Special Article

Prebiotic and probiotic treatment of nonalcoholic fatty liver disease: a systematic review and meta-analysis

Brett R. Loman, Diego Hernández-Saavedra, Ruopeng An, and R. Scott Rector

Context: *Nonalcoholic fatty liver disease (NAFLD) is a highly prevalent and underdiagnosed comorbidity of many chronic diseases that is associated with altered intestinal bacterial communities. This association has prompted research into alternative treatments aimed at modulating intestinal microbiota. Given the novelty of these treatments, scarce evidence regarding their effectiveness in clinical populations exists.* ***Objective:*** *This meta-analysis sought to systemically review and quantitatively synthesize evidence on prebiotic, probiotic, and synbiotic therapies for patients with NAFLD in randomized controlled trials.* ***Data sources:*** *PRISMA guidelines ensured transparent reporting of evidence. PICOS criteria defined the research question for the systematic review. A systematic keyword search in PubMed and EMBASE identified 25 studies: 9 assessed prebiotic, 11 assessed probiotic, and 7 assessed symbiotic therapies for a total of 1309 patients.* ***Data extraction:*** *Basic population characteristics, the primary variables of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (utilized for NAFLD diagnosis), and the secondary variables of body mass index (BMI), gamma-glutamyl transferase (γ-GT), tumor necrosis factor alpha (TNF-α), C-reactive protein (CRP), total cholesterol, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), and triglyceridges (TAG) were extracted. Pooled effect sizes of these variables were calculated by meta-analysis. No publication bias was identified using Begg's and Egger's tests or Cochrane bias assessment tool.* ***Results:*** *Meta-analysis indicated that microbial therapies significantly reduced BMI (−0.37 kg/m^2; 95% confidence interval [CI], −0.46 to −0.28; $P < 0.001$), hepatic enzymes (ALT, −6.9 U/L [95%CI, −9.4 to −4.3]; AST, −4.6 U/L [95%CI, −6.6 to −2.7]; γ-GT, −7.9 U/L [95%CI, −11.4 to −4.4]; $P < 0.001$), serum cholesterol (−10.1 mg/dL 95%CI, −13.6 to −6.6; $P < 0.001$), LDL-c (−4.5 mg/dL; 95%CI, −8.9 to −0.17; $P < 0.001$), and TAG (−10.1 mg/dL; 95%CI, −18.0 to −2.3; $P < 0.001$), but not inflammation (TNF-α, −2.0 ng/mL; [95%CI, −4.7 to 0.61]; CRP, −0.74 mg/L [95%CI, −1.9 to 0.37]). Subgroup analysis by treatment category indicated similar effects of prebiotics and probiotics on BMI and liver enzymes but not total cholesterol, HDL-c, and LDL-c.* ***Conclusion:*** *This meta-analysis supports the potential use of microbial therapies in the treatment of NAFLD and sheds light on their potential mode of action. Further research into these treatments should consider the limitations of biomarkers currently used for the diagnosis and progression of NAFLD, in addition to the inherent challenges of personalized microbial-based therapies.*

Affiliation: *B.R. Loman* was with the Division of Nutritional Sciences, College of Agricultural, Consumer and Environmental Sciences, University of Illinois, Urbana-Champaign, Illinois, USA, and is currently with the Center for Microbial Pathogenesis, The Research Institute at Nationwide Children's Hospital, Columbus, Ohio, USA. *D. Hernández-Saavedra* is with the Division of Nutritional Sciences, College of Agricultural, Consumer and Environmental Sciences, University of Illinois, Urbana-Champaign, Illinois, USA. *R. An* is with the Department of Kinesiology and Community Health, College of Applied Health Sciences, University of Illinois at Urbana-Champaign, Champaign, Illinois, USA. *R.S. Rector* is with the Research Service, Harry S. Truman Memorial VA Hospital and the Department of Medicine, Division of Gastroenterology and Hepatology, and Department of Nutrition and Exercise Physiology, University of Missouri–Columbia, Columbia, Missouri, USA.

Correspondence: *R.S. Rector*, 520 Hitt Street, University of Missouri, Columbia, MO 65211, USA. E-mail: rectors@health.missouri.edu.

Key words: alanine aminotransferase (ALT), aspartate aminotransferase (AST), microbiome, synbiotic, serum cholesterol.

doi: 10.1093/nutrit/nuy031

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most prevalent chronic liver disease, the second leading cause of liver transplantation, and a common comorbidity in patients with obesity, diabetes, and dyslipidemia.[1] Nonalcoholic fatty liver disease is characterized by excessive fat accumulation in the liver that promotes a chronic oxidative state and a proinflammatory environment, which can ultimately lead to liver failure. Nutritional, environmental, and genetic factors are known to contribute to the etiology of NAFLD, although these factors are not often integrated into the diagnosis, treatment, and monitoring of the disease.[2–4]

Serum concentrations of hepatic liver enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), are the clinical biomarkers of liver pathology, and abdominal ultrasound and liver biopsy are used for confirmation of NAFLD status.[5] Although elevated circulating levels of these enzymes often indicate hepatocellular inflammation or damage, the specificity and sensitivity of such markers present limitations for accurate clinical diagnosis.[6] Nevertheless, current intervention strategies that aim to improve NAFLD monitor circulating levels of AST and ALT to assess treatment effectiveness and progression.

