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# The role of gut microbiota augmentation in managing non-alcoholic fatty liver disease: an in-depth umbrella review of meta-analyses with grade assessment

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**Background and aim:** Currently, there are no authorized medications specifically for non-alcoholic fatty liver disease (NAFLD) treatment. Studies indicate that changes in gut microbiota can disturb intestinal balance and impair the immune system and metabolism, thereby elevating the risk of developing and exacerbating NAFLD. Despite some debate, the potential benefits of microbial therapies in managing NAFLD have been shown.

**Methods:** A systematic search was undertaken to identify meta-analyses of randomized controlled trials that explored the effects of microbial therapy on the NAFLD population. The goal was to synthesize the existing evidence-based knowledge in this field.

**Results:** The results revealed that probiotics played a significant role in various aspects, including a reduction in liver stiffness (MD: −0.38, 95% CI: [−0.49, −0.26]), hepatic steatosis (OR: 4.87, 95% CI: [1.85, 12.79]), decrease in body mass index (MD: −1.46, 95% CI: [−2.43, −0.48]), diminished waist circumference (MD: −1.81, 95% CI: [−3.18, −0.43]), lowered alanine aminotransferase levels (MD: −13.40, 95% CI: [−17.02, −9.77]), decreased aspartate aminotransferase levels (MD: −13.54, 95% CI: [−17.85, −9.22]), lowered total cholesterol levels (MD: −15.38, 95% CI: [−26.49, −4.26]), decreased fasting plasma glucose levels (MD: −4.98, 95% CI: [−9.94, −0.01]), reduced fasting insulin (MD: −1.32, 95% CI: [−2.42, −0.21]), and a decline in homeostatic model assessment of insulin resistance (MD: −0.42, 95% CI: [−0.72, −0.11]) ($P < 0.05$).

**Conclusion:** Overall, the results demonstrated that gut microbiota interventions could ameliorate a wide range of indicators including glycemic profile, dyslipidemia, anthropometric indices, and liver injury, allowing them to be considered a promising treatment strategy.

**Keywords:** meta-analysis, non-alcoholic fatty liver disease, prebiotic, probiotic, synbiotic, umbrella review

## Introduction

Overindulgence in fat accumulation in the liver is indicative of non-alcoholic fatty liver disease (NAFLD), which in turn triggers fibrosis and necroinflammation, ultimately resulting in liver failure[1–6]. Simple steatosis and non-alcoholic steatohepatitis (NASH) are among the many diseases that make up this disease[7–12]. Based on yearly increases in prevalence, NAFLD prevalence trended upward by 0.7%, with an estimated 29.8% prevalence worldwide. With a frequency of 35.7% and 35.3%, respectively, South America and North America were reported to have the highest rates of NAFLD, despite the fact that the condition is quite common throughout the world[13].

NAFLD is thought to be caused by a combination of genetic, environmental, and nutritional variables that alter glucose and lipid metabolism[14–19]. The exact pathophysiology of NAFLD is

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yet unknown. Recent research has identified the involvement of gut dysbiosis and its metabolites in the pathophysiology of NAFLD among the several risk factors[20–22]. According to recent studies, intestinal dysbiosis may have an impact on the innate immune system, gut permeability, and the fermentation of indigestible carbohydrates, all of which may contribute to NAFLD[23,24].

It has been shown that the gut microbiota of individuals with NAFLD is different from that of healthy individuals. Nutrition plays a crucial role in NAFLD because food can modify the gut microbiome. This means that changes in the gut microbiota caused by genetic and environmental factors can disrupt intestinal balance, impair the immune system, and impact metabolism. These factors can increase the risk of developing and exacerbating NAFLD[14,25–27].

The management of NAFLD mostly involves modifying lifestyle factors such as weight loss, physical activity, and diet, as there are currently limited pharmaceutical treatments available[20,28–31]. While there isn't a specific treatment for NAFLD, microbial therapies such as probiotics, prebiotics, and synbiotics are thought to offer a novel therapeutic approach by modifying the intestinal microbiota[32,33]. Live microorganisms that are beneficial to an individual's health and can manage their gut flora are known as probiotics[34]. Prebiotics are foods that are indigestible but have the ability to specifically stimulate the creation of certain bacteria in the human body[35], while synbiotics are a blend of probiotics and prebiotics[36].

Although the results of earlier studies were controversial, they demonstrated the potential benefits of microbial therapy on NAFLD; as a result, no drugs have been licensed for the treatment of NAFLD patients[20,37–41]. Therefore, our goal was to perform a comprehensive, evidence-based evaluation of meta-analysis studies in order to present a full picture of how microbial therapy affects the NAFLD population.

## Materials and methods

The study protocol for our umbrella review has been registered in PROSPERO under the designated registration code [CRD42024510147]. Throughout the research process, we adhered to standard methods outlined for umbrella reviews and reported our findings in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA, Supplemental Digital Content 1, http://links.lww.com/MS9/A507) guidelines[42]. The AMSTAR 2 checklist (Supplemental Digital Content 2, http://links.lww.com/MS9/A508) was utilized for the quality assurance of the current umbrella review[43].

### Search strategy

An extensive search was conducted across the PubMed, Scopus, and Web of Science databases until 1 January 2023, with the objective of identifying meta-analysis studies of randomized controlled trials (RCTs) that assess the impact of microbial treatments on NAFLD. The search strategy and keywords used are detailed in Table S1 in the supplementary material (Supplemental Digital Content 3, http://links.lww.com/MS9/A509).

### HIGHLIGHTS

- Non-alcoholic fatty liver disease (NAFLD) stands out as the predominant cause of liver diseases, intricately linked to a spectrum of metabolic disorders, notably obesity and diabetes.
- Extensive scrutiny has been directed toward the influence of gut microbiota on various metabolic conditions, yielding promising insights into their potential impacts.
- Previous meta-analyses have yielded conflicting conclusions regarding the efficacy of probiotics, prebiotics, and synbiotics in managing NAFLD, highlighting the imperative for a comprehensive umbrella review to reconcile existing findings.

### Study selection and eligibility criteria

The selection of studies was performed by three independent researchers, with any disagreements resolved through consensus. Our inclusion criteria for meta-analysis studies on RCTs were as follows: (1) the study population consisted of patients with NAFLD; (2) the intervention involved probiotics, prebiotics, or synbiotics; (3) at least one NAFLD-related outcome was evaluated, including radiologic characteristics, lipid profiles, hepatic enzymes, anthropometric measures, glycemic profiles, and inflammatory markers; and (4) studies were in English and available in full text.

### Data extraction

Three independent researchers extracted data from the selected studies, including the name of the authors, publication year, databases searched, and outcomes related to NAFLD associated with the consumption of probiotics, prebiotics, or synbiotics (Table 1).

### Quality assessment

The AMSTAR 2 checklist, comprising 16 questions aimed at assessing the methodological quality of systematic reviews, was utilized to evaluate the studies included in our review[43]. Three independent researchers conducted the quality assessment of the included studies independently, with any differences in evaluation resolved through consensus. Based on their scores, studies were categorized into four quality levels: high, moderate, low, and critically low. To determine the strength of the epidemiological evidence of the outcomes, we employed the GRADE criteria[54].

### Statistical analyses

The effect of microbial therapies (probiotics, prebiotics, and synbiotics) for each outcome was analyzed using Comprehensive Meta-Analyses (CMA) software version 3. When similar studies were available for the same intervention and outcome, the best study was selected based on sample size and AMSTAR score. The effect of each microbial therapy for the same outcomes was compared using mean difference and 95% confidence interval reported by meta-analysis studies and illustrated through forest plots. For some outcomes, the effect of the intervention was reported by the standard difference in mean in the meta-analysis studies, and the mean difference could not be calculated; hence, these outcomes could not be shown in the forest plots. Standard mean differences were used to compare the effect of one intervention (probiotics, prebiotics, and synbiotics) for different outcomes.

**Table 1**

**Characteristics of included meta-analyses of randomized control trial studies surveying the effect of microbial therapy on the NAFLD population**

| References | Intervention type | Number of total patients | Databases and date of search | The final number of included studies | Variables |
|---|---|---|---|---|---|
| Tang *et al.*[44] | Probiotics | 1356 | PubMed, Embase, the Cochrane Library, and the Web of Science; China National Knowledge Infrastructure (CNKI), Wan Fang Data, and VIP Databases (from inception up to 8 April 2019) | 18 | Weight, BMI, ALT, AST, GGT, TC, LDL, HDL, TG, FBS, Insulin, TNF-α, Leptin, DFI, ALKP, and HOMA-IR |
| Gao *et al.*[45] | Probiotics | 535 | Cochrane Library, PubMed/MEDLINE, EBSCO, OVID, SCI, CNKI, and VIP (from inception up to July 2015) | 9 | Weight, BMI, ALT, AST, TC, LDL, HDL, TG, FBS, Insulin, TNF-α, and HOMA-IR |
| Pan *et al.*[46] | Probiotics | 594 | Web of Science, PubMed, Embase, and Cochrane Library (from inception up to September 2019) | 11 | TNF-α, CRP, and IL-6 |
| Ma *et al.*[47] | Probiotics | 134 | PubMed, Medline, Embase, Web of Science, the Cochrane Library, and Chinese Biomedicine Database China Journal Full Text Database (search date not reported) | 4 | BMI, ALT, AST, TC, LDL, HDL, FBS, TNF-α, and HOMA-IR |
| Hadi *et al.*[40] | Synbiotics | 347 | PubMed, Scopus, ISI Web of Science, and Google Scholar (up to December 2017) | 11 | Weight, BMI, WC, ALT, AST, TC, LDL, HDL, TG, FBS, Insulin, TNF-α, CRP, and HOMA-IR |
| Sharpton *et al.*[48] | Probiotics and synbiotics | 1252 | PubMed/MEDLINE, EMBASE, and the Cochrane Library (from 1 January 2005 to 1 December 2018) | 21 | Liver stiffness management, Hepatic stenosis, BMI, ALT, TG, and HOMA-IR |
| Khan *et al.*[49] | Probiotics and synbiotics | 624 | PubMed, Medline, and Google Scholar (from inception up to 10 June 2018) | 12 | Liver stiffness management, ALT, AST, TC, LDL, HDL, TG, FBS, TNF-α, and HOMA-IR |
| Liu *et al.*[50] | Probiotics and synbiotics | 782 | PubMed, Cochrane, and Embase (from inception up to April 2018) | 15 | Hepatic stenosis, BMI, WC, AST, ALT, TC, LDL, HDL, TG, FBS, TNF-α, and HOMA-IR |
| Loman *et al.*[51] | Prebiotics and probiotics and synbiotics | 1309 | PubMed and EMBASE (from inception up to 14 December 2017) | 25 | BMI, ALT, AST, GGT, TC, LDL, HDL, TG, TNF-α, and CRP |
| Stachowska *et al.*[41] | Prebiotics | 242 | PubMed/MEDLINE, Embase, clinicaltrials.gov, Cinahl, and Web of Science of articles (from inception up to 20 March 2020) | 6 | Weight, BMI, WHR, AST, ALT, Insulin, and HOMA-IR |
| Xiao *et al.*[38] | Probiotics | 1555 | PubMed, Embase, Cochrane Library, Web of Science, OVID, China National Knowledge Infrastructure, VIP Database for Chinese Technical Periodicals, China Biology Medicine disc, and Wanfang Database (from inception up to April 2019) | 28 | BMI, AST, ALT, GGT, TC, LDL, HDL, TG, FBS, Insulin, TNF-α, and HOMA-IR |
| Yang *et al.*[52] | Probiotics | 352 | PubMed, Cochrane, Medline, Web of Science and Embase (from inception up to April 2021) | 9 | BMI, AST, ALT, TC, TNF-α, and HOMA-IR |
| Lavekar *et al.*[53] | Probiotics | 296 | PubMed, Cochrane, Embase (search date not reported) | 7 | Hepatic stenosis, BMI, AST, ALT, TG, and HOMA-IR |
| Koutnikova *et al.*[37] | Probiotics | 660 | Medline, EMBASE, and COCHRANE (from 1990 up to June 2018) | 12 | Weight, BMI, WC, AST, ALT, TG, FBS, Insulin, HbA1C, CRP, BFM, and HOMA-IR |

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BFM, body fat mass; BMI, body mass index; CRP, C-reactive protein; DFI, degree of fat infiltration; FBS, fasting blood sugar; GGT, gamma-glutamyl transferase; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; HS, hepatic steatosis; IL-6, interleukin 6; LDL, low-density lipoprotein; LS, liver stiffness; TC, total cholesterol; TG, triglyceride; TNF-α, tumor necrosis factor-alpha; WC, waist circumference; WHR, waist-to-hip ratio.

## Results

### *Study selection and characteristics of included studies*

The initial literature search was conducted, yielding 189 studies. After the removal of duplicates, 104 studies remained. The titles, abstracts, and full texts of these studies were then reviewed, resulting in the elimination of 91 studies for various reasons, as detailed in Supplementary Table S2 (Supplemental Digital Content 3, http://links.lww.com/MS9/A509). This process left 13 meta-analyses. The reference lists of these meta-analyses were also examined, leading to the identification and addition of one more meta-analysis study. Consequently, a total of 14 meta-analysis studies focusing on the effect of prebiotics, probiotics, or synbiotics on NAFLD were included (Fig. 1).

