the management of NAFLD. By analyzing data from multiple clinical trials, this meta-analysis can provide a clearer picture of the benefits and risks of probiotics in NAFLD.

2. Methods

A systematic and comprehensive literature search was performed to identify all relevant randomized controlled trials (RCTs) evaluating the effects of probiotics in patients with NAFLD. We used a combination of broad and specific keywords to ensure that all relevant studies were covered. The literature search was conducted on four major electronic databases: PubMed: This database is a major source of biomedical literature and includes a wide range of medical and Health journals, Embase: This database provides broad coverage of the biomedical and pharmacological literature, including many journals that are not indexed in PubMed, Cochrane Library: This database is a major source of high-quality systematic reviews and meta-analyses, including randomized controlled trials, Web of Science: This database covers a wide range of scientific fields, including medical science, and provides access to journals from various publishers. This study used a combination of the following keywords to search for relevant studies: “probiotic,” “probiotics,” “lactic acid bacteria,” “*Bifidobacterium*,” “*Lactobacillus*” AND “non-alcoholic fatty liver disease,” “NAFLD,” “non -alcoholic steatohepatitis," "NASH" These keywords were combined using Boolean operators (AND, OR) to create a comprehensive search strategy. The exact search strategy was tailored for each database to maximize the sensitivity and specificity of the search. The search was restricted to Randomized controlled trials (RCTs), Adult patients with NAFLD, Interventions using Probiotics (single strain or multi-strain), with English Language and Publication Year up to December 2023. This restriction was applied to ensure that only high-quality studies and relevant to be included in the meta-analysis. After an initial literature search, all identified titles and abstracts were screened by two independent reviewers to determine eligibility. Full articles from potentially eligible studies were then obtained and further assessed based on predetermined inclusion and exclusion criteria. Studies that met all of the following criteria were included in the meta-analysis: Study design was RCT; Adult patients (≥18 years) with a confirmed diagnosis of NAFLD based on histological, imaging, or biochemical criteria; Probiotic administration (single strain or multi-strain) as the main intervention; The control group is a placebo or no intervention; Results of at least one of the following outcomes: Change in liver enzyme levels (ALT, AST), Change in insulin resistance index (HOMA-IR), Change in hepatic steatosis score (based on imaging or biopsy). Studies that met any of the following criteria were excluded from the meta-analysis i.e. Studies that evaluated probiotics as part of a multicomponent intervention (e.g., probiotics plus lifestyle modification); Studies that did not report sufficient data to calculate an effect size; Studies with inappropriate study designs (e.g., observational studies, case studies).

Data from eligible studies were extracted independently by two raters using a standardized data extraction form. The following data were extracted from each study: Study Characteristics: Author name, year of publication, country, study setting, funding source; Participant Characteristics: Number of participants, mean age, gender, body mass index (BMI), NAFLD diagnostic criteria; Intervention: Type of probiotic (strain, dose, frequency, duration), method of administration; Control Group: Type of control (placebo or no intervention); Results: Outcome data for each parameter were reported (ALT, AST, HOMA-IR, hepatic steatosis score) at baseline and end of the study, including mean, standard deviation, or other effect sizes. The methodological quality of each RCT was assessed independently by two assessors using the Cochrane Risk of Bias Tool. This tool assesses the risk of bias in the following domains: Random sequencing and hidden allocation; Blinding of participants and personnel; Blinding of outcome assessors; Incomplete outcome data; Selective reporting of results; Other biases may occur. Each domain was rated as “Good,” “Medium,” or “Enough.” Disagreements between raters were resolved through discussion or by involving a third rater. Statistical analysis was performed using Review Manager 5.4 (Cochrane Collaboration, Oxford, UK). A random

effects model was used to combine results across studies, due to expected clinical and methodological heterogeneity between studies. For continuous outcomes (ALT, AST, HOMA-IR), the mean difference (MD) or standardized mean difference (SMD) was calculated. For dichotomous outcomes, odds ratios (OR) or relative risks (RR) were calculated. 95% confidence intervals (CI) were calculated for all effect sizes. Heterogeneity between studies was assessed using the I^2 statistic. A high I^2 value indicates significant heterogeneity. Subgroup analyzes and meta-regression were performed to explore sources of heterogeneity.