Healthy lifestyle[1,7–10] and pharmacological intervention strategies[11–15] have been proposed to ameliorate and/or reverse NAFLD pathogenesis. The most prominent among these strategies include supplementation of specific vitamins, weight reduction through diet and exercise, and drug therapies that increase insulin sensitivity and/or reduce inflammation.[16]

Recent investigations have explored the intestinal microbiota and how its manipulation can impact initiation, progression, and recession of the disease. This is mostly related to the intricate connection between the metabolites produced by intestinal microbes and the nutrient- and toxin-processing functions of the liver. Although changes in intestinal microbial composition are most pronounced in late stages of NAFLD,[17] animal models[18,19] and human studies[20,21] demonstrate that microbes can contribute to the development of the disease through multiple processes. Detrimentally, microbes can enhance efflux of free fatty acids and de novo lipogenesis in the liver, overcolonize the small intestine (small intestinal bacterial overgrowth), alter intestinal barrier function, promote inflammation, and induce insulin resistance.[22] These contributions are not, however, readily apparent by simple characterization of microbial abundances in the early stages of disease progression because there is substantial overlap of bacterial populations among patients with NAFLD and healthy individuals.[23] Furthermore, comparison of relative abundances of bacterial populations does not directly demonstrate activity or metabolite production of the taxa in question.[24]

Microbial therapies are treatments, including prebiotics and probiotics, that aim to manipulate the intestinal microbiota. Prebiotics are nondigestible food ingredients that promote growth of beneficial microorganisms in the intestine, although this definition is continuously under debate[25] and could arguably include conveyance of health benefit(s) to the host. Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit to the host. Additionally, synbiotics are a combination of prebiotics and probiotics, the use of which seeks to maximize the effect of both prebiotics and probiotics. Several mechanisms through which these treatments may positively impact progression of NAFLD are considered herein. Enhanced microbial production of short-chain fatty acids, particularly butyrate, alter energy metabolism in the intestine and systemically.[26,27] Production of antimicrobial compounds and acidification of the intestinal lumen by probiotics may limit the proliferation of pathogens.[28] Finally, various microbial products have been demonstrated to modulate host immune responses.[29]

Previous systematic reviews have explored the potential of various therapies in the treatment of NAFLD. A review with meta-analysis by Younossi and colleagues[30] investigated many current treatment options for patients with NALFD, but no attention was paid to microbial therapies. The authors concluded that more targeted and personalized treatment options should be explored. A systematic review by Tarantino and Finelli[31] focused primarily on aspects of obesity rather than NAFLD, and no meta-analysis was conducted. Finally, a review with meta-analysis by Ma and colleagues[32] included only 4 probiotic intervention trials, but reported a reduction in circulating hepatic aminotransferases and tumor necrosis factor alpha (TNF-α) with reduced insulin resistance following prebiotic (short-chain fructooligosaccharides) and probiotic (*Lactobacillus bulgaricus*, *Streptococcus thermophiles*, *Lactobacillus rhamnosus* GG, *Bifidobacterium longum*) supplementation. These reviews did not address via meta-analysis the effects of prebiotics and synbiotics in the treatment of NAFLD and did not include the numerous probiotic trials that have been conducted more recently. Furthermore, pertinent factors contributing to NAFLD etiology, such as body mass index (BMI), serum lipids, and systemic inflammation, also require attention. Given these limitations in the existing literature, the aim of this work was to conduct a

Table 1 **PICOS criteria for inclusion and exclusion of studies**

Criteria	Inclusion criteria	Exclusion criteria
Population	Male and female patients of any age that presented at least 1 of the following: NAFLD, steatosis, liver fibrosis, steatohepatitis	Patients that presented at least 1 of the following: alcoholic steatohepatitis, alcoholic fatty liver disease, cirrhosis, hepatocarcinoma, or hepatitis
Intervention	Any prebiotic or probiotic treatment, or a combination of both (synbiotic)	Pharmacological treatment, genetic predisposition (SNPs), liver transplant patients
Comparison	Compared with placebo	N/A
Outcomes	Changes after intervention in any of the following parameters: BMI, ALT, AST, γ-GT, CHOL, LDL-c, HDL-c, TAG, and inflammatory markers TNF-α and CRP	Values for AST and/or ALT not reported
Study design	Any randomized control clinical trial	Nonoriginal study or case report, and non–peer reviewed article

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CHOL, cholesterol; CRP, C-reactive protein; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; N/A, not applicable; NAFLD, nonalcoholic fatty liver disease; SNPs, single nucleotide polymorphisms; TAG, triglycerides; TNF-α, tumor necrosis factor alpha; γ-GT, gamma-glutamyl transferase.

systematic review and meta-analysis to assess the efficacy of microbial therapies, including prebiotics, probiotics, and synbiotics, to provide evidence regarding the prescription of these interventions to NAFLD patients. The hypothesis was that all 3 microbial therapies would improve serum hepatic aminotransferase concentrations, BMI, inflammatory markers, and serum lipids, which may help elucidate the underlying mechanisms by which these treatments improve symptoms of NAFLD.

METHODS

The Preferred Reporting Items for Systemic Reviews and Meta-Analysis statement (PRISMA) guidelines were used to ensure transparent reporting of the scientific evidence (Appendix S1 in the Supporting Information online).[33]

Study selection criteria

Studies that met all of the following criteria were included in the review—study design: randomized, controlled trial, cohort study, pre/post study, or cross-sectional study; study participants: patients with NAFLD, nonalcoholic steatohepatitis (NASH), steatosis, steatohepatitis, hepatic fibrosis, and/or type II diabetes/metabolic syndrome; main outcome: hepatic steatosis and function; article type: peer-reviewed publication; and language: English, Spanish, or Portuguese. PICOS criteria was used to define the research question for the systematic review (Table 1).