Data on 24 outcomes were extracted from the included studies, encompassing liver stiffness (LS) ($n=3$), hepatic steatosis (HS) ($n=3$), weight ($n=4$), body mass index (BMI) ($n=12$), waist circumference (WC) ($n=3$), waist-to-hip ratio

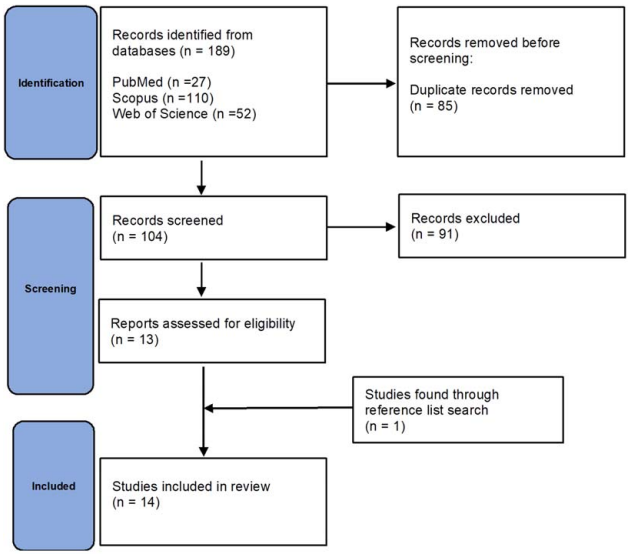


**Figure 1.** Study selection process.

(WHR) ($n=1$), alanine aminotransferase (ALT) ($n=13$), aspartate aminotransferase (AST) ($n=12$), gamma-glutamyl transferase (GGT) ($n=3$), total cholesterol (TC) ($n=9$), low-density lipoprotein (LDL) ($n=8$), high-density lipoprotein (HDL) ($n=8$), triglyceride (TG) ($n=10$), fasting blood sugar (FBS) ($n=8$), insulin level ($n=6$), hemoglobin A1c (HbA1c) ($n=1$), tumor necrosis factor-alpha (TNF-α) ($n=10$), leptin ($n=1$), degree of fat infiltration (DFI) ($n=1$), alkaline phosphatase (ALP) ($n=1$), homeostatic model assessment of insulin resistance (HOMA-IR) ($n=12$), C-reactive protein (CRP) ($n=5$), interleukin 6 (IL-6) ($n=1$), and body fat mass (BFM) ($n=1$). The average number of outcomes per meta-analysis was ten, with a range from 3 to 16 outcomes. The mean number of primary studies included in each meta-analysis was 13, ranging from 4 to 28 studies. Moreover, the mean number of cases per study was found to be 717, with a range from 134 to 1555. The characteristics of the included meta-analyses are depicted in Table 1.

### *Quality assessment of meta-analyses*

The AMSTAR 2 appraisal tool was utilized to assess the quality of the included studies. It was determined that 42.8% (6 studies) were of critically low quality, 50% (7 studies) of low quality, and 7.2% (1 study) of moderate quality. The predominant shortcomings identified in the included studies through the AMSTAR 2 evaluation included a lack of explanation for their selection of study designs for inclusion in their review, identified in all 14 meta-analyses (100%), an absence of reporting on the sources of funding for the studies included in the review, also found in all 14 meta-analyses (100%), and a failure to provide a list of excluded studies along with justification for these exclusions, observed in 13 meta-analyses (93%). These quality assessment findings are detailed in Supplementary Table S3 (Supplemental Digital Content 3, http://links.lww.com/MS9/A509).

## Findings

### *Radiological features*

Probiotics and synbiotics were found to significantly reduce liver stiffness (LS), as measured by elastography, with mean differences (MD) of – 0.38 kilopascals (kPa) (95% confidence interval [CI]: [ – 0.49, – 0.26], $P<0.01$, $I^2=0.0\%$) and – 0.84 kPa (95% CI: [ – 1.10, – 0.57], $P<0.01$, $I^2=85.2\%$), respectively. The impact of prebiotics on LS was not assessed in any of the studies (Fig. 2A). Additionally, hepatic steatosis showed significant resolution when treated with probiotics (odds ratio [OR]: 4.87, 95% CI: [1.85, 12.79], $P<0.01$, $I^2=9.5\%$) and synbiotics (OR: 1.91, 95% CI: [1.22, 2.96], $P<0.01$, $I^2=0.0\%$). No studies were identified that evaluated the effect of prebiotics on hepatic steatosis (Fig. 2B).

### *Anthropometric indices*

In the NAFLD population, both probiotics and synbiotics were found to significantly reduce weight, with MD of – 2.31 kg (95% CI: [ – 4.45, – 0.17], $P=0.03$, $I^2=0.0\%$) for probiotics and – 2.98 kg (95% CI: [ – 3.77, – 2.18], $P<0.01$, $I^2=0.0\%$) for synbiotics. Prebiotics, however, did not significantly affect weight change, with a standardized mean difference (SMD) of 0.05 (95% CI: [ – 0.78, 0.89], $P=0.89$, $I^2=88.26\%$) (Fig. 3A). Probiotics significantly reduced BMI by – 1.46 kg/m$^2$ (95% CI: [ – 2.43, – 0.48], $P<0.01$, $I^2=97.0\%$), and prebiotics also had a significant effect, reducing BMI by – 0.54 kg/m$^2$ (95% CI: [ – 0.86, – 0.21], $P<0.01$, $I^2=84.8\%$). However, the effect of synbiotics on BMI reduction was not significant (MD: – 0.85 kg/m$^2$, 95% CI: [ – 2.16, 0.46], $P=0.20$, $I^2=96.6\%$) (Fig. 3B).

Waist circumference (WC) was significantly reduced by probiotic consumption (MD: – 1.81 cm, 95% CI: [ – 3.18, – 0.43], $P=0.01$, $I^2=0.0\%$), but this relationship was not significant for synbiotics (MD: – 0.01 kg/m$^2$, 95% CI: [ – 0.04, 0.02], $P=0.51$, $I^2=0.0\%$). No study assessed the effect of prebiotics on WC (Fig. 3C). In this umbrella review, only one study examined the relationship between prebiotics and WHR in NAFLD patients, finding the effect of prebiotics on reducing WHR to be insignificant (SMD: – 0.04, 95% CI: [ – 0.40, 0.32], $P=0.82$, $I^2=1.18\%$). There were no studies available that surveyed the relationship between probiotics or synbiotics and WHR in the NAFLD population (Fig. 3D).

### *Liver enzymes*

ALT levels were significantly reduced by the administration of probiotics, prebiotics, and synbiotics in NAFLD patients. A MD of – 13.40 (95% CI: [ – 17.02, – 9.77], $P<0.01$, $I^2=88.0\%$) was observed with probiotics, – 9.75 (95% CI: [ – 15.75, – 3.74], $P<0.01$, $I^2=89.0\%$) with prebiotics, and – 10.09 (95% CI: [ – 14.43, – 5.74], $P<0.01$, $I^2=88.9\%$) with synbiotics (Fig. 4A). Similarly, significant reductions were recorded in AST levels, with probiotics showing a MD of – 13.54 (95% CI: [ – 17.85, – 9.22], $P<0.01$, $I^2=0.0\%$), prebiotics a MD of – 5.73 (95% CI: [ – 8.04, – 3.41], $P<0.01$, $I^2=87.3\%$), and synbiotics a MD of – 13.17 (95% CI: [ – 18.41, – 7.93], $P<0.01$, $I^2=75.0\%$) (Fig. 4B).

GGT levels were significantly reduced by probiotics (MD: – 9.88, 95% CI: [ – 17.77, – 1.99], $P=0.01$, $I^2=98.0\%$) in NAFLD patients (Fig. 4C). The effects of prebiotics and synbiotics on GGT levels were not assessed in the studied NAFLD population. A significant reduction in ALP levels was also

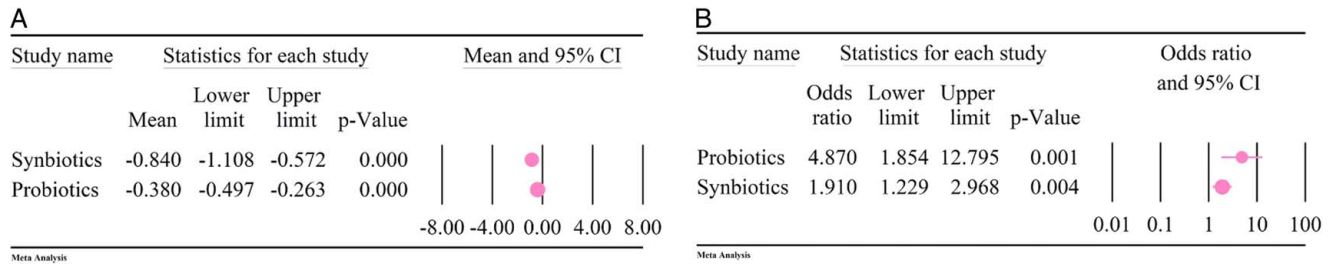


**Figure 2.** (A) Forest plot for the relationship between microbial therapy and liver stiffness (LS); (B) forest plot for the relationship between microbial therapy and hepatic steatosis (HS).

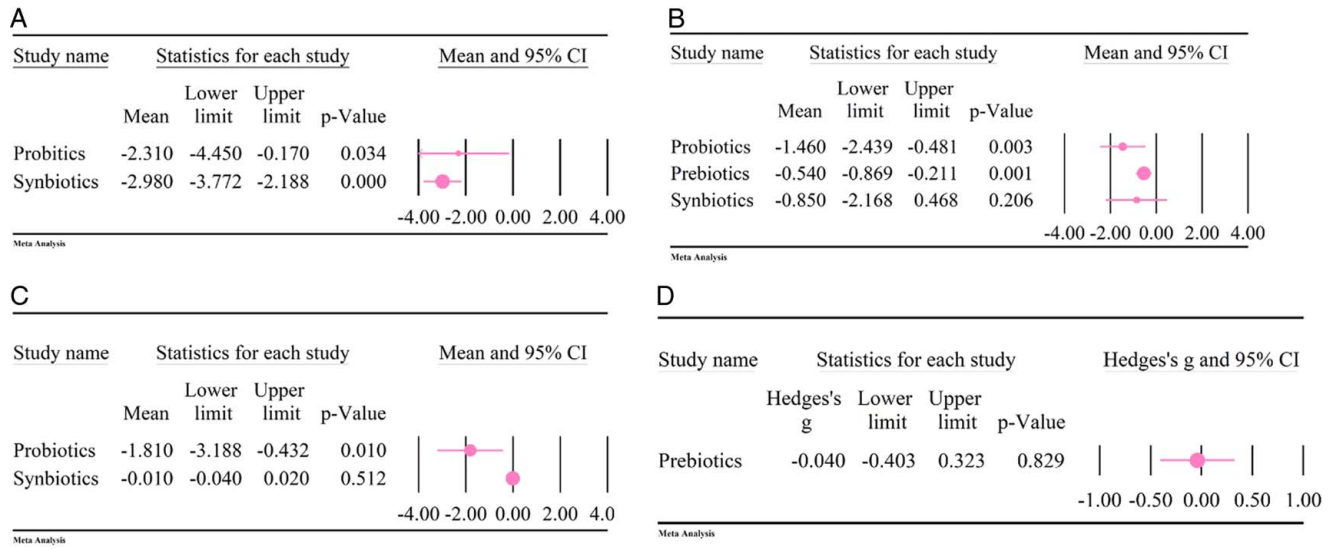


**Figure 3.** (A) Forest plot for the relationship between microbial therapy and weight; (B) forest plot for the relationship between microbial therapy and body mass index (BMI); (C) forest plot for the relationship between microbial therapy and waist circumference (WC); (D) forest plot for the relationship between microbial therapy and waist-to-hip ratio (WHR).

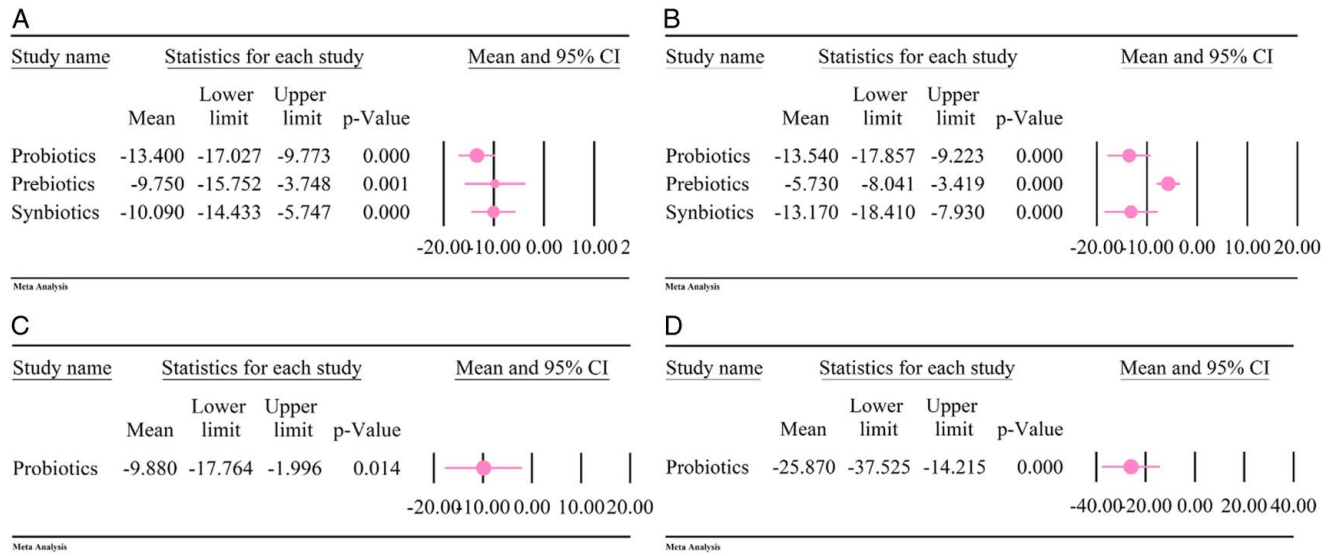


**Figure 4.** (A) Forest plot for the relationship between microbial therapy and alanine aminotransferase (ALT); (B) forest plot for the relationship between microbial therapy and aspartate aminotransferase (AST); (C) forest plot for the relationship between microbial therapy and gamma-glutamyl transferase (GGT); (D) forest plot for the relationship between microbial therapy and alkaline phosphatase (ALP).

observed to be caused by probiotics (MD: −25.87, 95% CI: [−37.52, −14.21], $P<0.01$, $I^2=0.0\%$), with no studies available to assess the impact of prebiotics and synbiotics on ALP levels (Fig. 4D).

### *Lipid profile*

The impact of probiotics, prebiotics, and synbiotics on TC levels was found to be controversial. It was shown that significant reductions in TC levels were achieved by probiotics and synbiotics, with MD of −15.38 (95% CI: [−26.49, −4.26], $P<0.01$, $I^2=0.0\%$) and −14.89 (95% CI: [−17.32, −12.44], $P<0.01$, $I^2=0.0\%$), respectively. However, a significant effect on lowering TC by prebiotics was not observed, with an MD of −5.56 (95% CI: [−12.59, 1.47], $P=0.12$, $I^2=86.0\%$) (Fig. 5A).