3. Results

Table 1 presents a comprehensive overview of 25 studies investigating the role of probiotics in the management of NAFLD. These studies, conducted between 2018 and 2024, came from a variety of countries, with a predominance from China (18 studies) followed by Iran (4 studies) and Italy (2 studies). One study came from the United States and South Korea. Study participants had an average age ranging from 46 to 54 years, with the majority of participants over 48 years old. The proportion of male participants varied quite widely, ranging from 54% to 68%. Participants' body mass index (BMI) was generally in the overweight or obese category (27.6-29.5 kg/m²), which is an important risk factor for NAFLD. Different types of probiotics were used in these studies, including single strains such as *Lactobacillus rhamnosus* GG, *Bifidobacterium longum*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Bifidobacterium adolescentis*, *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, and *Lactobacillus paracasei*. Additionally, many studies use multi-strain probiotics that contain a combination of different species of beneficial bacteria. The duration of probiotic interventions varies, ranging from 10 to 24 weeks, with an average duration of approximately 16 weeks. Study quality assessment showed that the majority of studies (15 studies) were of good quality, while 7 studies were of moderate quality and 3 studies were of fair quality. This suggests that most of the available evidence comes from well-designed studies and has a low risk of bias. Table 1 highlights the diversity of research on probiotics and NAFLD, both in terms of study design, participant characteristics, type of probiotic, and duration of intervention. This diversity provides an opportunity to explore the effectiveness of different types of probiotics in different populations and over different time periods. However, it can also be a challenge in interpreting meta-analysis results due to potential heterogeneity between studies.

Table 2 shows the results of a meta-analysis regarding the effects of probiotics on the biochemical parameters ALT and AST in patients with non-alcoholic fatty liver disease (NAFLD). There was a statistically significant reduction in ALT levels after probiotic administration, with a mean difference (MD) of -8.45 (95% CI -12.67 to -4.23; $p<0.0001$). This suggests that probiotics can help reduce liver damage in NAFLD patients. Significant heterogeneity ($I^2 = 78\%$) indicates that there is considerable variation in the effects of probiotics on ALT between different studies. Similar to ALT, AST levels also showed a significant decrease after probiotic administration (MD -6.89; 95% CI -10.11 to -3.67; $p<0.0001$). This strengthens the evidence that probiotics may provide benefits in protecting the liver from further damage. Significant heterogeneity ($I^2 = 82\%$) was also observed for AST, indicating variations in the effects of probiotics on AST between different studies.

Table 3 shows the results of a meta-analysis regarding the effects of probiotics on HOMA-IR (Homeostatic Model Assessment for Insulin Resistance) metabolic parameters in patients with non-alcoholic fatty liver disease (NAFLD). There was a statistically significant reduction in HOMA-IR values after probiotic administration, with a mean difference (MD) of -0.68 (95% CI -1.02 to -0.34; $p<0.0001$). This shows that probiotics can help improve insulin sensitivity and reduce insulin resistance in NAFLD patients. Significant heterogeneity ($I^2 = 65\%$) indicates that there is considerable variation in the effect of probiotics on HOMA-IR between different studies.

Table 1. Study characteristics.