Studies were excluded from the review for meeting any of the following criteria: patients with alcoholic steatohepatitis, alcoholic fatty liver disease, cirrhosis, or hepatocarcinoma; patients receiving additional drug therapy or with genetic predisposition (single nucleotide polymorphisms); liver transplant patients; nonoriginal study or case report; and non–peer reviewed article (eg, dissertation or conference proceeding) (Table 1).

Search strategy

The selection of an adequate search algorithm was adapted according to the medical subject heading (MeSH) terms implemented in PubMed and adapted for EMBASE on December 14, 2017. The term "prebiotic" is a relatively new concept; as such, dietary fiber was included in the algorithm to account for those papers that did not mention the concept of prebiotic. Unlike "prebiotic," "probiotic" is harder to assess given the ample selection of terms that could identify probiotic agents. However, certain probiotic foods were included in the search algorithm to account for the MeSH definition. Given the broad application of prebiotic and probiotic treatments, together with evidence supporting the synergic or additive effect of prebiotic and probiotics, this analysis also included studies that implemented a synbiotic formulation. A keyword search was performed in PubMed (Table S1 in the Supporting Information online). The search algorithm included all possible combinations of the MeSH terms from the following 4 groups: (1) NAFLD; (2) prebiotic; (3) probiotics; and (4) synbiotics. These algorithms were also adapted for searching in EMBASE (Table S2 in the Supporting Information online). Articles with ≥1 of the following keywords were excluded: "alcoholic steatohepatitis," "alcoholic fatty liver disease," "cirrhosis," "hepatocarcinoma," "drug therapy," and "hepatitis." Titles and abstracts of the articles identified through the keyword search were screened against the study selection criteria. Potentially relevant articles were retrieved for evaluation of the full texts. Two reviewers independently conducted title and abstract screening and identified potentially relevant articles. Inter-rater

agreement was assessed using Cohen's kappa ($\kappa = 0.753 \pm 0.0544$; $P < 0.0001$). Discrepancies were resolved through discussion between the 2 reviewers.

A cited reference search (ie, forward reference search) and a reference list search (ie, backward reference search) were conducted based on the articles identified from the keyword search. Articles found through forward/backward reference searches were further screened and evaluated using the same study selection criteria. The reference search was repeated on all newly identified articles until no additional relevant article was found. The 2 reviewers jointly determined the inclusion/exclusion of all articles retrieved in full texts, and discrepancies were resolved through discussion.[34]

Data extraction

A standardized data extraction form was used to collect the following methodological and outcome variables from each included study (Table 1): author(s), publication year, study design, type of treatment (prebiotic, probiotic, synbiotic), sample size, participant characteristics (ie, sex, age, and country), and NAFLD marker and treatment effect (ie, increase, decrease, or neutral change on physiological and biochemical parameters). Hepatic function was assessed through the parameters identified during the data extraction process: BMI, ALT, AST, gamma-glutamyl transferase (γ-GT), total cholesterol (CHOL), low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), triglycerides (TAG), and inflammation (TNF-α, interleukin 6, and C-reactive protein [CRP]).

Quantitative data synthesis

A meta-analysis was performed to estimate the pooled effect size for NAFLD status, assessed by steatosis/fibrosis and functional hepatic enzymes. A priori subgroup analyses by treatment type (prebiotic, probiotic, and synbiotic) and NAFLD confirmation (confirmed by ultrasound or biopsy vs no confirmation) were performed for all included studies. Study heterogeneity was assessed using the I^2 index. The level of heterogeneity represented by I^2 was interpreted as modest ($I^2 \leq 25\%$), moderate ($25\% < I^2 \leq 50\%$), substantial ($50\% < I^2 \leq 75\%$), or considerable ($I^2 > 75\%$). A fixed-effect model was estimated when modest to moderate heterogeneity was present, and a random-effect model was estimated when substantial to considerable heterogeneity was present. Publication bias was assessed by a visual inspection of the funnel plot and Begg's and Egger's tests, as well as by the Cochrane bias assessment tool.[35] All statistical analyses were conducted using the Stata 14.2 SE version (StataCorp, College Station, TX, USA). All analyses used 2-sided tests, and $P < 0.05$ was considered statistically significant.

Study quality assessment

The assessment was adapted from Littell et al[36] using the following criteria: 1) research question was clearly stated; 2) inclusion and exclusion criteria were clearly defined; 3) study participants represented the pathological population; 4) study findings were appropriately reported; 5) age and sex distribution were matched between healthy and pathological group; (6) hepatic function was clearly defined; (7) assessment assay was uniformly applied; (8) appropriate methodology was used to measure hepatic function; (9) sample size was justified using a power analysis; and (10) potential confounders were controlled for. Scores for each criterion range from 0 to 2, depending on whether the criterion was unmentioned or unmet (0), partially met (1), or completely met (2). The possible total study score ranges between 0 and 20. The study quality score helped measure the strength of study evidence but was not used to determine the inclusion of studies. The 2 evaluators of this review independently scored each study based on these 10 criteria.