Regarding LDL levels in patients with NAFLD, significant reductions were not achieved by any of the interventions – probiotics, prebiotics, or synbiotics. The MDs were reported as −6.14 (95% CI: [−21.70, 9.42], $P=0.43$, $I^2=63.9\%$) for probiotics, −4.97 (95% CI: [−10.93, 0.99], $P<0.01$, $I^2=92.0\%$) for prebiotics, and −17.22 (95% CI: [−34.54, 0.10], $P=0.05$, $I^2=91.6\%$) for synbiotics (Fig. 5B).

A significant positive effect on HDL levels in NAFLD patients was observed with prebiotics, evidenced by an MD of 2.25 (95% CI: [0.69, 3.80], $P<0.01$, $I^2=96.0\%$). Conversely, significant associations with increased HDL levels were not demonstrated by probiotics and synbiotics, with MDs of 1.32 (95% CI: [−1.99, 4.63], $P=0.43$, $I^2=88.0\%$) and 1.08 (95% CI: [−6.65, 8.81], $P=0.78$ $I^2=76.4\%$), respectively (Fig. 5C).

Moreover, it was found that TG levels in NAFLD patients were not significantly reduced by any of the interventions. The MDs recorded were −9.60 (95% CI: [−22.12, 2.92], $P=0.13$, $I^2=0.0\%$) for probiotics, −8.15 (95% CI: [−30.96, 14.66], $P=0.48$, $I^2=94.6\%$) for prebiotics, and −15.78 (95% CI: [−33.13, 1.57], $P=0.07$, $I^2=83.9\%$) for synbiotics (Fig. 5D).

### *Glycemic profile*

A marginally significant reduction in FBS levels in patients with NAFLD was observed following probiotic supplementation, with an MD of −4.98 (95% [CI]: [−9.94, −0.01], $P=0.04$, $I^2=10.8\%$). Synbiotic supplementation was found to significantly decrease FBS levels in the NAFLD population, with an MD of −8.23 (95% CI: [−14.03, −2.42], $P<0.01$, $I^2=83.7\%$). The impact of prebiotics on FBS levels in NAFLD was not evaluated in any of the reviewed studies (Fig. 6A).

Significant decreases in fasting insulin concentrations were reported in the NAFLD population with the use of probiotics (MD: −1.32, 95% CI: [−2.42, −0.21], $P=0.02$, $I^2=0.0\%$), prebiotics (SMD: −0.70, 95% CI: [−1.11, −0.29], $P<0.01$, $I^2=0.0\%$), and synbiotics (MD: −0.83, 95% CI: [−1.53, −0.12], $P=0.02$, $I^2=84.0\%$) (Fig. 6B).

Among the included studies, only one assessed the effect of probiotics on HbA1c levels, reporting a non-significant mean difference of −0.17 (95% CI: [−0.39, 0.05], $P=0.13$, $I^2=0.0\%$) (Fig. 6C).

Probiotics (MD: −0.42, 95% CI: [−0.72, −0.11], $P<0.01$, $I^2=40.1\%$) and prebiotics (SMD: −0.61, 95% CI: [−1.02, −0.21], $P<0.01$, $I^2=0.0\%$) were found to significantly reduce the HOMA-IR index, indicating an improvement in insulin sensitivity. However, no significant change in HOMA-IR was observed with synbiotic supplementation (MD: −0.07, 95% CI: [−1.41, 1.27], $P=0.91$, $I^2=99.6\%$) (Fig. 6D).

### *Inflammatory markers*

The relationship between probiotics and TNF-α levels in the NAFLD population was found to be insignificant, with an MD of −0.65 (95% CI: [−1.56, 0.26], $P=0.16$, $I^2=15.8\%$). In contrast, synbiotics were observed to significantly reduce this inflammatory marker, with an MD of −1.12 (95% CI: [−1.97, −0.26], $P=0.01$, $I^2=86.1\%$) (Fig. 7A).

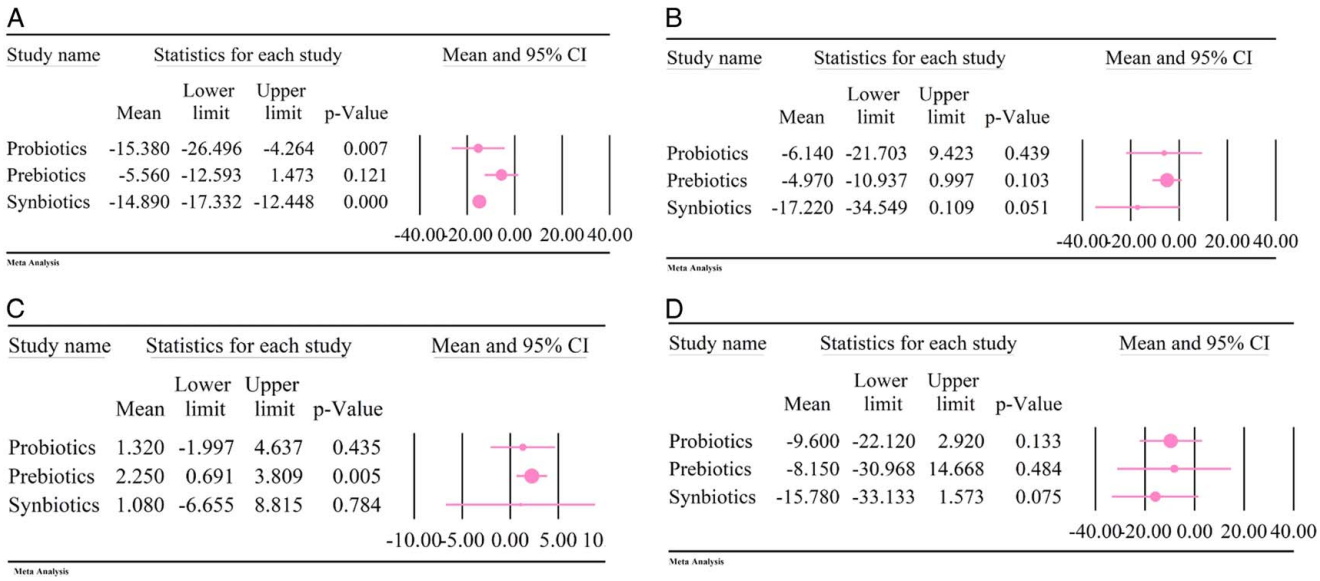


**Figure 5.** (A) Forest plot for the relationship between microbial therapy and total cholesterol (TC); (B) forest plot for the relationship between microbial therapy and low-density lipoprotein (LDL); (C) forest plot for the relationship between microbial therapy and high-density lipoprotein (HDL); (D) forest plot for the relationship between microbial therapy and triglyceride (TG).

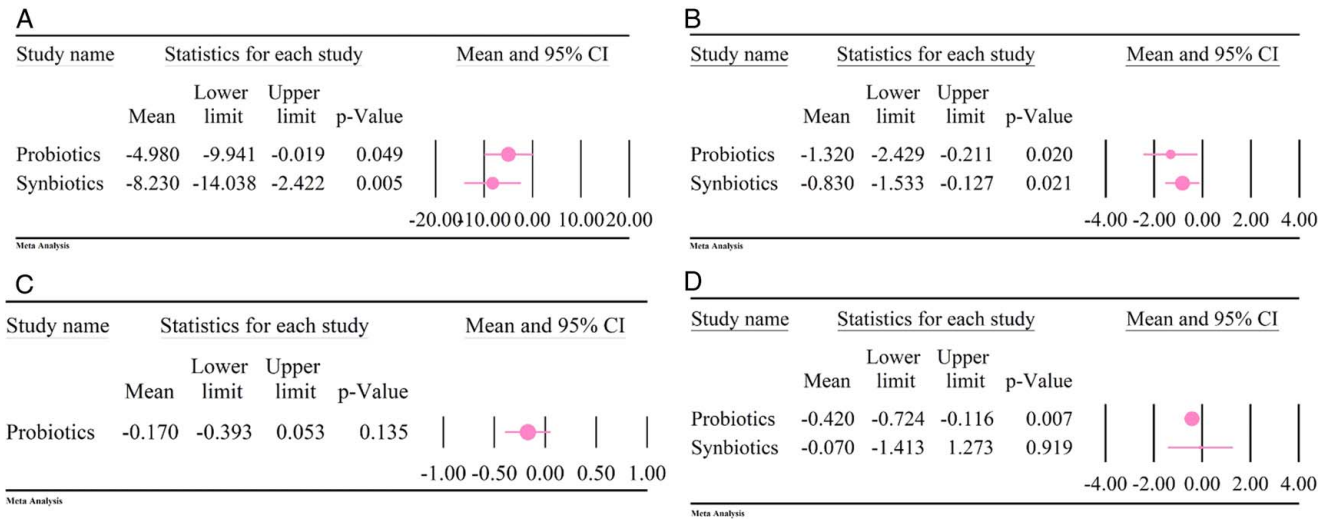


**Figure 6.** (A) Forest plot for the relationship between microbial therapy and fasting blood sugar (FBS); (B) forest plot for the relationship between microbial therapy and insulin; (C) forest plot for the relationship between microbial therapy and hemoglobin A1c (HbA1c); (D) forest plot for the relationship between microbial therapy and homeostatic model assessment of insulin resistance (HOMA-IR).

Similarly, the effect of probiotics on CRP levels in the NAFLD population did not reach statistical significance, with an MD of −0.41 (95% CI: [−1.84, 1.02], $P=0.57$, $I^2=46.8\%$). On the other hand, synbiotics demonstrated a significant reduction in CRP levels, with an MD of −0.58 (95% CI: [−1.01, −0.14], $P<0.01$, $I^2=62.8\%$). The impact of prebiotics on both TNF-α and CRP levels in NAFLD patients was not evaluated in any of the included studies (Fig. 7B).

Regarding the impact on IL-6 levels, only one of the included studies assessed the effect of probiotics in the NAFLD population, resulting in an SMD of −0.72 (95% CI: [−1.90, 0.46], $P=0.23$, $I^2=93.0\%$), indicating no significant effect. There were no studies assessing the relationship between prebiotics or synbiotics and IL-6 levels in NAFLD patients (Fig. 7C).

### *Other variables*

A significant reduction in leptin levels in the NAFLD population was observed, showing an SMD of −1.14 (95% CI: [−1.52, −0.76], $P<0.01$, $I^2=46.8\%$). The relationship between prebiotics and synbiotics with leptin levels in NAFLD patients was not examined in any study (Fig. 8A).

In the meta-analyses reviewed, only one study assessed the impact of probiotics on DFI. Probiotics were found to significantly reduce DFI in patients experiencing NAFLD, with a relative risk (RR) of 2.42 (95% CI: [1.69, 3.44], $P<0.01$, $I^2=51.4\%$). The effects of prebiotics and synbiotics on DFI were not explored in any study (Fig. 8B).

The relationship between BFM and probiotics was found to be insignificant, with an MD of −0.86 (95% CI: [−2.14, 0.42],

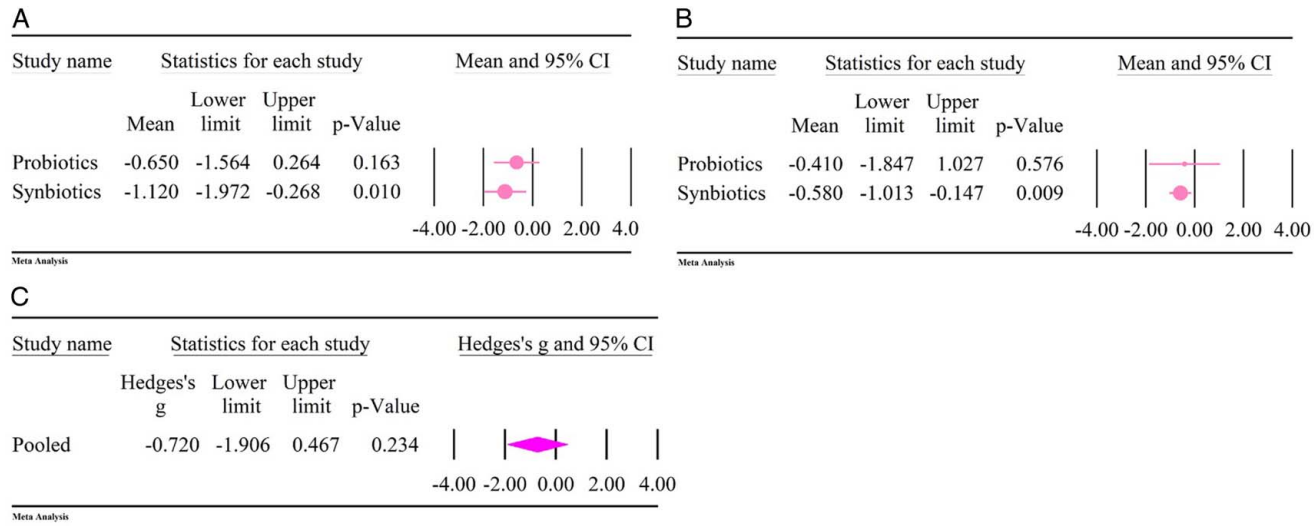


**Figure 7.** (A) Forest plot for the relationship between microbial therapy and tumor necrosis factor-alpha (TNF-α); (B) forest plot for the relationship between microbial therapy and C-reactive protein (CRP); (C) forest plot for the relationship between microbial therapy and interleukin 6 (IL-6).