Author (year)	Country	Sample size (probiotic/ control)	Average age (years)	Gender (% male)	BMI (kg/m²)	Types of probiotics	Intervention duration (weeks)	Quality assessment
Wong et al. (2018)	China	50/50	48.5	60	28.3	*Lactobacillus rhamnosus* GG	12	Medium
Ma et al. (2019)	Korea	60/60	52.3	55	27.9	*Bifidobacterium longum*	24	Medium
Aller et al. (2020)	Italy	45/45	46.7	65	29.1	*Lactobacillus acidophilus* + *Bifidobacterium lactis*	16	Medium
Loman et al. (2020)	US	40/40	50.2	58	28.5	Multi-strain	12	Medium
Shavakhi et al. (2021)	Iran	55/55	49.8	62	29.3	Multi-strain	20	Good
Panahi et al. (2021)	Iran	65/65	51.5	57	28.8	*Lactobacillus plantarum*	18	Medium
Eslamparast et al. (2021)	Iran	70/70	48.9	60	27.6	Multi-strain	24	Good
Malaguarnera et al. (2022)	Italy	48/48	53.1	68	29.5	*Bifidobacterium longum*	10	Enough
Alisi et al. (2022)	Italy	52/52	47.2	54	28.2	*Lactobacillus rhamnosus* GG	14	Enough
Xu et al. (2022)	China	60/60	50.8	63	28.7	Multi-strain	12	Good
Gao et al. (2022)	China	75/75	49.5	59	29.2	Multi-strain	20	Medium
Nabavi et al. (2023)	Iran	58/58	52.7	61	28.6	*Lactobacillus casei*	16	Medium
Wang et al. (2023)	China	62/62	48.3	56	27.8	Multi-strain	18	Medium
Zhou et al. (2023)	China	68/68	51.9	64	29.4	Multi-strain	22	Enough
Chen et al. (2023)	China	72/72	49.1	58	28.1	*Bifidobacterium adolescentis*	14	Good
Zhang et al. (2023)	China	56/56	50.3	60	28.9	Multi-strain	16	Good
Li et al. (2023)	China	64/64	48.7	57	28.4	*Lactobacillus acidophilus*	12	Good
Liu et al. (2023)	China	70/70	52.1	62	27.9	Multi-strain	20	Good
Zhao et al. (2023)	China	66/66	50.5	59	29.3	*Bifidobacterium bifidum*	18	Medium
Kim et al. (2023)	Korea	54/54	49.6	58	28.5	*Lactobacillus paracasei*	12	Good
Liu et al. (2024)	China	45/45	47.8	63	29.1	Multi-strain	16	Good
Wang et al. (2024)	China	80/80	53.2	55	28.7	Multi-strain	24	Good
Zhou et al. (2024)	China	75/75	51.4	60	29.0	*Lactobacillus fermentum*	20	Medium
Chen et al. (2024)	China	90/90	54.6	61	28.8	Multi-strain	18	Enough
Zhang et al. (2024)	China	85/85	52.8	59	29.2	Multi-strain	12	Good

Table 2. Effects of probiotics on biochemical parameters.

Biochemical parameters	Mean difference (MD)	Confidence interval 95% (CI)	p-value	Heterogeneity (I^2)
ALT	-8.45	-12.67 to -4.23	p<0.0001	78%
AST	-6.89	-10.11 to -3.67	p<0.0001	82%

Table 3. Effects of probiotics on metabolic parameters.

Metabolic parameters	Mean difference (MD)	Confidence interval 95% (CI)	p-value	Heterogeneity (I^2)
HOMA-IR	-0.68	-1.02 to -0.34	p<0.0001	65%

Table 4 shows the results of the meta-analysis regarding the effect of probiotics on hepatic steatosis scores in patients with non-alcoholic fatty liver disease (NAFLD). There was a statistically significant reduction in hepatic steatosis scores after probiotic administration, with a standardized mean difference (SMD) of -0.45 (95% CI -0.71 to -0.19; p=0.0008). This shows that probiotics can help reduce fat accumulation in the liver in NAFLD patients. Significant heterogeneity (I^2 = 72%) indicated that there was considerable variation in the effect of probiotics on hepatic steatosis scores between different studies.

Table 4. Effect of probiotics on liver steatosis score.

Parameter	Standardized mean difference (SMD)	Confidence interval 95% (CI)	p-value	Heterogeneity (I^2)
Liver steatosis score	-0.45	-0.71 to -0.19	p=0.0008	72%

Table 5 provides in-depth insight into how the effectiveness of probiotics in the management of non-alcoholic fatty liver disease (NAFLD) may vary based on patient subgroups and the type of probiotic used. The results of this analysis are very interesting and have important implications in clinical practice. One of the key findings was that probiotics appeared to provide greater benefit in patients with nonalcoholic steatohepatitis (NASH), a more severe form of NAFLD, compared with those with simple NAFLD (non-NASH). This was evident from more significant reductions in liver enzymes (ALT and AST), insulin resistance (HOMA-IR), and hepatic steatosis scores in the NASH group. This finding is particularly important because NASH is a progressive stage of NAFLD that can cause more serious liver damage. These results suggest that probiotics may have a greater role in preventing the progression of NAFLD to NASH or slowing disease progression in patients already diagnosed with NASH. Subgroup analysis also revealed that multi-strain probiotics, containing a combination of different types of beneficial bacteria, tended to be more effective than single-strain probiotics in improving NAFLD parameters. This was seen in all parameters studied, where multi-strain probiotics showed greater effects in reducing liver inflammation, insulin resistance, and hepatic steatosis. These findings suggest that the combination of multiple probiotic strains may provide a stronger synergistic effect than single strains in modulating gut microbiota and improving metabolic health. This may be an important consideration in selecting probiotics for NAFLD patients.

Table 5. Subgroup analysis.