RESULTS

Study selection

Figure 1 shows the study selection flow chart. A total of 3111 unduplicated articles were identified through the keyword and reference search, from which 2720 nonhuman studies were excluded. The remaining 391 articles went through title and abstract screening, and 357 of them were excluded. The remaining 34 articles were assessed in full texts. Nine articles were excluded after full-text review due to the following reasons: not available in English, Spanish, or Portuguese; studied a different population that was not relevant to NAFLD or NASH-related pathologies; was conducted in combination with transplant therapy; and/or had no prebiotic, probiotic, or relevant treatment that interacted directly with the microbiome (eg, fiber). In addition, 2 study protocols were eliminated because no experiment was conducted. Finally, 25 articles were included in the review.

Basic characteristics of the selected studies

Table 2[37–51,53–62] reports the basic characteristics of the selected studies. Of the 25 articles included in the review, 9 assessed prebiotic, 11 assessed probiotic, and 7 assessed symbiotic therapies. Notably, Javadi et al[54]

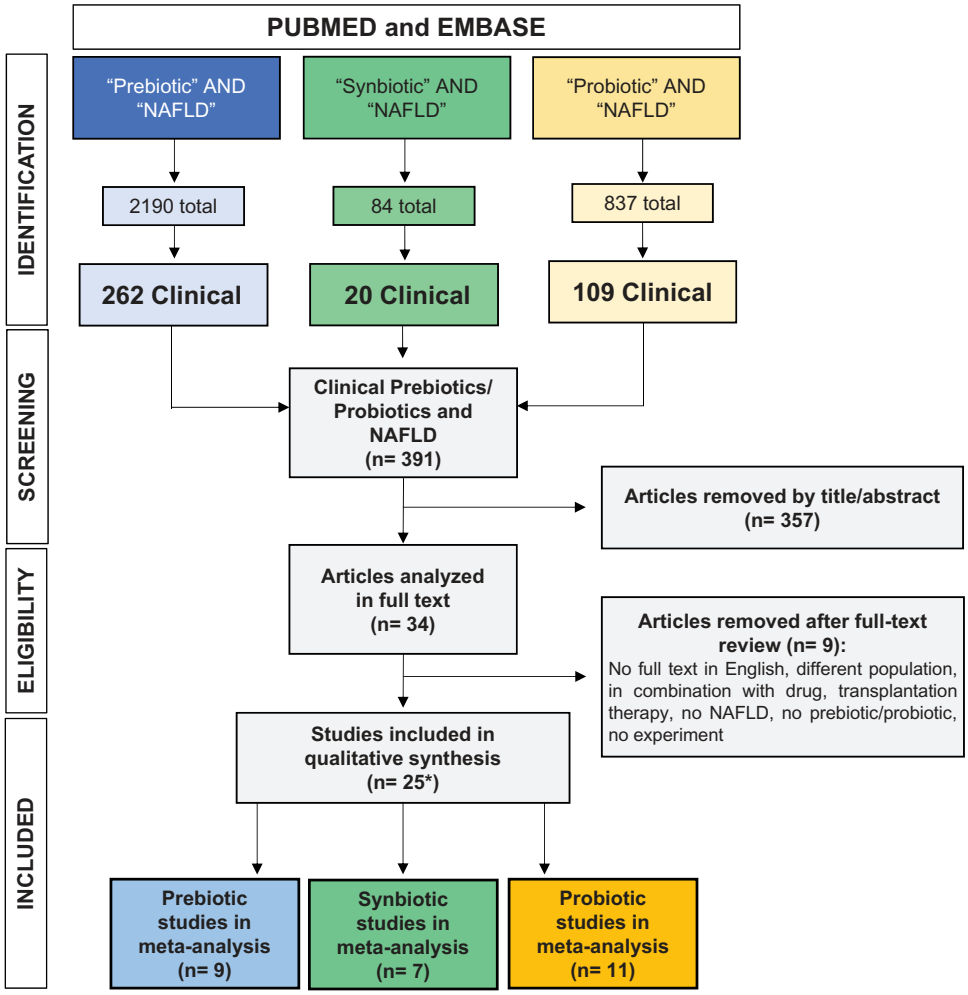

Figure 1 **Study selection flow chart.** *One study investigated 1 prebiotic, 1 probiotic, and their synbiotic combination. *Abbreviation*: NAFLD, nonalcoholic fatty liver disease.

investigated 1 prebiotic, 1 probiotic, and their synbiotic combination (displayed as "Javadi . . . (PRE)," "Javadi . . .(PRO)," and "Javadi . . .(SYN)" on the figures) and compared each with placebo control. Mobini et al[57] investigated 2 separate doses of the same probiotic (displayed as "Mobini . . .(LOW)" and "Mobini . . .(HIGH)" on the figures). The effect sizes of these different treatments were separated to investigate their variable contributions to overall effect size. Regarding the age of the participants included in the studies, probiotic studies tended to enroll a younger population, with a mean value of 35.4 ± 19.6 years, than did prebiotic and synbiotic studies (47.7 ± 15.9 and 47.6 ± 11.1, respectively). All patients included in the selected studies were ambulatory and did not require hospitalization. Sex distribution from all of the studies was approximately 59% males (n = 772) and 41% females (n = 537). Sample sizes were different between treatment types, with probiotic and synbiotic studies yielding a higher number of total participants (treatment and control combined) per study (n = 124.9 ± 40.9 and 103.1 ± 39.5, respectively) than prebiotic studies (n = 70.6 ± 30.6). Studies included in the review were conducted globally, with at least 1 study from each continent except Oceania included.

Patients receiving probiotic treatments represented the majority of the total patient population (43.3% for probiotic vs 34.0% and 16.5% for synbiotic and prebiotic, respectively), and most had confirmed cases of NAFLD or NASH by ultrasound or liver biopsy (68.0%). The average intervention duration was 2.9 ± 1.4 months.