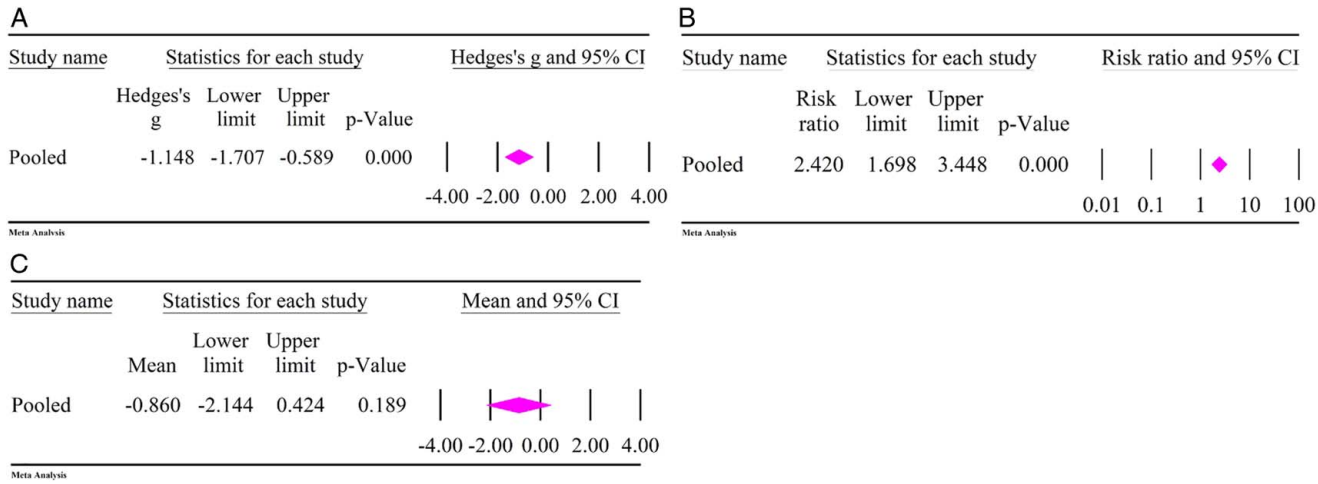


**Figure 8.** (A) Forest plot for the relationship between microbial therapy and leptin; (B) forest plot for the relationship between microbial therapy and degree of fat infiltration (DFI); (C) forest plot for the relationship between microbial therapy and body fat mass (BFM).

$P = 0.18$, $I^2 = 0.0\%$). No study assessed the relationship between prebiotics or synbiotics and BFM in the NAFLD population (Fig. 8C).

### *The total effect of probiotics, prebiotics, and synbiotics on NAFLD-related indices and biomarkers*

To depict the impact of probiotics on various biomarkers in patients with NAFLD, the SMD was utilized as a measure of effect size. It was found that probiotics significantly influenced 15 out of the surveyed outcomes. Notably, the effect on DFI was measured using RR, due to which it could not be visualized in Figure 9A alongside other outcomes measured by SMD.

The comprehensive impact of prebiotics on NAFLD was captured in Figure 9B, which illustrated their effects on 11 variables. Out of these, six variables showed significant changes following the consumption of prebiotics, highlighting their potential benefits in the management of NAFLD.

Synbiotic consumption was evaluated against 16 variables in the NAFLD population, with Figure 9C showcasing these effects. Remarkably, 10 variables were found to be significantly associated with synbiotic intake, indicating a substantial impact of synbiotics on NAFLD biomarkers.

### *Strength of epidemiologic evidence*

For the assessment of the strength of epidemiological evidence, the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) methodology was utilized, and the GRADE Profiler software version 3.6 was also employed. The included studies were evaluated based on their risk of bias, inconsistency, indirectness, imprecision, and publication bias. The GRADE scores for the outcomes are presented in Table 2, and Supplementary Tables S4 (Supplemental Digital Content 3, http://links.lww.com/MS9/A509), S5 (Supplemental Digital Content 3, http://links.lww.com/MS9/A509), and S6 (Supplemental Digital Content 3, http://links.lww.com/MS9/A509).

### Publication bias

The information regarding publication bias is detailed in Table 3. Among the analyzed outcomes, the effects of prebiotics on HDL and probiotics on FBS exhibited significant publication bias according to Egger's regression test ($P < 0.05$). However, the results for other variables did not show a significant association with publication bias.

## Discussion

This umbrella review of 14 meta-analyses investigated the impact of microbial therapy (including probiotics, prebiotics, and synbiotics) on patients with NAFLD. It offered a comprehensive, quantitative summary of the current, evidence-based data on the potential of microbial treatment to affect NAFLD-related outcomes. Although the effects of microbial therapy on NAFLD outcomes varied across different meta-analyses, to identify the most reliable evidence-based results, we selected the highest-quality study for each outcome, considering the total sample size, the AMSTAR checklist, and the year of publication.

In this study, 24 outcomes were evaluated: LS, HS, BMI, weight, WC, WHR, ALT, AST, GGT, TC, LDL, HDL, TG, FBS, insulin, HbA1c, TNF-α, leptin, DFI, ALP, HOMA-IR, CRP, IL-6, and BFM. Of these, 15 outcomes were significantly influenced by probiotics (LS, HS, weight, BMI, WC, ALT, AST, GGT, ALP, TC, FBS, insulin, leptin, DFI, and HOMA-IR), 6 outcomes by prebiotics (BMI, ALT, AST, HDL, insulin, and HOMA-IR), and 10 outcomes by synbiotics (LS, HS, weight, ALT, AST, TC, FBS, insulin, TNF-α, and CRP).

The term 'gut microbiota' refers to the population of microorganisms residing in the gastrointestinal tract and is considered a crucial component of an individual's health[55]. This ecosystem maintains a symbiotic relationship with its host, contributing to the homeostasis of various organs' physiology[56]. Furthermore, this microbial community plays a vital role in infection prevention, immune response regulation, and the provision of nutrients to the host[57,58]. An imbalance in the gut microbiota, known as dysbiosis, is associated with the pathogenesis of various intra-

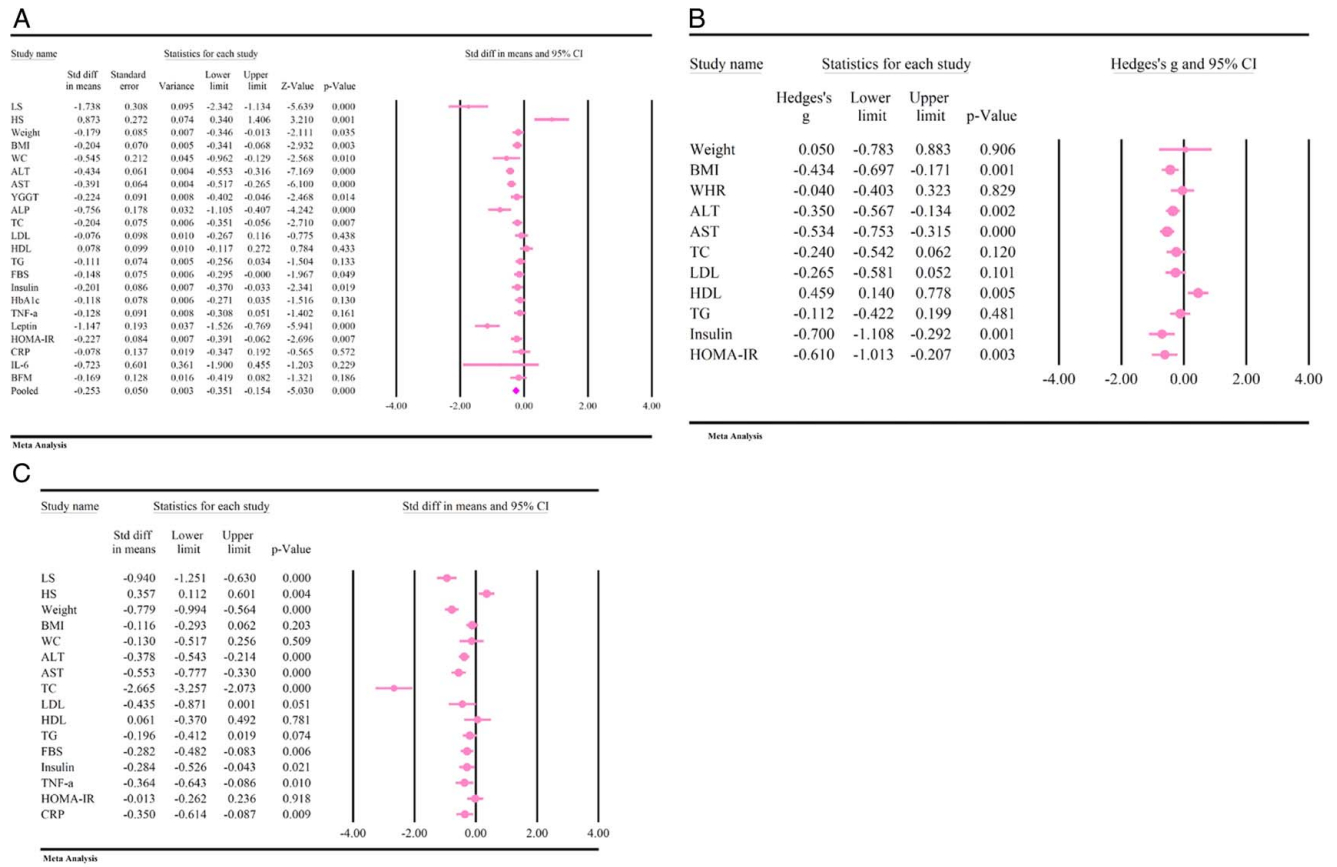


**Figure 9.** (A) Total effects of probiotics on non-alcoholic fatty liver disease (NAFLD)-related outcomes; (B) total effects of prebiotics on NAFLD-related outcomes; (C) total effects of synbiotics on NAFLD-related outcomes.

intestinal and extra-intestinal conditions, including inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), celiac disease (CD), diabetes mellitus (DM), obesity, cardiovascular diseases (CVD), and cerebrovascular diseases[21,58–68].

NAFLD, the most prevalent hepatic disease, is closely associated with the gut microbiota[58,69–71]. Among its risk factors, alterations in the gut microbiome are considered the most prevalent[72–76]. Previous studies have indicated an increase in the genera of *Lactobacillus*, *Bifidobacterium*, and *Streptococcus* in patients with NAFLD[77]. Recent research suggests that NAFLD may progress to NASH due to gut microbial dysbiosis, which affects carbohydrate and lipid metabolism pathways[78]. Furthermore, dysbiosis in the gut microbiota can disrupt the balance between pro-inflammatory and anti-inflammatory markers, leading to NAFLD progression[78,79]. Although the precise mechanisms by which the gut microbiota contributes to the pathogenesis of NAFLD remain incompletely understood, this relationship highlights potential avenues for NAFLD treatment[21,78].

Hepatic steatosis, characterized by lipid accumulation in the liver[80], is intricately linked to gut microbiota dysbiosis, though the precise mechanisms remain somewhat elusive. The gut–liver axis plays a pivotal role in this interaction[81,82]. It is posited that in individuals with NAFLD, the tight junctions between enterocytes are compromised, leading to increased gut permeability[82]. Additionally, small intestine bacterial overgrowth stands out as a significant concern in these patients[83]. The theory suggests that the combination of heightened gut permeability and bacterial overgrowth contributes to the accumulation of fat in the liver[82]. This study's findings indicate that microbial therapies can effectively improve NAFLD's radiological features. Notably, there was a significant reduction in hepatic steatosis and liver stiffness following treatment with these compounds.

Research has established a link between anthropometric indices and NAFLD, highlighting these indices as markers of obesity, which is intimately associated with NAFLD[84–89]. The intricate relationship raises questions about whether NAFLD merely coexists with obesity or if obesity significantly contributes to the pathogenesis of NAFLD[44]. Studies have demonstrated the effectiveness of microbial therapy in managing obesity[90,91]. This study further supports the potential of microbial therapy in reducing anthropometric indices, underscoring its promising role in treating NAFLD. Specifically, probiotics were found to decrease WC, BMI, and weight; synbiotics contributed to weight reduction; and prebiotics significantly lowered BMI.

Increases in serum levels of hepatic enzymes, such as AST, ALT, ALP, and GGT, are indicative of liver damage[92]. The progression of NAFLD, leading to elevated hepatic enzymes, aligns with the 'two-hit theory' hypothesis, where fat accumulation serves as the initial insult ('first hit'), and subsequent liver injury from necroinflammation and oxidative stress constitutes the 'second hit'[93–95]. This study demonstrates the efficacy of microbial therapy in reducing levels of hepatic enzymes in individuals with NAFLD.

**Table 2**

**Grading of recommendations assessment, development and evaluation (GRADE) scoring of outcomes**

| Outcome | Probiotics | Prebiotics | Synbiotics |
|---|---|---|---|
| Liver stiffness | Low | NS | Very low |
| Hepatic steatosis | Low | NS | Low |
| Weight | High | Low | Moderate |
| BMI | Low | Very low | Very low |
| WC | Low | NS | Moderate |
| WHR | NS | Moderate | NS |
| ALT | Moderate | Very low | Moderate |
| AST | High | Very low | Moderate |
| YGGT | Low | NS | NS |
| ALP | Moderate | NS | NS |
| TC | Moderate | Very low | Very low |
| LDL | High | Very low | Very low |
| HDL | Moderate | Very low | Very low |
| TG | Low | Very low | Moderate |
| FBS | Moderate | NS | High |
| Insulin | Moderate | Moderate | Moderate |
| HbA1c | Low | NS | NS |
| TNF-a | Moderate | NS | Low |
| Leptin | Moderate | NS | NS |
| DFI | High | NS | NS |
| HOMA-IR | Moderate | Moderate | Very low |
| CRP | Moderate | NS | Low |
| IL-6 | Very low | NS | NS |
| BFM | moderate | NS | NS |

NS, no study was available for the outcome.

Treatment with probiotics specifically lowered levels of GGT and ALP, with GGT recognized as a highly sensitive marker for liver damage and a novel indicator of inflammation and oxidative stress[96]. The beneficial impact of microbial therapy on hepatic enzyme reduction extends beyond the NAFLD population to include patients with hepatitis and alcoholic fatty liver disease[97,98].

Dyslipidemia, characterized by elevated serum TG and LDL levels, along with reduced HDL levels, is closely linked to NAFLD and its comorbidities[99–101]. The gut microbiota plays a vital role in lipid metabolism, a process influenced by microbiota-derived metabolites such as short-chain fatty acids and lipopolysaccharides[102–104]. Findings from this study indicate that probiotics and synbiotics can significantly lower TC, whereas prebiotics effectively increase HDL levels.

Previous research has explored the beneficial impacts of probiotics on inflammatory markers[105,106]. Elevated levels of inflammatory markers, such as CRP and TNF-α, have been linked with NAFLD[107–110]. However, the findings from this study indicate that while probiotic consumption did not significantly affect these markers, synbiotics were effective in reducing CRP levels significantly. The effects of prebiotics on these markers have yet to be studied. Additionally, a meta-analysis investigated the levels of leptin and IL-6, identifying leptin as a recent mediator of inflammation[111]. Notably, probiotic therapy led to a decrease in leptin levels among NAFLD patients.