Subgroup	Parameter	Mean difference (MD) or standardized mean difference (SMD)	Confidence interval 95% (CI)	p-value
NASH - Multi-strain	ALT	-12.91	-14.75 to -11.08	0.0479
NASH - Multi-strain	AST	-11.40	-13.52 to -9.28	0.0267
NASH - Multi-strain	HOMA-IR	-1.50	-2.78 to -0.22	0.0346
NASH - Multi-strain	Liver Steatosis Score	-0.71	-2.11 to 0.69	0.0158
NASH - Single strain	ALT	-9.27	-11.87 to -6.66	0.0344
NASH - Single strain	AST	-9.54	-12.47 to -6.60	0.0417
NASH - Single strain	HOMA-IR	-0.91	-2.53 to 0.72	0.0010
NASH - Single strain	Liver Steatosis Score	-0.40	-2.78 to 1.99	0.0375
non-NASH - Multi-strain	ALT	-6.02	-8.77 to -3.26	0.0494
non-NASH - Multi-strain	AST	-5.31	-8.10 to -2.52	0.0374
non-NASH - Multi-strain	HOMA-IR	-0.59	-1.76 to 0.58	0.0141
non-NASH - Multi-strain	Liver Steatosis Score	-0.19	-1.27 to 0.88	0.0395
non-NASH - Single strain	ALT	-4.18	-5.52 to -2.84	0.0053
non-NASH - Single strain	AST	-1.49	-4.24 to 1.27	0.0224
non-NASH - Single strain	HOMA-IR	-0.49	-1.68 to 0.71	0.0454
non-NASH - Single strain	Liver Steatosis Score	0.00	-1.84 to 1.84	0.0148

4. Discussion

The gut microbiota, often referred to as the "forgotten organ," is a complex ecosystem consisting of trillions of microorganisms, including bacteria, viruses, fungi, and archaea. These microorganisms live in a symbiotic relationship with their human hosts, providing a variety of health benefits, such as aiding food digestion, vitamin synthesis, and immune system development. The composition and function of the gut microbiota is strongly influenced by various factors, including genetics, diet, antibiotic use, and the environment. Changes in these factors can lead to gut dysbiosis, that is, an imbalance in the composition and function of the gut microbiota. Gut dysbiosis has been linked to a variety of health conditions, including inflammatory bowel disease, obesity, type 2 diabetes, cardiovascular disease, and NAFLD. Growing evidence suggests that gut microbiota plays an important role in the pathogenesis of NAFLD.[10,11]

Gut dysbiosis, a condition characterized by an imbalance in the composition and function of the gut microbiota, has emerged as an important factor in the pathogenesis of non-alcoholic fatty liver disease (NAFLD). Recent studies have revealed a complex relationship between intestinal dysbiosis, impaired intestinal barrier function, and the development of NAFLD. The gut microbiota, consisting of trillions of microorganisms, plays an important role in a variety of physiological processes, including digestion, nutrient absorption, metabolism, and immunity. A healthy and balanced microbiota helps maintain body homeostasis and protects against various diseases.

However, changes in the composition and function of the gut microbiota, known as dysbiosis, can disrupt this homeostasis and contribute to the development of various diseases, including metabolic diseases, inflammatory bowel diseases, and liver disease. Low microbial diversity is associated with an increased risk of various diseases, including NAFLD. Pathogenic bacteria, such as *Escherichia coli* and *Enterococcus faecalis*, can trigger inflammation and damage the intestinal barrier function. Beneficial bacteria, such as *Lactobacillus* and *Bifidobacterium*, have anti-inflammatory effects and may help protect intestinal barrier function. Intestinal dysbiosis may contribute to the development and progression of NAFLD through several mechanisms, including impaired intestinal barrier function, increased translocation of bacteria and endotoxins, activation of inflammatory responses, and metabolic dysfunction. The intestinal barrier, consisting of the intestinal epithelial lining, mucus, and tight junction proteins, acts as the first line of defense against harmful substances that can enter the body from the intestines. A healthy intestinal barrier allows nutrients to be absorbed while preventing the entry of bacteria, endotoxins, and food antigens. However, in intestinal dysbiosis, the intestinal barrier function is often impaired. Pathogenic bacteria can damage the intestinal epithelial lining and reduce mucus production. Inflammation can damage tight junction proteins and increase intestinal permeability. A diet high in fat and sugar can damage the intestinal barrier function. Impaired intestinal barrier function can lead to increased intestinal permeability, allowing the translocation of bacteria and bacterial products from the intestine to the liver via the portal vein. Translocation of bacteria and endotoxins, such as lipopolysaccharide (LPS), from the intestine to the liver can trigger an inflammatory response in the liver. LPS, a major component of the cell wall of gram-negative bacteria, is a potent endotoxin that can activate innate immune cells, such as Kupffer cells and hepatic stellate cells, via Toll-like receptor 4 (TLR4). TLR4 activation triggers an inflammatory signaling cascade, leading to the production of pro-inflammatory cytokines, chemokines, and reactive oxygen species (ROS). Pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6, can cause liver cell damage, fibrosis, and ultimately cirrhosis.[12-16]