The dose and characteristics of the treatments were more variable across the prebiotic class. Treatments included beta-glucan–supplemented cereals, psyllium husk, fructooligosaccharides (FOS), xylooligosaccharides (XOS), chicory inulin, and fiber extracts (ie, *Chlorella vulgaris*). For the synbiotic group of studies, the main source of prebiotic was FOS (n = 5 of 7 studies); the other 2 studies used inulin. Similar to the prebiotic studies, the probiotic

Table 2 **Basic characteristics of studies included in the review in chronological order**

References	Treatment type	Age, y; Sex distribution	Study design	Country	Prebiotic/probiotic dose	NAFLD marker/effect	Sample size
Cho et al. (2005)[43]	PRE	57.2 ± 2.8; M: 35.3%, F: 64.7%	Double-blind, randomized clinical trial	South Korea	2 packs of *Cassia tora* fiber supplement (2 g of *C. tora* fiber, 200 mg of tocopherol, 500 mg of ascorbic acid, and 300 mg of maltodextrin), or placebo (3.0 g of maltodextrin), for 2 mo	AST (−), ALT (−), CHOL (↓), LDL-c (↓), HDL-c (↑), TAG (−), BMI (↓)	PRE = 15 (n = 42)
Daubioul et al. (2005)[44]	PRE	54.6 ± 8.7; M: 46.7%, F: 53.3%	Double-blind, crossover, placebo-controlled design	Belgium	2 packs/d, breakfast and dinner 8 g of OFS (raftilose P95), or placebo (maltodextrine), supplied in 2 bouts of 8 wk	AST (−), ALT (−), γ-GT (−), CHOL (↑), LDL-c (↑), HDL-c (↑), TAG (−)	PRE = 7
Rocha et al. (2007)[59]	PRE	40.3 ± 8.7; M: 100.0%	Open-label clinical trial	Brazil	10 g/d of *Psyllum plantago* husk (Ispaghula husk) for 3 mo	AST (−), ALT (−), γ-GT (↓), CHOL (↓), TAG (↑), BMI (↓)	PRE = 12
Sheu et al. (2008)[60]	PRE	65.7 ± 7.7; M: 73.0%, F: 27.0%	Double-blind, randomized, placebo-controlled trial	Taiwan	Supplementation with 4 g/d of xylooligosaccharides (Xylooligo 95P, Suntory Co.), or placebo for 8 wk	AST (−), ALT (−), CHOL (↓), LDL-c (↓), HDL-c (−), TAG (−), BMI (−)	PRE = 14 (n = 26)
Chang et al. (2013)[42]	PRE	38.6 ± 11.1; M: 91.7%, F: 8.3%	Double-blind, randomized clinical trial	Taiwan	37.5 g cereal pack containing beta-glucan (3.7 g of fiber and 1.5 g of beta-glucan), or placebo (with a similar external but without beta-glucan) for 12 wk	BMI (↑), AST (↓), ALT (↓), γ-GT(↓), CHOL (↓), LDL-c (↓), HDL-c (−), TAG (↓)	PRE = 16 (n = 34)
Ebrahimi-Mameghani et al. (2014)[47]	PRE	20–50; M: 54.4%, F: 45.4%	Double-blind, randomized, controlled clinical trial	Iran	400 mg/d of vitamin E plus 4 300-mg tablets of *Chorella vulgaris* (ALGOMED) before breakfast (1 tablet), lunch (2 tablets), and dinner (1 tablet); placebo received 400 mg/d of vitamin E and 4 placebos/d for 8 wk	AST (–), ALT (−), CHOL (↓), LDL-c (↓), HDL-c (↑), TAG (−), BMI (↓)	PRE = 29 (n = 55)
Akbarzadeh et al. (2015)[37]	PRE	18–77; M: 53.0%, F: 46.0%	Single-blind, placebo-controlled, parallel, randomized clinical trial	Iran	10 g of psyllium (*Plantago ovata*) or 10 g of crushed wheat as placebo for 4 mo	AST (↓), ALT (↓), BMI (−)	PRE = 38 (n = 75)
Farhangi et al. (2016)[51]	PRE	48.07 ± 8.70; F: 100.0%	Randomized, placebo-controlled trial	Iran	Daily dose of 10 g of chicory inulin enriched with oligofructose (Frutafit IQ); placebo group received 10 g of maltodextrin (Jiujiang Hurirong Trade Co.) for 2 mo	AST (−), ALT (−)	PRE = 27 (n = 49)
Javadi et al. (2017)[54]	PRE, PRO, SYN	42.0 ± 8.9; M: 80.0%, F: 20.0%	Double-blind, placebo-controlled, clinical trial	Iran	Probiotic group received capsules (*Bifidobacterium longum* and *Lactobacillus acidophilus*: 2×10^7 CFU) and placebo of prebiotics (maltodextrin powder). Prebiotic received powder (inulin HP: 10 g/d) and a placebo of probiotics (fat- and lactose-free milk capsules). Synbiotic group received the probiotic and the prebiotic (*B. longum* and *L. acidophilus*: 2 × 107 CFU + inulin HP: 10 g) daily. Group 4 received placebos of prebiotics and probiotics. Dosage of supplements was 2 capsules at 250 mg/d of probiotics, 5-g sachet of prebiotic twice/day during morning and evening for 3 mo	AST (↓), ALT (↓), BMI (↓), γ-GT (−)	PRO = 20, PRE = 19, SYN = 17 (n = 75)