The intricate link between NAFLD and DM is well-documented, with insulin resistance and hyperinsulinism serving as hallmark features of NAFLD[112–114]. Studies have indicated elevated fasting insulin levels in NAFLD patients, even in the absence of diabetes[113]. HOMA-IR, a key indicator of insulin resistance, is particularly useful in evaluating NAFLD among diabetic patients[115,116] and has been identified as an independent predictor for advanced liver fibrosis in NAFLD cases[117]. Furthermore, FBS levels, another indicator of glycemic control, are significantly higher in individuals with NAFLD[118–120]. This study demonstrates that microbial therapy can significantly reduce insulin, HbA1c, and FBS levels, showcasing the potential of these interventions in improving insulin resistance among the NAFLD population.

The results of meta-analysis studies on the effects of prebiotics, probiotics, and synbiotics on NAFLD were marked by significant

**Table 3**

**Results of publication bias based on Begg's and Egger's regression test**

| | Publication bias based on Begg's regression test | | | Publication bias based on Egger's regression test | | |
|---|---|---|---|---|---|---|
| Variable | Probiotics | Synbiotics | Prebiotics | Probiotics | Synbiotics | Prebiotics |
| Liver stiffness | LN | 0.73 | NS | LN | 1.00 | NS |
| Hepatic Steatosis | LN | 0.22 | NS | LN | 0.54 | NS |
| Weight | 0.67 | 0.62 | 0.08 | 0.84 | 0.10 | 0.06 |
| Body mass index | 0.09 | 0.72 | 1.00 | 0.12 | 0.33 | 0.87 |
| Waist circumference | LN | 1.00 | NS | LN | 0.53 | NS |
| Waist-to-hip ratio | NS | NS | LN | NS | NS | LN |
| Alanin aminotransferase | 0.49 | 0.59 | 0.53 | 0.13 | 0.89 | 0.11 |
| Aspartate aminotransferase | 0.80 | 1.00 | 0.90 | 0.63 | 0.57 | 0.86 |
| Total cholesterol | 0.85 | LN | 0.46 | 0.17 | LN | 0.34 |
| Low-density lipoprotein | 0.16 | LN | 1 | 0.29 | LN | 0.92 |
| High-density lipoprotein | 0.26 | LN | 0.73 | 0.48 | LN | 0.04 |
| Triglyceride | 0.19 | 1 | 1 | 0.23 | 0.56 | 0.18 |
| Fasting blood sugar | 0.13 | 0.33 | NS | 0.04 | 0.19 | NS |
| Fasting insulin | 0.27 | 0.32 | NS | 0.16 | 0.19 | NS |
| Glycated hemoglobin | 0.15 | NS | NS | 0.34 | NS | NS |
| Homeostatic model assessment for insulin resistance | 0.16 | 0.73 | LN | 0.28 | 0.43 | LN |
| Tumor necrosis factor-alpha | 0.22 | 1 | NS | 0.74 | 0.47 | NS |
| Leptin | 1 | NS | NS | 0.98 | NS | NS |
| Alkaline phosphatase | 0.91 | NS | NS | 0.73 | NS | NS |

LN, low number of studies for assessing publication bias; NS, no study was available for the outcome.

heterogeneity, which can impact the generalizability and robustness of the findings. This heterogeneity can be attributed to various factors in the primary studies included, such as the type and dosage of interventions, the duration of follow-up periods, and the specific bacterial genera used in the treatments, all contributing to the diversity of the data.

To assess the quality of the evidence, we applied the GRADE approach to the outcomes reviewed. This methodology helped us categorize the evidence quality, resulting in 14 outcomes being assessed as very low quality, 12 as low quality, 19 as moderate quality, and 5 as high quality. This stratification aids in understanding the confidence level we can place in each result and highlights areas where further research is necessary to strengthen the evidence base.

Despite statistical significance in the relationship between these microbial therapies and certain outcomes, the epidemiological evidence remains inconclusive for several reasons, including small sample sizes, unexplained heterogeneity, and the inclusion of randomized controlled trials with a high risk of bias. To enhance our understanding of the effects of microbial therapies on NAFLD, we recommend conducting more rigorous, well-powered RCTs with larger sample sizes. Additionally, there's a call for high-quality meta-analyses incorporating results from newly published RCTs. Moreover, employing power analysis to identify sources of heterogeneity in meta-analyses, a step not taken in the studies reviewed, is advised.

In this study, although the results of publication bias were not significant for most of the outcomes, it is important to note that the publication bias of the effects of probiotics on LS, HS, WC, and WHR; the effects of synbiotics on leptin, WHR, TC, LDL, HDL, HbA1c, and ALP, and the effects of prebiotics on LS, HS, leptin, WC, WHR, FBS, FI, HbA1c, TNF-α, and ALP were not assessed due to the low number of studies evaluated or the absence of available studies for these outcomes. Future RCTs are necessary to fill this gap and provide a more comprehensive understanding of these effects.

The precise mechanisms by which microbial treatment might impact NAFLD have not yet been fully understood. However, several mechanisms have been identified. Regarding its effect on anthropometric indices, among the suggested processes, decreasing appetite, reducing cholesterol absorption and synthesis, and increasing lipolysis and fatty acid oxidation are mentioned. Short-chain fatty acids (SCFAs) produced by probiotics increase gut peptides such as peptide YY and glucagon-like peptide 1 (GLP-1), which regulate appetite[121]. Hormones that reduce appetite reduce calorie intake and, as a result, decrease weight[91,122]. In addition, cholesterol absorption in the gut occurs via Niemann-Pick C1-like receptors on enterocytes[123]. Previous research indicates that probiotics may decrease receptor expression, resulting in decreased cholesterol absorption. Low cholesterol absorption could result in weight reduction[124,125]. Probiotic-produced SCFAs, which include propionate and butyrate, may lower blood cholesterol by inhibiting the enzyme hydroxymethylglutaryl-coenzyme A reductase (HMG-CoA) reductase, which is crucial for cholesterol production[126,127]. Furthermore, probiotics decrease fat cell size by reducing fatty acid intake and increasing fatty acid oxidation gene expression[128]. They also lead to adenosine monophosphate kinase phosphorylation, which reduces hepatic fat accumulation[129] (Fig. 10).

Concerning microbial treatment's impact on liver enzymes, one of the suggested methods for reducing liver damage is lowering endotoxemia. The reduction of endotoxemia may be achieved in several ways, such as the production of antimicrobial molecules, prevention of pathogen adherence, enhancement of secretory IgA, and enhancement of epithelial cell junctions. Probiotics may boost host-cell antimicrobial peptide production. Additionally, these agents may create antimicrobial compounds such as SCFAs, microcins, and bacteriocins[130,131]. These chemicals destroy pathogens by altering their outer membranes, pore development, and enzyme activity[131–133]. Probiotics also lower luminal pH by producing acetic and lactic acids, which eliminate certain bacteria[134]. Also, certain probiotic strains may attach to epithelial cells and mucous membranes, limiting pathogen invasion[135,136]. Additionally, probiotics may suppress virulence factors that promote pathogen adhesion[133,137]. Besides, certain probiotic strains may boost pathogen-specific secretory IgA activity without affecting probiotic-specific IgA[138–140]. Pathogens cannot colonize or multiply due to secretory IgA[141,142]. This capacity was also seen in prebiotics and synbiotics. In addition, enterocytes connect via several junctions, such as adherence junctions, tight junctions, desmosome, and gap junctions[143,144]. Previous research indicates that infections may impair intestinal barrier function by altering intercellular connections[145,146]. Probiotics may prevent tight-junction changes from infections and inflammation[147,148] (Fig. 10).

Regarding lipids, some processes by which microbial treatment might improve lipid profiles in NALFD include bile salt deconjugation, increased hepatic LDL receptors, cholesterol reduction, and inhibition of Niemann-Pick C1 like 1 expression. Most bile salt in the intestinal lumen is reabsorbed during the enterohepatic cycle, but 400–800 mg remains for gut bacteria to deconjugate[149,150]. Deconjugation occurs via the action of the bile salt hydrolase (BSH) enzyme[150]. Conjugated bile salt has antibacterial characteristics, so deconjugated bile is more effective for gut microbiota replication[151]. Deconjugated bile salt has decreased solubility, leading to poor reabsorption and increased excretion in stool[151]. Lower bile salt absorption from intestinal barriers reduces cholesterol delivery to the liver, causing a decrease in denovo synthesis. The liver compensates by increasing hepatocyte LDL receptor and absorption of serum LDL, resulting in lower serum LDL concentration[152]. Introducing probiotics may reduce blood TC levels due to their favorable BSH effects[153]. The cholesterol content of the medium may be converted into coprostanol and, to a lesser level, coprostanone. This transition relies on the enzymatic activity of cholesterol reductase. Certain probiotics possess cholesterol-lowering effects. Coprostanol and coprostanone have minimal intestinal absorption[150]. The absorption of cholesterol particles occurs via Niemann-Pick C1-like 1 (NPC1L1) transporters, which are situated on the membrane of intestinal cells[123,154]. Prior in-vitro research has shown that some probiotics can lower the expression of NPC1L1 on the surface of cells, resulting in a reduction in cholesterol absorption[124,125] (Fig. 10).

When it comes to the usefulness of gut microbial therapy for glycemic parameters, a significant amount of information has been gathered on individuals with NAFLD. However, getting a more exact understanding of how gut microbial activity specifically contributes to improving glycemic parameters is necessary. Here, we provide a summary of some probable pathways that are involved: Improving intestinal barrier and inflammation reduction, Production of insulin-sensitizing hormone, Adiponectin, and Immunomodulation. Probiotics enhance glucose tolerance, insulin secretion, and reduce inflammation by controlling the permeability of the gut via the maintenance of tight junctions,

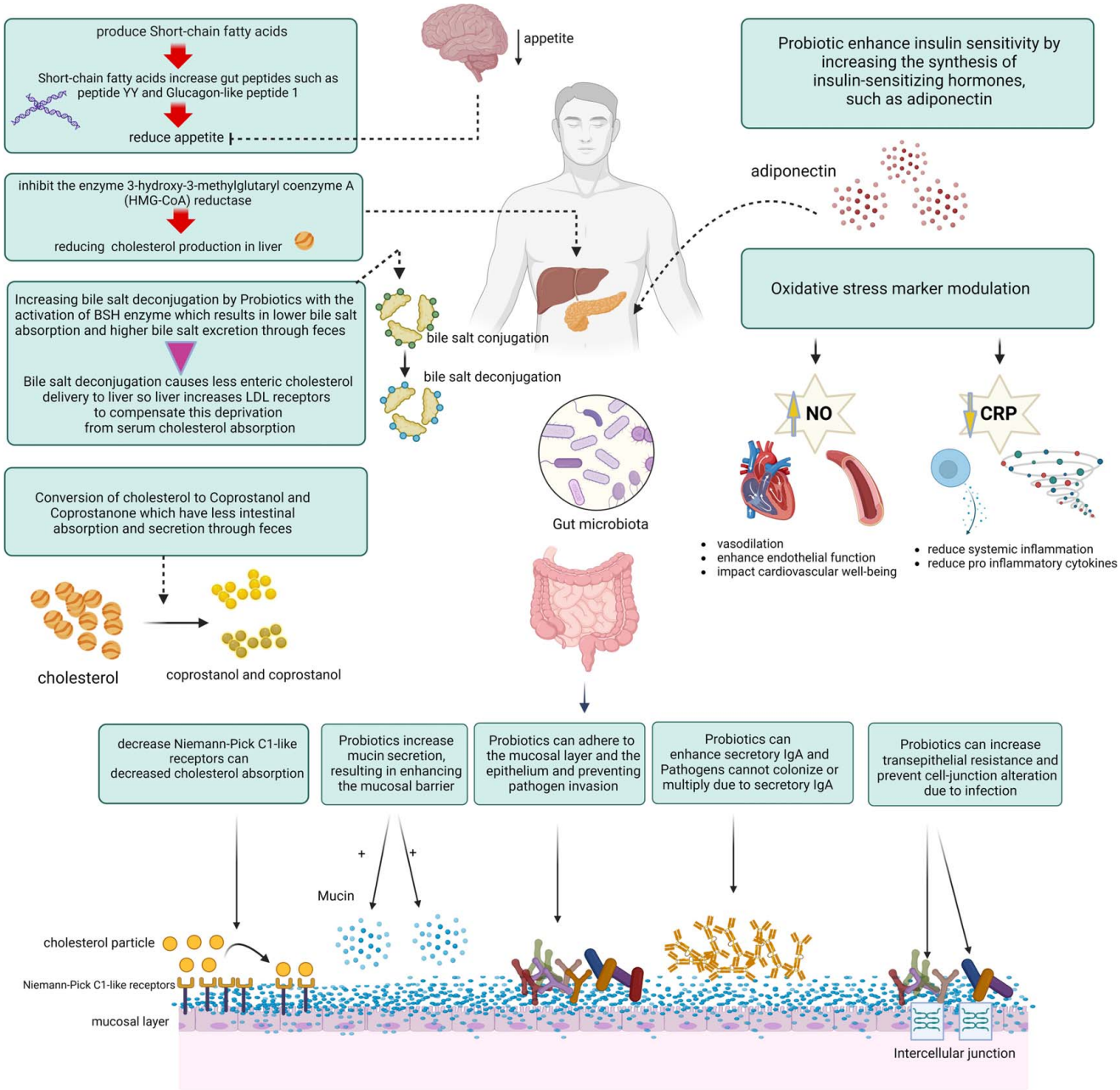


**Figure 10.** Mechanisms of action of the gut microbiota in patients with non-alcoholic fatty liver disease.

altering the ratio of gram-negative to gram-positive microbiomes, boosting GLP2 release, and supporting the well-being of intestinal cells by supplying essential nutrients[112,155,156]. Also, probiotic therapy has been shown to enhance insulin sensitivity by increasing the synthesis of insulin-sensitizing hormones, such as adiponectin[157]. Previous research found that therapy with *Lactobacillus rhamnosus* GG (LGG) resulted in better glucose tolerance and increased insulin sensitivity via the release of adiponectin and activation of adenosine monophosphate-activated protein kinase (AMPK)[158]. Moreover, probiotics are linked to improved immune function, lower levels of pro-inflammatory cytokines, and reduced oxidative stress[159]. During a randomized, double-blinded, controlled clinical research, individuals who consumed yogurt containing probiotics (*L. acidophilus* La5 and *Bifidobacterium lactis* Bb12) had a decrease in FBS and HbA1C levels, as well as an increase in total antioxidant levels, as compared to the control group[160] (Fig. 10).