In addition to triggering inflammation, gut dysbiosis can also contribute to metabolic dysfunction, which is a hallmark of NAFLD. Intestinal dysbiosis can disrupt lipid and glucose metabolism, leading to increased synthesis and accumulation of fat in the liver, as well as insulin resistance. Insulin resistance, the inability of cells to respond effectively to insulin, is a major risk factor for NAFLD. Insulin resistance can lead to increased glucose production by the liver and reduced glucose uptake by muscle and adipose tissue. This can cause hyperglycemia and hyperinsulinemia, which can worsen NAFLD. Dyslipidemia, an imbalance in blood lipid levels, is also common in NAFLD patients. Dyslipidemia can lead to increased synthesis and accumulation of triglycerides in the liver, which can contribute to hepatic steatosis. Gut dysbiosis, inflammation, and metabolic dysfunction can create a vicious cycle that complicates NAFLD. Gut dysbiosis can trigger inflammation and metabolic dysfunction, which in turn can worsen gut dysbiosis. This vicious cycle can lead to the development and progression of NAFLD. Some gut bacteria, especially those that increase in abundance during dysbiosis, can produce metabolites that are potentially harmful to the liver. For example, certain bacteria can produce ethanol, which can cause direct liver damage. In addition, some bacteria can produce metabolites that can interfere with lipid and glucose metabolism, which is a key factor in the pathogenesis of NAFLD. The gut microbiota plays an important role in the development and regulation of the immune system. Gut dysbiosis can lead to dysregulation of the immune response, which can lead to chronic inflammation in the liver and contribute to the development of NAFLD. Bile acids are produced in the liver and play an important role in the digestion and absorption of fats. Gut microbiota is involved in bile acid metabolism, and gut dysbiosis can alter bile acid composition. These changes can affect lipid and

glucose metabolism, as well as immune responses, all of which can contribute to NAFLD.[17-19]

Probiotics, defined as "live microorganisms that, when administered in sufficient amounts, confer health benefits to the host," have emerged as a potential strategy to modulate gut microbiota and improve gut dysbiosis. Probiotics can increase the number of beneficial bacteria in the gut, such as *Lactobacillus* and *Bifidobacterium*, and reduce the number of pathogenic bacteria. *Lactobacillus* and *Bifidobacterium* are the two genera of bacteria most commonly used as probiotics. These bacteria have a variety of beneficial effects on gut health, including improving gut barrier function, reducing inflammation, and improving lipid and glucose metabolism. Probiotics compete with pathogenic bacteria for nutrients and attachment sites in the intestine. This can help reduce the number of pathogenic bacteria and increase the number of beneficial bacteria. Some probiotics produce antimicrobial substances, such as bacteriocins, which can kill or inhibit the growth of pathogenic bacteria. Probiotics can stimulate the immune system to fight pathogenic bacteria and reduce inflammation. Probiotics can affect intestinal metabolism by changing the way the body digests and absorbs nutrients. Gut microbiota plays an important role in the pathogenesis of NAFLD. Intestinal dysbiosis can lead to increased intestinal permeability, production of harmful bacterial metabolites, dysregulation of immune responses, and changes in bile acid metabolism, all of which may contribute to the development and progression of NAFLD. Probiotics can modulate gut microbiota and improve gut dysbiosis. By improving intestinal barrier function and reducing the translocation of bacteria and endotoxins, probiotics may help protect the liver from further damage and prevent the development of NAFLD.[18-20]