(continued)

Table 2 **Continued**

References	Treatment type	Age, y; Sex distribution	Study design	Country	Prebiotic/probiotic dose	NAFLD marker/effect	Sample size
Malaguarnera et al. (2012)[55]	SYN	48.3 ± 5.8; M: 50.0%, F: 50.0%	Double-blind, randomized clinical trial	Italy	2.5-g sachet containing *Bifidobacterium longum* W11 (5 billion per sachet) and FOS (Zirfos, Alfa Wassermann) and lifestyle modification, of placebo of only lifestyle modification for 24 wk	BMI (↑), AST (↑), ALT (↑), TNF-α (↓), CRP (↓), CHOL (↓), LDL-c (↓), HDL-c (↑), TAG (↓)	SYN = 34 (n = 63)
Wong et al. (2013)[62]	SYN	48.5 ± 11.1; M: 65.0%, F: 35.0%	Open-label, randomized controlled trial	China	10-g sachet of lepicol probiotic (with 200 million CFU of 5 probiotic cultures including *Lactobacillus plantarum*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, and *Bifidobacterium bifidum*) and prebiotic (3 g of FOS, cellulose, magnesium stearate, silica, and milk) twice a day for 6 mo, or usual care group for 6 mo	BMI (−), AST (↓), ALT (↓), CHOL (−), LDL-c (−), HDL-c (−), TAG (−)	SYN = 10 (n = 20)
Eslamparast et al. (2014)[49]	SYN	46.0 ± 9.1; M: 48.1%, F: 51.9%	Randomized, double-blind, placebo-controlled pilot trial	Iran	2 synbiotic capsules (Protexin) that contained 200 million CFU of 7 strains (*Lactobacillus casei, L. rhamnosus, Streptococcus thermophilus, Bifidobacteriu breve, L. acidophilus, B. longum*, and *L. bulgaricus*) and prebiotic (fructooligosaccharide) and probiotic cultures (magnesium stearate [source: mineral and vegetable]), or placebo (vegetable capsule [hydroxypropyl methyl cellulose]) for 28 wk	AST (↓), ALT (−), γ-GT (↓), TNF-α (−), CRP (↓)	SYN = 26 (n = 52)
Asgharian et al. (2016)[41]	SYN	Mean age 46–47; M: 25.6%, F: 74.3%	Double-blind, randomized, placebo-controlled, clinical trial	Iran	500-mg symbiotic capsule (Familact) containing 7 species of probiotic bacteria (*L. casei*, *L. acidophilus*, *L. rhamnosus*, *L. bulgaricus*, *B. breve*, *B. longum*, *S. thermophilus*) and fructooligosaccharides, or a placebo capsule (containing 120 mg of starch) for 8 wk	AST (−), ALT (−), CRP (−), BMI (−)	SYN = 38 (n = 74)
Asemi et al. (2017)[40]	SYN	35–70; Sex not specified	Randomized, crossover, clinical trial	Iran	Synbiotic food (Sekkeh Gaz Company) consisted of *Lactobacillus sporogenes* (1×10^7 CFU), 0.04 g of inulin (HPX) with 0.38 g of isomalt, 0.36 g of sorbitol, and 0.05 g of stevia as sweetener per 1 g for 6 wk	AST (−), ALT (−), BMI (−)	SYN = 62 (n = 124)
Ekhlasi et al. (2017)[48]	SYN	25–64; M: 80.0%, F: 20.0%	Double-blind, randomized, clinical trial	Iran	2 synbiotic capsules per day (Protexin Probiotics International Ltd.) that contained *L. casei*, *L. rhamnosus*, *S. thermophilus*, *B. breve*, *L. acidophilus*, *B. longum*, *L. bulgaricus* (2×10^8 CFU/g per capsule of each probiotic), and prebiotic fructooligosaccharide, or 2 placebo capsules of identical appearance to synbiotic (corn starch, Zahravi Pharmaceutical; alpha-tocopherol Zahravi Pharmaceutical Co) at a daily dosage of 400 IU for 8 wk	AST (↓), ALT (↓), TNF-α (↓), BMI (−)	SYN = 15 (n = 30)

(continued)