With attention to the anti-inflammatory effect of gut microbial therapy, several mechanisms have been developed, including Intestinal chemical barrier modulation, Immune cells modulation, and oxidative stress marker modulation. The gut includes several chemicals, including antimicrobial peptides (AMPs), mucin (MUC), lysozymes, and antibacterial substances[161,162]. The mucus coating of the gastrointestinal epithelium layer is the

primary barrier against harmful chemicals in the gut. Inflammation in the colon may disrupt the mucus barrier, allowing bacteria to adhere to the epithelium[163]. Research indicates that probiotics like lactobacilli enhance MUC 2 and MUC 3 mucin production and inhibit pathogenic bacteria adherence[164,165]. Antigen-presenting cells (APCs) are necessary for gastrointestinal immunological balance. Macrophages, monocytes, and dendritic cells (DCs) produce cytokines and chemokines that help T cells activate, proliferate, and differentiate[166]. According to prior research, probiotics may internalize to APCs and activate them, releasing cytokines that activate T lymphocytes[167]. Oxidative stress occurs when there is an imbalance in favor of oxidants over antioxidants. This disturbs redox signaling, changes molecules, and leads to molecular damage. Oxidative stress may be evaluated by assessing the overall antioxidant capacity (TAC), nitric oxide (NO), CRP, glutathione (GSH), and other markers of stress[168]. Nitric oxide (NO) can enhance oxidative stress, induce vasodilation, enhance endothelial function, and positively impact cardiovascular well-being[169]. Probiotics may improve endothelial function and reduce the risk of cardiovascular disease and metabolic dysfunction via altering gut microbiota and NO bioavailability[170]. CRP is primarily synthesized in the liver and is used to indicate inflammation inside the body. Probiotics have been shown to regulate CRP levels by raising the synthesis of SCFA, reducing the expression of pro-inflammatory cytokines, and enhancing the presence of antioxidants and free radical scavengers such as GSH[171,172] (Fig. 10).

This study's interpretations are subject to certain limitations. Notably, the included meta-analyses did not assess several outcomes related to the impact of prebiotics on LS, HS, WC, GGT, ALP, FBS, HbA1c, TNF-α, leptin, DFI, CRP, IL-6, and BFM, as well as the effects of synbiotics on WHR, GGT, ALP, HbA1c, leptin, DFI, IL-6, and BFM. Additionally, there was a lack of evidence regarding the association between probiotics and WHR. In addition, while the meta-analyses identified the type of bacteria utilized within their original study, they failed to perform subgroup analyses or dose–response assessments. Consequently, they did not delineate the most effective bacterial strains, optimal dosages, or the most beneficial treatment durations.

Another limitation of our current study was the lack of consideration for the impact of ethnicity and age on the effects of gut microbial modulation on host responses. The studies included did not conduct sufficient investigation or subgroup analyses based on geographical distribution and the characteristics of participants in their original studies. Future meta-analyses should take this aspect into account, as ethnicity has been shown to influence the function of probiotics, prebiotics, and synbiotics[173,174]. Recognizing and addressing these variables are crucial for tailoring microbial therapies to diverse populations and enhancing the precision of our understanding in this field.

In our umbrella review, we specifically analyzed meta-analyses evaluating the impact of probiotics, prebiotics, and synbiotics on the clinical parameters of NAFLD. However, we observed a notable discrepancy in the volume of research across these interventions. Most of the included studies focused on probiotics and synbiotics, while relatively fewer studies explored the effects of prebiotics. This imbalance highlights a significant gap in the current literature. Acknowledging this, we suggest that future research, particularly RCTs, should prioritize investigating prebiotics. Exploring this underrepresented area could provide a more comprehensive understanding of how different microbial therapies affect NAFLD, thereby enhancing the scope and applicability of treatment strategies for this condition.

Several priority areas for future research in microbial therapy for NAFLD can be outlined to refine treatment strategies and address existing knowledge gaps. First, future studies should focus on identifying which specific strains of probiotics, prebiotics, or synbiotics are most effective for NAFLD management, as understanding the strain-specific mechanisms and their interactions with host metabolism could tailor more effective treatments. Additionally, there is a need for long-term clinical trials to assess the sustained efficacy and safety of these therapies in managing NAFLD. Longitudinal data would help in understanding the long-term impacts and potential side effects. Also, more research is needed to explore the interaction between microbial therapies and standard pharmacological treatments to determine synergistic effects that could enhance treatment efficacy. Furthermore, studies should aim to include diverse patient populations to ensure the generalizability of findings. Lastly, exploring the economic aspects of integrating microbial therapies into standard care practices could provide insights into their cost-effectiveness and potential for widespread adoption.

## Conclusion

Our findings suggest that microbial therapy could serve as a beneficial adjunct to lifestyle interventions, which is the current mainstay for managing NAFLD. Microbial therapy has shown significant improvements in liver stiffness, hepatic steatosis, and metabolic markers like body mass index, liver enzymes, and lipid profiles. These changes could potentially reduce the risk of liver fibrosis and cirrhosis, offering a less burdensome alternative to strict lifestyle changes, which many patients find challenging to maintain. Compared to existing treatments, which primarily focus on symptom management through dietary and exercise modifications, microbial therapy could provide a targeted approach that directly influences the gut–liver axis. Translating the findings of microbial therapy for NAFLD into clinical practice involves several challenges. First, there is a lack of standardization in probiotic and synbiotic formulations. The efficacy of microbial therapies can vary significantly depending on the strains used, their concentrations, and the combinations in which they are administered, making it crucial to develop standardized products with consistent quality and proven efficacy. Additionally, regulatory approval processes can be complex, as probiotics and synbiotics are often categorized as dietary supplements rather than medications, which may limit rigorous clinical testing and oversight. Another major challenge is the integration into existing treatment protocols, requiring clinicians to be educated on the benefits and limitations of microbial therapies to effectively incorporate these options into patient management plans. Moreover, long-term safety and effectiveness need to be established through extensive clinical trials to ensure these therapies can be safely recommended for routine use. Lastly, the cost-effectiveness of implementing microbial therapies on a larger scale needs to be evaluated to ensure that they provide a viable economic option for health systems and patients.

## Ethical approval

Since we conducted an umbrella meta-analysis, we did not have any human or animal subjects and our study did not require an Ethical approval.

## Consent

Since we did not have any human or animal subject, conducting this section is not applicable.


## Source of funding

Not applicable.


## Author contribution

G.M., A.M., and S.O.: concept development (provided the idea for the research); A.M., R.R.K. and S.J.: design (planned the methods to generate the results); G.M., A.M., F.V., and D.P.: supervision (provided oversight, responsible for organization and implementation, and writing of the manuscript); N.C.T. and R.R.K.: data collection/processing; G.M. and A.M.: analysis/interpretation (responsible for statistical analysis, evaluation, and presentation of the results); A.M. and F.V.: literature search (performed the literature search); All authors: writing (responsible for writing a substantive part of the manuscript).

## Conflicts of interest disclosure

The authors declare no conflict of interest.

## Research registration unique identifying number (UIN)

The study protocol was registered in PROSPERO with the registration code CRD42024510147.

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## Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Provenance and peer review

Not commissioned, externally peer-reviewed.


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## References


[1] Puri P, Sanyal AJ. Nonalcoholic fatty liver disease: definitions, risk factors, and workup. Clin Liver Dis 2012;1:99–103.

[2] Abd El-Kader SM, El-Den Ashmawy EM. Non-alcoholic fatty liver disease: the diagnosis and management. World J Hepatol 2015;7:846–58.

[3] Angulo P, Machado MV, Diehl AM. Fibrosis in nonalcoholic fatty liver disease: mechanisms and clinical implications. Semin Liver Dis 2015;35:132–45.

[4] Cooper J, Baumgartner K, Smith A, *et al*. Liver disease: nonalcoholic fatty liver disease. FP Essent 2021;511:29–35.

[5] Ipsen DH, Lykkesfeldt J, Tveden-Nyborg P. Molecular mechanisms of hepatic lipid accumulation in non-alcoholic fatty liver disease. Cell Mol Life Sci 2018;75:3313–27.

[6] Islam R, Kundu S, Jha SB, *et al*. Cirrhosis and coagulopathy: mechanisms of hemostasis changes in liver failure and their management. Cureus 2022;14:e23785.

[7] Tevar AD, Clarke C, Wang J, *et al*. Clinical review of nonalcoholic steatohepatitis in liver surgery and transplantation. J Am Coll Surg 2010;210:515–26.

[8] Collier J. Non-alcoholic fatty liver disease. Medicine 2007;35:86–8.

[9] Paschos P, Paletas K. Non alcoholic fatty liver disease and metabolic syndrome. Hippokratia 2009;13:9.

[10] Moore JB. Non-alcoholic fatty liver disease: the hepatic consequence of obesity and the metabolic syndrome. Proc Nutr Soc 2010;69:211–20.

[11] Mazzolini G, Sowa JP, Atorrasagasti C, *et al*. Significance of simple steatosis: an update on the clinical and molecular evidence. Cells 2020;9:2458.

[12] Amini-Salehi E, Hassanipour S, Joukar F, *et al*. Risk factors of non-alcoholic fatty liver disease in the Iranian adult population: a systematic review and meta-analysis. Hepat Mon 2023;23:e131523.

[13] Le MH, Yeo YH, Li X, *et al*. 2019 Global NAFLD prevalence: a systematic review and meta-analysis. Clin Gastroenterol Hepatol 2022;20:2809–17.

[14] Koopman N, Molinaro A, Nieuwdorp M, *et al*. Can bugs be drugs? The potential of probiotics and prebiotics as treatment for non-alcoholic fatty liver disease,. Aliment Pharmacol Ther 2019;50:628–39.

[15] Sookoian S, Pirola CJ. Genetic predisposition in nonalcoholic fatty liver disease. Clin Mol Hepatol 2017;23:1.

[16] Mokhtari Z, Gibson DL, Hekmatdoost A. Nonalcoholic fatty liver disease, the gut microbiome, and diet. Adv Nutr 2017;8:240–52.

[17] Wajsbrot NB, Leite NC, Salles GF, *et al*. Non-alcoholic fatty liver disease and the impact of genetic, epigenetic and environmental factors in the offspring. World J Gastroenterol 2022;28:2890–9.

[18] Hassanipour S, Amini-Salehi E, Joukar F, *et al*. The prevalence of non-alcoholic fatty liver disease in Iranian children and adult population: a systematic review and meta-analysis. Iran J Public Health 2023;52:1600–12.

[19] Islam H, Puttagunta SM, Islam R, *et al*. Risk of stroke with mitral stenosis: the underlying mechanism, treatment, and prevention. Cureus 2022;14:e23784.

[20] Meroni M, Longo M, Dongiovanni P. The role of probiotics in non-alcoholic fatty liver disease: a new insight into therapeutic strategies. Nutrients 2019;11:2642.

[21] Khan A, Ding Z, Ishaq M, *et al*. Understanding the effects of gut microbiota dysbiosis on nonalcoholic fatty liver disease and the possible probiotics role: recent updates. Int J Biol Sci 2021;17:818–33.

[22] Fang J, Yu C-H, Li X-J, *et al*. Gut dysbiosis in nonalcoholic fatty liver disease: pathogenesis, diagnosis, and therapeutic implications. Front Cell Infect Microbiol 2022;12:997018.

[23] Moschen AR, Kaser S, Tilg H. Non-alcoholic steatohepatitis: a microbiota-driven disease. Trends Endocrinol Metab 2013;24:537–45.

[24] Tripathi A, Debelius J, Brenner DA, *et al*. The gut–liver axis and the intersection with the microbiome,. Nat Rev Gastroenterol Hepatol 2018;15:397–411.

[25] Carpi RZ, Barbalho SM, Sloan KP, *et al*. The effects of probiotics, prebiotics and synbiotics in non-alcoholic fat liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH): a systematic review. Int J Mol Sci 2022;23:8805.

[26] Amini-Salehi E, Mahapatro A, Korsapati RR, *et al*. Exploring the relationship between gut microbiome modulation and blood pressure in type 2 diabetes: an umbrella review. Nutr Metab Cardiovasc Dis 2024;S0939–4753(24)00200-X. doi:10.1016/j.numecd.2024.05.017. Epub ahead of print.

[27] Vakilpour A, Amini-Salehi E, Soltani Moghadam A, *et al*. The effects of gut microbiome manipulation on glycemic indices in patients with non-alcoholic fatty liver disease: a comprehensive umbrella review. Nutr Diabetes 2024;14:25.