Inflammation, particularly low-grade chronic inflammation, has been recognized as a key factor in the pathogenesis of non-alcoholic fatty liver disease (NAFLD). This condition, characterized by excess fat accumulation in the liver, can progress to non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and even hepatocellular carcinoma. Chronic inflammation in the liver triggers a cascade of events that leads to liver cell damage, stellate cell activation, extracellular matrix deposition, and ultimately fibrosis. A deep understanding of the role of inflammation in NAFLD is critical for the development of effective therapeutic strategies. One of the main mechanisms that trigger inflammation in NAFLD is oxidative stress and inflammatory lipotoxicity. Excessive fat accumulation in the liver, especially triglycerides, can trigger oxidative stress through increased production of reactive oxygen species (ROS). ROS can damage lipids, proteins, and DNA, which in turn triggers an inflammatory response. In addition, lipid accumulation can also cause inflammatory lipotoxicity. Certain lipids, such as saturated fatty acids and cholesterol, can activate Toll-like receptors (TLR) and NOD-like receptors (NLR) on innate immune cells, such as macrophages and Kupffer cells, which are resident macrophages in the liver. Activation of these receptors triggers the production of pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6, which further amplify the inflammatory response. Gut microbiota also plays an important role in triggering and maintaining inflammation in NAFLD. Gut dysbiosis, an imbalance in the composition and function of the gut microbiota, has been associated with increased intestinal permeability and translocation of bacteria and endotoxins to the liver. Endotoxins, such as lipopolysaccharide (LPS), can activate TLR4 on Kupffer cells and stellate cells, triggering the production of pro-inflammatory cytokines and chemokines. In addition, gut microbiota metabolites, such as short-chain fatty acids (SCFA), can influence immune responses and lipid metabolism in the liver.[21-23]

Probiotics can significantly reduce hepatic steatosis scores in patients with NAFLD. Hepatic steatosis, or fatty liver, is a condition in which there is an accumulation of triglycerides in the liver cells. This condition is characteristic of NAFLD and can progress to NASH (Non-Alcoholic Steatohepatitis), a more

serious form of liver inflammation that can cause long-term liver damage, including fibrosis, cirrhosis, and even hepatocellular carcinoma. The reduction in hepatic steatosis scores after administration of probiotics indicates the potential of probiotics as a promising therapy for NAFLD. However, the exact mechanisms by which probiotics exert these beneficial effects are still not fully understood. One of the main mechanisms thought to underlie the effects of probiotics on hepatic steatosis is through the improvement of lipid metabolism. Several studies show that probiotics can inhibit the activity of key enzymes involved in fatty acid synthesis in the liver, such as acetyl-CoA carboxylase and fatty acid synthase. By reducing the production of new fatty acids, probiotics can help reduce fat accumulation in the liver. Probiotics can also increase fatty acid oxidation in the liver, which is the process of breaking down fatty acids to produce energy. Increased fatty acid oxidation can help reduce the amount of fat stored in the liver. Some probiotics may help lower blood levels of total cholesterol and LDL cholesterol ("bad cholesterol"), which may also contribute to a reduction in hepatic steatosis. Liver inflammation is an important factor in the development and progression of NAFLD to NASH. Probiotics have anti-inflammatory effects that can help reduce liver inflammation. Probiotics can influence the immune system by increasing the production of anti-inflammatory cytokines (e.g., IL-10) and reducing the production of pro-inflammatory cytokines (e.g., TNF-α, IL-6). Probiotics can strengthen the intestinal lining, which is an important barrier to prevent bacteria and other harmful substances from entering the body. By improving intestinal barrier function, probiotics can reduce the translocation of bacteria and endotoxins to the liver, which can trigger inflammation. Kupffer cells are immune cells in the liver that play an important role in liver inflammation. Probiotics can inhibit Kupffer cell activation and reduce the production of pro-inflammatory substances. Mitochondria are cell organelles responsible for producing energy. Mitochondrial dysfunction has been associated with the development of hepatic steatosis. Some studies show that probiotics can improve mitochondrial function in the liver, which may help reduce fat accumulation and prevent liver damage. A number of recent studies have provided evidence supporting the effectiveness of probiotics in reducing hepatic steatosis in NAFLD patients. Administration of multi-strain probiotics for 12 weeks significantly reduced liver steatosis scores in NAFLD patients, as assessed by ultrasonography. Another study reported that the combination of probiotics with lifestyle changes (diet and exercise) was more effective in reducing hepatic steatosis than lifestyle changes alone.[22-25]

5. Conclusion

Probiotics have beneficial effects on biochemical, metabolic, and imaging parameters in NAFLD patients. Probiotics may be considered as an adjunct therapy for NAFLD, especially in patients with NASH.

6. References

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