Table 2 **Continued**

References	Treatment type	Age, y; Sex distribution	Study design	Country	Prebiotic/probiotic dose	NAFLD marker/effect	Sample size
Aller et al. (2011)[39]	PRO	46.8 ± 13.4; M: 71.4%, F: 28.6%	Double-blind, randomized, clinical trial	Spain	1 tablet per day of probiotic mix (500 million CFU *L. bulgaricus* and *S. thermophiles*), or placebo (Starch), for 3 mo	BMI (−), AST (↓), ALT (↓), γ-GT(↓), TNF-α (−), IL-6 (↑), CHOL (−), LDL-c (−), HDL-c (↓), TAG (↓)	PRO = 14 (n = 24)
Vajro et al. (2011)[61]	PRO	10.7 ± 2.1; M: 100.0%	Double-blind, placebo-controlled, pilot study	Italy	Oral *Lactobacillus* Gorbach-Goldin (12 billion CFU/d), or placebo for 8 wk	ALT (↓), TNF-α (−), IL-6 (↑)	PRO = 10 (n = 20)
Alisi et al. (2014)[38]	PRO	10.7 ± 1.3; M: 35.1%, F: 64.9%	Double-blind, randomized controlled trial	Italy	1 sachet/d of VSL#3 (*S. thermophilus*, *B. breve*, *Bifidobacterium infantis*, *B. longum*, *L. acidophilus*, *L. plantarum*, *L. paracasei*, and *L. delbrueckii* spp. *Bulgaricus*) if the participant was aged <10 y, or 2 sachets of VSL#3 if aged >10 y, or placebo for 4 mo	BMI (↓), ALT (↓), TAG (−)	PRO = 22, males = 10 (n = 44)
Dönmez et al. (2014)[45]	PRO	33.6 ± 3.1; M: 54.8%, F: 45.2%	Randomized controlled trial	Turkey	350 mL of koumiss per d for 15 d (fresh homemade koumiss), with or without exercise, or just exercise for 2 wk	AST (↓), ALT (↓), γ-GT (↓), CHOL (−), TAG (↑)	PRO = 6 (n = 18)
Nabavi et al. (2014)[58]	PRO	43.4 ± 8.4; M: 48.6%, F: 51.4%	Double-blind, randomized controlled trial	Iran	300 g daily of probiotic yogurt (*L. acidophilus* La5 4.42×10^6 CFU/g, and *B. lactis* Bb12 $\times$ 10^6 CFU/g), or placebo (conventional yogurt) for 8 wk	BMI (↓), AST (−), ALT (↓), CHOL (↓), LDL-c (↓), HDL-c (↓), TAG (↓)	PRO = 36, males = 17 (n = 72)
Firouzi et al. (2015)[53]	PRO	56.7 ± 9.1; M: 66.7%, F: 33.3%	Randomized, double-blind, parallel-group, controlled, clinical trial	Malaysia	1 sachet of probiotic (*L. acidophilus*, *L. casei*, *L. lactis*, *B. bifidum*, *B. longum*, and *B. infantis* with a daily dose of 6×10^{10} CFU [Hexbio B-Crobes Laboratory]), or placebo for 12 wk	AST (↓), ALT (↓)	PRO = 68 (n = 136)
Miccheli et al. (2015)[56]	PRO	10.7 ± 1.3; M: 90.0%, F: 10.0%	Double-blind randomized controlled trial	Italy	1 sachet VSL#3 if the participant was aged <10 y, or 2 sachets of VSL#3 if aged >10 y, or placebo for 4 mo	BMI (↓), AST (−), ALT (−), CHOL (−), LDL-c (↑), HDL-c (−), TAG (↑)	PRO = 15, males = 8 (n = 31)
Dugan et al. (2016)[46]	PRO	N/A; M: 54.5%, F: 45.5%	Randomized crossover study	United States	16-wk crossover study of low-fat dairy products (10 oz of 1% milk, 6 oz of nonfat yogurt, and 2 oz of 2% cheese, providing ≈3 dairy servings/d) or control foods (1.5 oz of granola bar and 12 oz of juice) into their usual diet for 6 wk	BMI (−), AST (↓), ALT (↓), γ-GT(↓), TNF-α(−), CRP (↑), CHOL (↓), LDL-c (−), HDL-c (↓), TAG (↓)	PRO = 37, males = 13

(continued)

Table 2 **Continued**

References	Treatment type	Age, *y*; Sex distribution	Study design	Country	Prebiotic/probiotic dose	NAFLD marker/effect	Sample size
Mobini et al. (2017)[57]	PRO	65 ± 6; M: 23.0%, F: 77.0%	Double-blind, randomized, placebo-controlled trial	Sweden	3 parallel groups of participants receiving 10^8 or 10^{10} CFU of *Lactobacillus reuteri* DSMZ 17 938 (BioGaia) or placebo for 12 wk	AST (−), ALT (−), CHOL (−), LDL-c (−), HDL-c (−), TAG (−), CRP (−), BMI (↑ low dose)	PRO (low dose) = 15, PRO (high dose) = 14 (n = 44)
Famouri et al. (2017)[50]	PRO	12.6 ± 1.9; M: 50.0%, F: 50.0%	Randomized, triple-blind controlled trial	Iran	1 capsule/d (*L. acidophilus* ATCC B3208, 3×10^9 CFU; *B. lactis* DSMZ 32 269, 6×10^9 CFU; *B. bifidum* ATCC SD6576, 2×10^9 CFU; *L. rhamnosus* DSMZ 21 690, 2×10^9 CFU), or placebo for 12 wk	AST (−), ALT (↓), CHOL (↓), LDL-c (↓), HDL-c (−), TAG (↓)	PRO = 32 (n = 64)

For effect on measurable outcomes, a positive or upward effect is denoted by (↑), a negative or downward effect is denoted by (↓), and no effect is denoted by (−).

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ATCC, American Type Culture Collection; BMI, body mass index; CFU, colony-forming unit; CHOL, cholesterol; CRP, C-reactive protein; DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen; F, female; FOS, fructooligosaccharide; HDL-c, high-density lipoprotein cholesterol; IL-6, interleukin 6; LDL-c, low-density lipoprotein cholesterol; M, male; N/A, not applicable; NAFLD, nonalcoholic fatty liver disease; OFS, oligofructosaccharides; PRE, patients with prebiotic treatment; PRO, patients with probiotic treatment; SYN, patients with simultaneous prebiotic and probiotic treatment; TAG, triglycerides; TNF-α, tumor necrosis factor alpha; γ-GT, gamma-glutamyl transferase.

studies were highly divergent by the species of microorganisms supplemented (*Lactobacillus reuteri*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus lactis*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus sporogenes*, *Lactobacillus delbrueckii*, *Bifidobacterium bifidum*, *Bifidobacterium longum*, *Bifidobacterium infantis*, *Bifidobacterium breve*, and *Streptococcus thermophilus*), and most studies supplemented multiple organisms. *Lactobacillus acidophilus* was the most commonly used species in both probiotic and synbiotic treatments.