[28] Pouwels S, Sakran N, Graham Y, *et al*. Non-alcoholic fatty liver disease (NAFLD): a review of pathophysiology, clinical management and effects of weight loss. BMC Endocr Disord 2022;22:63.
[29] Zelber-Sagi S, Ratziu V, Oren R. Nutrition and physical activity in NAFLD: an overview of the epidemiological evidence. World J Gastroenterol 2011;17:3377–89.
[30] Hallsworth K, Adams LA. Lifestyle modification in NAFLD/NASH: facts and figures. JHEP Rep 2019;1:468–79.
[31] Naghipour M, Joukar F, Amini Salehi E, *et al*. The association between age at first pregnancy and number of deliveries with metabolic syndrome and its components: results from Persian Guilan Cohort Study (PGCS). Iran J Obstet Gynecol Infertil 2022;25:1–11.
[32] Naghipour A, Amini-Salehi E, Orang Gorabzarmakhi M, *et al*. Effects of gut microbial therapy on lipid profile in individuals with non-alcoholic fatty liver disease: an umbrella meta-analysis study. Syst Rev 2023;12:144.
[33] Amini-Salehi E, Hassanipour S, Keivanlou M-H, *et al*. The impact of gut microbiome-targeted therapy on liver enzymes in patients with non-alcoholic fatty liver disease: an umbrella meta-analysis. Nutr Rev 2024;82:815–30.
[34] Lynch SV, Pedersen O. The human intestinal microbiome in health and disease. N Engl J Med 2016;375:2369–79.
[35] Davani-Davari D, Negahdaripour M, Karimzadeh I, *et al*. Prebiotics: definition, types, sources, mechanisms, and clinical applications. Foods (Basel, Switzerland) 2019;8:92.
[36] de Vrese M, Schrezenmeir J. Probiotics, prebiotics, and synbiotics. Adv Biochem Eng Biotechnol 2008;111:1–66.
[37] Koutnikova H, Genser B, Monteiro-Sepulveda M, *et al*. Impact of bacterial probiotics on obesity, diabetes and non-alcoholic fatty liver disease related variables: a systematic review and meta-analysis of randomised controlled trials. BMJ Open 2019;9:e017995.
[38] Xiao M-W, Lin S-X, Shen Z-H, *et al*. Systematic review with meta-analysis: the effects of probiotics in nonalcoholic fatty liver disease. Gastroenterol Res Practice 2019;2019:1484598.
[39] Khalesi S, Johnson DW, Campbell K, *et al*. Effect of probiotics and synbiotics consumption on serum concentrations of liver function test enzymes: a systematic review and meta-analysis. Eur J Nutr 2018;57:2037–53.
[40] Hadi A, Mohammadi H, Miraghajani M, *et al*. Efficacy of synbiotic supplementation in patients with nonalcoholic fatty liver disease: a systematic review and meta-analysis of clinical trials: synbiotic supplementation and NAFLD. Crit Rev Food Sci Nutr 2019;59:2494–505.
[41] Stachowska E, Portincasa P, Jamioł-Milc D, *et al*. The relationship between prebiotic supplementation and anthropometric and biochemical parameters in patients with NAFLD-a systematic review and meta-analysis of randomized controlled trials. Nutrients 2020;12:3460.
[42] Page MJ, McKenzie JE, Bossuyt PM, *et al*. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. Int J Surg 2021;88:105906.
[43] Shea BJ, Reeves BC, Wells G, *et al*. AMSTAR 2: a critical appraisal tool for systematic reviews that include randomised or non-randomised studies of healthcare interventions, or both. BMJ 2017;358:j4008.
[44] Tang Y, Huang J, Zhang WY, *et al*. Effects of probiotics on nonalcoholic fatty liver disease: a systematic review and meta-analysis. Therap Adv Gastroenterol 2019;12:1756284819878046.
[45] Gao XL, Zhu Y, Wen Y, *et al*. Efficacy of probiotics in non-alcoholic fatty liver disease in adult and children: a meta-analysis of randomized controlled trials. Hepatol Res 2016;46:1226–33.
[46] Pan X, Wen SW, Kaminga AC, *et al*. Gut metabolites and inflammation factors in non-alcoholic fatty liver disease: a systematic review and meta-analysis. Sci Rep 2020;10:8848.
[47] Ma YY, Li L, Yu CH, *et al*. Effects of probiotics on nonalcoholic fatty liver disease: a meta-analysis. World J Gastroenterol 2013;19:6911–8.
[48] Sharpton SR, Maraj B, Harding-Theobald E, *et al*. Gut microbiome-targeted therapies in nonalcoholic fatty liver disease: a systematic review, meta-analysis, and meta-regression. Am J Clin Nutr 2019;110:139–49.
[49] Khan MY, Mihali AB, Rawala MS, *et al*. The promising role of probiotic and synbiotic therapy in aminotransferase levels and inflammatory markers in patients with nonalcoholic fatty liver disease - a systematic review and meta-analysis. Eur J Gastroenterol Hepatol 2019;31:703–15.
[50] Liu L, Li P, Liu YQ, *et al*. Efficacy of probiotics and synbiotics in patients with nonalcoholic fatty liver disease: a meta-analysis. Dig Dis Sci 2019;64:3402–12.
[51] Loman BR, Hernandez-Saavedra D, An RP, *et al*. Prebiotic and probiotic treatment of nonalcoholic fatty liver disease: a systematic review and meta-analysis. Nutr Rev 2018;76:822–39.
[52] Yang RW, Shang JY, Zhou YR, *et al*. Effects of probiotics on non-alcoholic fatty liver disease: a systematic review and meta-analysis. Expert Rev Gastroenterol Hepatol 2021;15:1401–9.
[53] Lavekar AS, Raje DV, Manohar T, *et al*. Role of probiotics in the treatment of nonalcoholic fatty liver disease: a meta-analysis. Euroasian J Hepatogastroenterol 2017;7:130.
[54] Guyatt G, Oxman AD, Akl EA, *et al*. GRADE guidelines: 1. Introduction—GRADE evidence profiles and summary of findings tables. J Clin Epidemiol 2011;64:383–94.
[55] Guinane CM, Cotter PD. Role of the gut microbiota in health and chronic gastrointestinal disease: understanding a hidden metabolic organ. Therap Adv Gastroenterol 2013;6:295–308.
[56] Hou K, Wu Z-X, Chen X-Y, *et al*. Microbiota in health and diseases. Signal Transduct Target Ther 2022;7:135.
[57] Markowiak P, Śliżewska K. Effects of probiotics, prebiotics, and synbiotics on human health. Nutrients 2017;9:1021.
[58] Mahapatro A, Bawna F, Kumar V, *et al*. Anti-inflammatory effects of probiotics and synbiotics on patients with non-alcoholic fatty liver disease: an umbrella study on meta-analyses. Clin Nutr ESPEN 2023;57:475–86.
[59] DeGruttola AK, Low D, Mizoguchi A, *et al*. Current understanding of dysbiosis in disease in human and animal models. Inflamm Bowel Dis 2016;22:1137–50.
[60] Chong PP, Chin VK, Looi CY, *et al*. The microbiome and irritable bowel syndrome - a review on the pathophysiology, current research and future therapy. Front Microbiol 2019;10:1136.
[61] Sharma S, Tripathi P. Gut microbiome and type 2 diabetes: where we are and where to go? J Nutr Biochem 2019;63:101–8.
[62] Pecora F, Persico F, Gismondi P, *et al*. Gut microbiota in celiac disease: is there any role for probiotics? Front Immunol 2020;11:957.
[63] Menees S, Chey W. The gut microbiome and irritable bowel syndrome, F1000Research 2018;7:F1000 Faculty Rev-1029.
[64] Trøseid M, Andersen GØ, Broch K, *et al*. The gut microbiome in coronary artery disease and heart failure: current knowledge and future directions. EBioMedicine 2020;52:102649.
[65] Tonomura S, Ihara M, Friedland RP. Microbiota in cerebrovascular disease: a key player and future therapeutic target. J Cereb Blood Flow Metab 2020;40:1368–80.
[66] Toor D, Wasson MK, Kumar P, *et al*. Dysbiosis disrupts gut immune homeostasis and promotes gastric diseases. Int J Mol Sci 2019;20:2432.
[67] Flores Monar GV, Islam H, Puttagunta SM, *et al*. Association between type 1 diabetes mellitus and celiac disease: autoimmune disorders with a shared genetic background. Cureus 2022;14:e22912.
[68] Rivera AP, Flores Monar GV, Islam H, *et al*. Ulcerative colitis-induced colorectal carcinoma: a deleterious concatenation. Cureus 2022;14:e22636.
[69] Lau E, Carvalho D, Freitas P. Gut microbiota: association with NAFLD and metabolic disturbances. BioMed Res Int 2015;2015:979515.
[70] Cheemerla S, Balakrishnan M. Global epidemiology of chronic liver disease. Clin Liver Dis 2021;17:365–70.
[71] Amini-Salehi E, Samethadka Nayak S, maddineni G, *et al*. Can modulation of gut microbiota affect anthropometric indices in patients with non-alcoholic fatty liver disease? An umbrella meta-analysis of randomized controlled trials. Ann Med Surg (Lond) 2024;86:2900–10.
[72] Jia W, Rajani C. The influence of gut microbial metabolism on the development and progression of non-alcoholic fatty liver disease. Adv Exp Med Biol 2018;1061:95–110.
[73] Schwimmer JB, Johnson JS, Angeles JE, *et al*. Microbiome signatures associated with steatohepatitis and moderate to severe fibrosis in children with nonalcoholic fatty liver disease. Gastroenterology 2019;157:1109–22.
[74] Amabebe E, Robert FO, Agbalalah T, *et al*. Microbial dysbiosis-induced obesity: role of gut microbiota in homoeostasis of energy metabolism. Br J Nutr 2020;123:1127–37.
[75] Kapil S, Duseja A, Sharma BK, *et al*. Small intestinal bacterial overgrowth and toll-like receptor signaling in patients with non-alcoholic fatty liver disease. J Gastroenterol Hepatol 2016;31:213–21.

[76] Boursier J, Mueller O, Barret M, *et al*. The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. Hepatology 2016;63:764–75.
[77] Sharpton SR, Ajmera V, Loomba R. Emerging role of the gut microbiome in nonalcoholic fatty liver disease: from composition to function . Clin Gastroenterol Hepatol 2019;17:296–306.
[78] Kolodziejczyk AA, Zheng D, Shibolet O, *et al*. The role of the microbiome in NAFLD and NASH. EMBO Mol Med 2019;11:e9302.
[79] Jiang W, Wu N, Wang X, *et al*. Dysbiosis gut microbiota associated with inflammation and impaired mucosal immune function in intestine of humans with non-alcoholic fatty liver disease. Sci Rep 2015;5:1–7.
[80] AlShaalan R, Aljiffry M, Al-Busafi S, *et al*. Nonalcoholic fatty liver disease: noninvasive methods of diagnosing hepatic steatosis. Saudi J Gastroenterol 2015;21:64–70.
[81] Gil-Gómez A, Brescia P, Rescigno M, *et al*. Gut–liver axis in nonalcoholic fatty liver disease: the impact of the metagenome, end products, and the epithelial and vascular barriers. Semin Liver Dis 2021;41: 191–205.
[82] Abenavoli L, Scarpellini E, Rouabhia S, *et al*. Probiotics in non-alcoholic fatty liver disease: which and when. Ann Hepatol 2013;12:357–63.
[83] Miele L, Valenza V, La Torre G, *et al*. Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. Hepatology 2009;49:1877–87.
[84] Mansour-Ghanaei R, Mansour-Ghanaei F, Naghipour M, *et al*. The role of anthropometric indices in the prediction of non-alcoholic fatty liver disease in the PERSIAN Guilan Cohort study (PGCS). J Med Life 2018;11:194–202.
[85] Singh A, Parida S, Narayan J, *et al*. Simple anthropometric indices are useful for predicting non-alcoholic fatty liver disease [NAFLD] in Asian Indians. J Clin Exp Hepatol 2017;7:310–5.
[86] Zhang Y, Li B, Liu N, *et al*. Evaluation of different anthropometric indicators for screening for nonalcoholic fatty liver disease in elderly individuals. Int J Endocrinol 2021;2021:6678755.
[87] Fabbrini E, Sullivan S, Klein S. Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. Hepatology 2010;51:679–89.
[88] Ononamadu CJ, Ezekwesili CN, Onyeukwu OF, *et al*. Comparative analysis of anthropometric indices of obesity as correlates and potential predictors of risk for hypertension and prehypertension in a population in Nigeria. Cardiovasc J Africa 2017;28:92–9.
[89] Piqueras P, Ballester A, Durá-Gil JV, *et al*. Anthropometric indicators as a tool for diagnosis of obesity and other health risk factors: a literature review. Front Psychol 2021;12:631179.
[90] Mazloom K, Siddiqi I, Covasa M. Probiotics: How effective are they in the fight against obesity? Nutrients 2019;11:258.
[91] Wiciński M, Gębalski J, Gołębiewski J, *et al*. Probiotics for the treatment of overweight and obesity in humans-a review of clinical trials. Microorganisms 2020;8:1148.
[92] Giannini EG, Testa R, Savarino V. Liver enzyme alteration: a guide for clinicians. CMAJ 2005;172:367–79.
[93] Imajo K, Yoneda M, Kessoku T, *et al*. Rodent models of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. Int J Mol Sci 2013;14: 21833–57.
[94] Adams LA, Angulo P, Lindor KD. Nonalcoholic fatty liver disease. CMAJ 2005;172:899–905.
[95] Dowman JK, Tomlinson JW, Newsome PN. Pathogenesis of non-alcoholic fatty liver disease. QJM 2009;103:71–83.
[96] Musazadeh V, Roshanravan N, Dehghan P, *et al*. Effect of probiotics on liver enzymes in patients with non-alcoholic fatty liver disease: an umbrella of systematic review and meta-analysis. Front Nutr 2022;9: 844242.
[97] Gratz SW, Mykkanen H, El-Nezami HS. Probiotics and gut health: a special focus on liver diseases. World J Gastroenterol 2010;16:403–10.
[98] Kirpich IA, Solovieva NV, Leikhter SN, *et al*. Probiotics restore bowel flora and improve liver enzymes in human alcohol-induced liver injury: a pilot study. Alcohol (Fayetteville NY) 2008;42:675–82.
[99] Chatrath H, Vuppalanchi R, Chalasani N. Dyslipidemia in patients with nonalcoholic fatty liver disease. Semin Liver Dis 2012;32:22–9.
[100] Katsiki N, Mikhailidis DP, Mantzoros CS. Non-alcoholic fatty liver disease and dyslipidemia: an update. Metabolism 2016;65:1109–23.
[101] Zhang QQ, Lu LG. Nonalcoholic fatty liver disease: dyslipidemia, risk for cardiovascular complications, and treatment strategy. J Clin Transl Hepatol 2015;3:78–84.
[102] Jia X, Xu W, Zhang L, *et al*. Impact of gut microbiota and microbiota-related metabolites on hyperlipidemia. Front Cell Infect Microbiol 2021;11:634780.
[103] Yoshida N, Yamashita T, Hirata K-i. Gut microbiome and cardiovascular diseases. Diseases 2018;6:56.
[104] Rabot S, Membrez M, Bruneau A, *et al*. Germ-free C57BL/6J mice are resistant to high-fat-diet-induced insulin resistance and have altered cholesterol metabolism. FASEB J 2010;24:4948–59.
[105] Milajerdi A, Mousavi SM, Sadeghi A, *et al*. The effect of probiotics on inflammatory biomarkers: a meta-analysis of randomized clinical trials. Eur J Nutr 2020;59:633–49.
[106] Ding L-N, Ding W-Y, Ning J, *et al*. Effects of probiotic supplementation on inflammatory markers and glucose homeostasis in adults with type 2 diabetes mellitus: a systematic review and meta-analysis. Front Pharmacol 2021;12:770861.
[107] Yoneda M, Mawatari H, Fujita K, *et al*. High-sensitivity C-reactive protein is an independent clinical feature of nonalcoholic steatohepatitis (NASH) and also of the severity of fibrosis in NASH. J Gastroenterol 2007;42:573–82.
[108] Foroughi M, Maghsoudi Z, Khayyatzadeh S, *et al*. Relationship between non-alcoholic fatty liver disease and inflammation in patients with non-alcoholic fatty liver. Adv Biomed Res 2016;5:28.
[109] Kakino S, Ohki T, Nakayama H, *et al*. Pivotal role of TNF-α in the development and progression of nonalcoholic fatty liver disease in a murine model. Horm Metab Res 2018;50:80–7.
[110] Seo YY, Cho YK, Bae JC, *et al*. Tumor necrosis factor-α as a predictor for the development of nonalcoholic fatty liver disease: a 4-year follow-up study,. Endocrinol Metab (Seoul, Korea) 2013;28:41–5.
[111] Pérez-Pérez A, Sánchez-Jiménez F, Vilariño-García T, *et al*. Role of leptin in inflammation and vice versa. Int J Mol Sci 2020;21:5887.
[112] Dharmalingam M, Yamasandhi PG. Nonalcoholic fatty liver disease and type 2 diabetes mellitus. Indian J Endocrinol Metab 2018;22: 421–8.
[113] Bril F, McPhaul MJ, Kalavalapalli S, *et al*. Intact fasting insulin identifies nonalcoholic fatty liver disease in patients without diabetes. J Clin Endocrinol Metab 2021;106:e4360–71.
[114] Bril F, Lomonaco R, Orsak B, *et al*. Relationship between disease severity, hyperinsulinemia, and impaired insulin clearance in patients with nonalcoholic steatohepatitis. Hepatology 2014;59:2178–87.
[115] Diniz M, Beleigoli AMR, Schmidt MI, *et al*. Homeostasis model assessment of insulin resistance (HOMA-IR) and metabolic syndrome at baseline of a multicentric Brazilian cohort: ELSA-Brasil study. Cad Saude Publica 2020;36:e00072120.
[116] Gutierrez-Buey G, Núñez-Córdoba JM, Llavero-Valero M, *et al*. Is HOMA-IR a potential screening test for non-alcoholic fatty liver disease in adults with type 2 diabetes? Eur J Intern Med 2017;41:74–8.
[117] Fujii H, Imajo K, Yoneda M, *et al*. HOMA-IR: an independent predictor of advanced liver fibrosis in nondiabetic non-alcoholic fatty liver disease. J Gastroenterol Hepatol 2019;34:1390–5.
[118] Mansour-Ghanaei R, Mansour-Ghanaei F, Naghipour M, *et al*. Biochemical markers and lipid profile in nonalcoholic fatty liver disease patients in the PERSIAN Guilan cohort study (PGCS), Iran. J Fam Med Prim Care 2019;8:923–8.
[119] Pardhe BD, Shakya S, Bhetwal A, *et al*. Metabolic syndrome and biochemical changes among non-alcoholic fatty liver disease patients attending a tertiary care hospital of Nepal. BMC Gastroenterol 2018; 18:109.
[120] Suresh S, Rajanbabu B, Veetil VM, *et al*. A study on the altered glycemic and lipid parameters and prevalence of insulin resistance in nonalcoholic fatty liver disease. J Family Med Prim Care 2018;7:93–7.
[121] Tremaroli V, Bäckhed F. Functional interactions between the gut microbiota and host metabolism. Nature 2012;489:242–9.
[122] Hansen TT, Mead BR, García-Gavilán JF, *et al*. Is reduction in appetite beneficial for body weight management in the context of overweight and obesity? Yes, according to the SATIN (Satiety Innovation) study. J Nutr Sci 2019;8:e39.
[123] Davis HR Jr, Zhu LJ, Hoos LM, *et al*. Niemann-Pick C1 Like 1 (NPC1L1) is the intestinal phytosterol and cholesterol transporter and a key modulator of whole-body cholesterol homeostasis. J Biol Chem 2004;279:33586–92.
[124] Huang Y, Wu F, Wang X, *et al*. Characterization of *Lactobacillus plantarum* Lp27 isolated from Tibetan kefir grains: a potential probiotic bacterium with cholesterol-lowering effects. J Dairy Sci 2013;96: 2816–25.

[125] Huang Y, Zheng Y. The probiotic *Lactobacillus acidophilus* reduces cholesterol absorption through the down-regulation of Niemann-Pick C1-like 1 in Caco-2 cells. Br J Nutr 2010;103:473–8.
[126] Zhuang G, Liu X-M, Zhang Q-X, *et al*. Research advances with regards to clinical outcome and potential mechanisms of the cholesterol-lowering effects of probiotics. Clin Lipidol 2012;7:501–7.
[127] Cani PD, Neyrinck AM, Fava F, *et al*. Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. Diabetologia 2007;50:2374–83.
[128] Wu Y, Zhang Q, Ren Y, *et al*. Effect of probiotic *Lactobacillus* on lipid profile: a systematic review and meta-analysis of randomized, controlled trials. PLoS One 2017;12:e0178868.
[129] Zhang M, Wang C, Wang C, *et al*. Enhanced AMPK phosphorylation contributes to the beneficial effects of *Lactobacillus rhamnosus* GG supernatant on chronic-alcohol-induced fatty liver disease. J Nutr Biochem 2015;26:337–44.
[130] Collins SM, Bercik P. The relationship between intestinal microbiota and the central nervous system in normal gastrointestinal function and disease. Gastroenterology 2009;136:2003–14.
[131] Alakomi HL, Skyttä E, Saarela M, *et al*. Lactic acid permeabilizes gram-negative bacteria by disrupting the outer membrane. Appl Environ Microbiol 2000;66:2001–5.
[132] Duquesne S, Petit V, Peduzzi J, *et al*. Structural and functional diversity of microcins, gene-encoded antibacterial peptides from enterobacteria. J Mol Microbiol Biotechnol 2007;13:200–9.
[133] Ohland CL, Macnaughton WK. Probiotic bacteria and intestinal epithelial barrier function. Am J Physiol Gastrointest Liver Physiol 2010; 298:G807–19.
[134] Ogawa M, Shimizu K, Nomoto K, *et al*. Inhibition of in vitro growth of Shiga toxin-producing *Escherichia coli* O157:H7 by probiotic *Lactobacillus* strains due to production of lactic acid. Int J Food Microbiol 2001;68:135–40.
[135] Johnson-Henry KC, Donato KA, Shen-Tu G, *et al*. *Lactobacillus rhamnosus* strain GG prevents enterohemorrhagic *Escherichia coli* O157:H7-induced changes in epithelial barrier function. Infect Immun 2008;76:1340–8.
[136] Johnson-Henry KC, Hagen KE, Gordonpour M, *et al*. Surface-layer protein extracts from *Lactobacillus helveticus* inhibit enterohaemorrhagic *Escherichia coli* O157:H7 adhesion to epithelial cells. Cell Microbiol 2007;9:356–67.
[137] Wu X, Vallance BA, Boyer L, *et al*. *Saccharomyces boulardii* ameliorates *Citrobacter rodentium*-induced colitis through actions on bacterial virulence factors. Am J Physiol Gastrointest Liver Physiol 2008;294: G295–306.
[138] Leblanc J, Fliss I, Matar C. Induction of a humoral immune response following an *Escherichia coli* O157:H7 infection with an immunomodulatory peptidic fraction derived from *Lactobacillus helveticus*-fermented milk. Clin Diagn Lab Immunol 2004;11:1171–81.
[139] Ogawa M, Shimizu K, Nomoto K, *et al*. Protective effect of *Lactobacillus casei* strain Shirota on Shiga toxin-producing *Escherichia coli* O157:H7 infection in infant rabbits. Infect Immun 2001;69: 1101–8.
[140] Roller M, Rechkemmer G, Watzl B. Prebiotic inulin enriched with oligofructose in combination with the probiotics *Lactobacillus rhamnosus* and *Bifidobacterium lactis* modulates intestinal immune functions in rats. J Nutr 2004;134:153–6.
[141] Macpherson AJ, McCoy KD, Johansen FE, *et al*. The immune geography of IgA induction and function. Mucosal Immunol 2008;1: 11–22.
[142] Monteiro RC, Van De Winkel JG. IgA Fc receptors. Annu Rev Immunol 2003;21:177–204.
[143] Groschwitz KR, Hogan SP. Intestinal barrier function: molecular regulation and disease pathogenesis. J Allergy Clin Immunol 2009;124: 3–20; quiz 21-2.
[144] Marchiando AM, Graham WV, Turner JR. Epithelial barriers in homeostasis and disease. Annu Rev Pathol 2010;5:119–44.
[145] Fedwick JP, Lapointe TK, Meddings JB, *et al*. Helicobacter pylori activates myosin light-chain kinase to disrupt claudin-4 and claudin-5 and increase epithelial permeability. Infect Immun 2005;73: 7844–52.
[146] Lapointe TK, O'Connor PM, Buret AG. The role of epithelial malfunction in the pathogenesis of enteropathogenic *E. coli*-induced diarrhea. Lab Invest 2009;89:964–70.
[147] Resta-Lenert S, Barrett KE. Live probiotics protect intestinal epithelial cells from the effects of infection with enteroinvasive *Escherichia coli* (EIEC). Gut 2003;52:988–97.
[148] Resta-Lenert S, Barrett KE. Probiotics and commensals reverse TNF-alpha- and IFN-gamma-induced dysfunction in human intestinal epithelial cells. Gastroenterology 2006;130:731–46.
[149] Ridlon JM, Kang DJ, Hylemon PB. Bile salt biotransformations by human intestinal bacteria. J Lipid Res 2006;47:241–59.
[150] Gérard P. Metabolism of cholesterol and bile acids by the gut microbiota. Pathogens 2013;3:14–24.
[151] Kumar M, Nagpal R, Kumar R, *et al*. Cholesterol-lowering probiotics as potential biotherapeutics for metabolic diseases. Exp Diabetes Res 2012;2012:902917.
[152] Lecerf JM, de Lorgeril M. Dietary cholesterol: from physiology to cardiovascular risk. Br J Nutr 2011;106:6–14.
[153] Ishimwe N, Daliri EB, Lee BH, *et al*. The perspective on cholesterol-lowering mechanisms of probiotics. Mol Nutr Food Res 2015;59: 94–105.
[154] Altmann SW, Davis HR Jr, Zhu LJ, *et al*. Niemann-Pick C1 Like 1 protein is critical for intestinal cholesterol absorption. Science 2004; 303:1201–4.
[155] Younossi ZM, Golabi P, Paik JM, *et al*. The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): a systematic review. Hepatology 2023;77: 1335–47.
[156] Hossain N, Afendy A, Stepanova M, *et al*. Independent predictors of fibrosis in patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 2009;7:1224–9; 1229.e1-2.
[157] Delzenne NM, Neyrinck AM, Cani PD. Modulation of the gut microbiota by nutrients with prebiotic properties: consequences for host health in the context of obesity and metabolic syndrome. Microb Cell Fact 2011;10(suppl 1):S10.
[158] Kim SW, Park KY, Kim B, *et al*. *Lactobacillus rhamnosus* GG improves insulin sensitivity and reduces adiposity in high-fat diet-fed mice through enhancement of adiponectin production. Biochem Biophys Res Commun 2013;431:258–63.
[159] Ma D, Forsythe P, Bienenstock J. Live *Lactobacillus rhamnosus* [corrected] is essential for the inhibitory effect on tumor necrosis factor alpha-induced interleukin-8 expression. Infect Immun 2004;72: 5308–14.
[160] Ejtahed HS, Mohtadi-Nia J, Homayouni-Rad A, *et al*. Probiotic yogurt improves antioxidant status in type 2 diabetic patients. Nutrition 2012; 28:539–43.
[161] Ren Z, Guo C, Yu S, *et al*. Progress in mycotoxins affecting intestinal mucosal barrier function. Int J Mol Sci 2019;20:2777.
[162] Ge P, Luo Y, Okoye CS, *et al*. Intestinal barrier damage, systemic inflammatory response syndrome, and acute lung injury: a troublesome trio for acute pancreatitis. Biomed Pharmacother 2020;132: 110770.
[163] Swidsinski A, Loening-Baucke V, Theissig F, *et al*. Comparative study of the intestinal mucus barrier in normal and inflamed colon. Gut 2007;56: 343–50.
[164] Kim Y, Kim SH, Whang KY, *et al*. Inhibition of *Escherichia coli* O157: H7 attachment by interactions between lactic acid bacteria and intestinal epithelial cells. J Microbiol Biotechnol 2008;18:1278–85.
[165] Caballero-Franco C, Keller K, De Simone C, *et al*. The VSL#3 probiotic formula induces mucin gene expression and secretion in colonic epithelial cells. Am J Physiol Gastrointest Liver Physiol 2007;292: G315–22.
[166] Guermonprez P, Valladeau J, Zitvogel L, *et al*. Antigen presentation and T cell stimulation by dendritic cells. Annu Rev Immunol 2002;20: 621–67.
[167] Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature 1998;392:245–52.
[168] Kwok KO, Fries LR, Silva-Zolezzi I, *et al*. Effects of probiotic intervention on markers of inflammation and health outcomes in women of reproductive age and their children. Front Nutr 2022;9:889040.
[169] Bondonno CP, Croft KD, Hodgson JM. Dietary nitrate, nitric oxide, and cardiovascular health. Crit Rev Food Sci Nutr 2016;56: 2036–52.
[170] Vasquez EC, Pereira TMC, Peotta VA, *et al*. Probiotics as beneficial dietary supplements to prevent and treat cardiovascular diseases: uncovering their impact on oxidative stress. Oxid Med Cell Longev 2019;2019:3086270.

[171] Asemi Z, Zare Z, Shakeri H, *et al*. Effect of multispecies probiotic supplements on metabolic profiles, hs-CRP, and oxidative stress in patients with type 2 diabetes. Ann Nutr Metab 2013;63:1–9.
[172] Hegazy SK, El-Bedewy MM. Effect of probiotics on pro-inflammatory cytokines and NF-kappaB activation in ulcerative colitis,. World J Gastroenterol 2010;16:4145–51.
[173] Bubier JA, Chesler EJ, Weinstock GM. Host genetic control of gut microbiome composition. Mamm Genome 2021;32:263–81.
[174] Lopera-Maya EA, Kurilshikov A, van der Graaf A, *et al*. LifeLines Cohort Study. Effect of host genetics on the gut microbiome in 7,738 participants of the Dutch Microbiome Project. Nat Genet 2022;54: 143–51.