Fourteen of 25 studies included in the review showed an effect on at least 1 liver enzyme (ie, AST, ALT, or γ-GT). The proportion of success was similar between treatments: 75% among prebiotic studies, 70% among probiotic studies, and 75% among synbiotic studies. Other parameters, such as anthropometric measurements (eg, waist-to-hip ratio), insulin sensitivity, and serum very-low-density lipoprotein cholesterol (VLDL-c), were not analyzed because very few of the included studies measured or reported them.

Effect on body mass index

The results of the meta-analysis on BMI are displayed in Figure 2.[37–42, 46–49, 54–60,62] All 3 treatment types combined reduced BMI by 0.37 kg/m^2 (95% confidence interval [CI], −0.46 to −0.28; $P < 0.001$). Furthermore, each treatment type individually reduced BMI: prebiotics reduced BMI by 0.54 kg/m^2 (95%CI, −0.87 to −0.21; $P < 0.001$) (Figure 2B), probiotics reduced BMI by 0.51 kg/m^2 (95%CI, −0.86 to −0.16; $P < 0.001$) (Figure 2C), and synbiotics reduced BMI by 0.13 kg/m^2 (95%CI, −0.22 to −0.05; $P < 0.001$) (Figure 2D). Subgroup analyses of studies that confirmed NAFLD diagnosis for all treatment types combined was performed. Because the study performed by Malaguarnera et al[55] does not include a treatment-free or no-exercise control group (reference group was dietary intervention plus exercise), this study was excluded from all subgroup analyses. Consistently, microbial therapies decreased BMI in confirmed NAFLD studies by 0.55 kg/m^2 (95%CI, −0.69 to −0.41; $P < 0.001$); although studies without NAFLD confirmation did not produce a change in BMI (−0.12 kg/m^2; 95%CI, −0.27 to 0.03).

Effect on liver function and inflammation

Figures 3–5[37–51,53–62] display the results of the meta-analysis on serum liver enzymes. All 3 treatment types combined reduced ALT by 6.85 U/L (95%CI, −9.37 to −4.33; $P < 0.001$) (Figure 3A). Prebiotics reduced ALT by 9.75 U/L (95%CI, −15.77 to −3.72; $P < 0.001$) (Figure 3B), and probiotics reduced ALT by 6.60 U/L

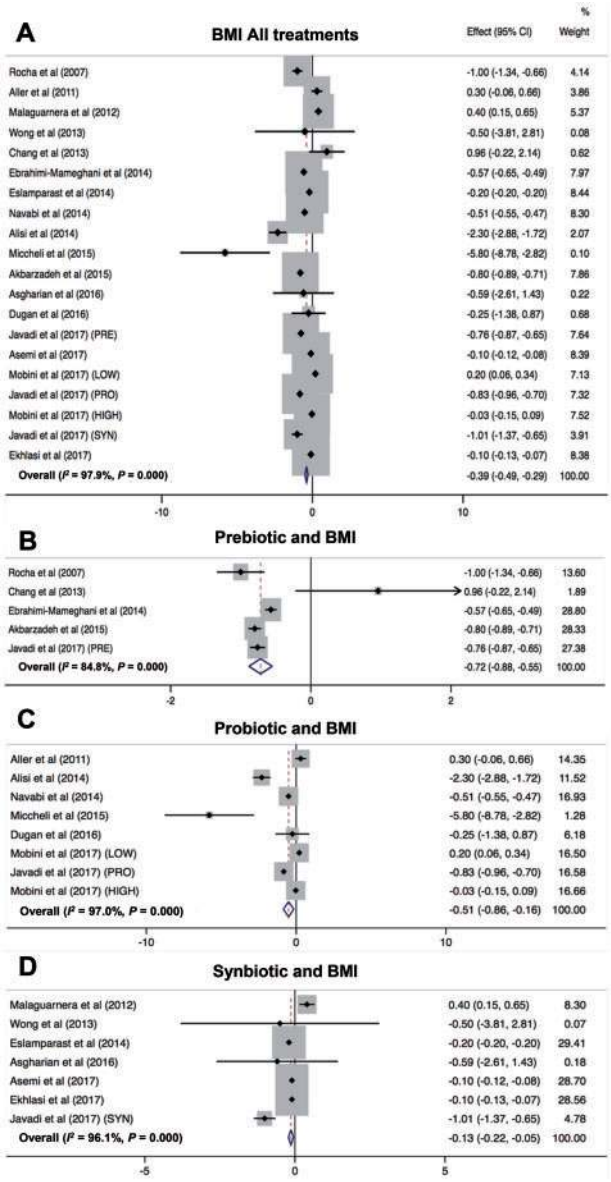

Figure 2 **Effect of prebiotic, probiotic, and synbiotic therapies on body mass index (BMI).** A, Forest plot for meta-analysis of BMI for all treatments. Subgrouping of prebiotic only (B), probiotic only (C), and synbiotic (D). Overall estimate is pooled estimate of BMI values. A dashed line indicates an average of the control group, and a solid line indicates an average of the overall pooled estimate. Weights are from random effects; DerSimonian-Laird estimator. *Abbreviations*: BMI, body mass index; CI, confidence interval.

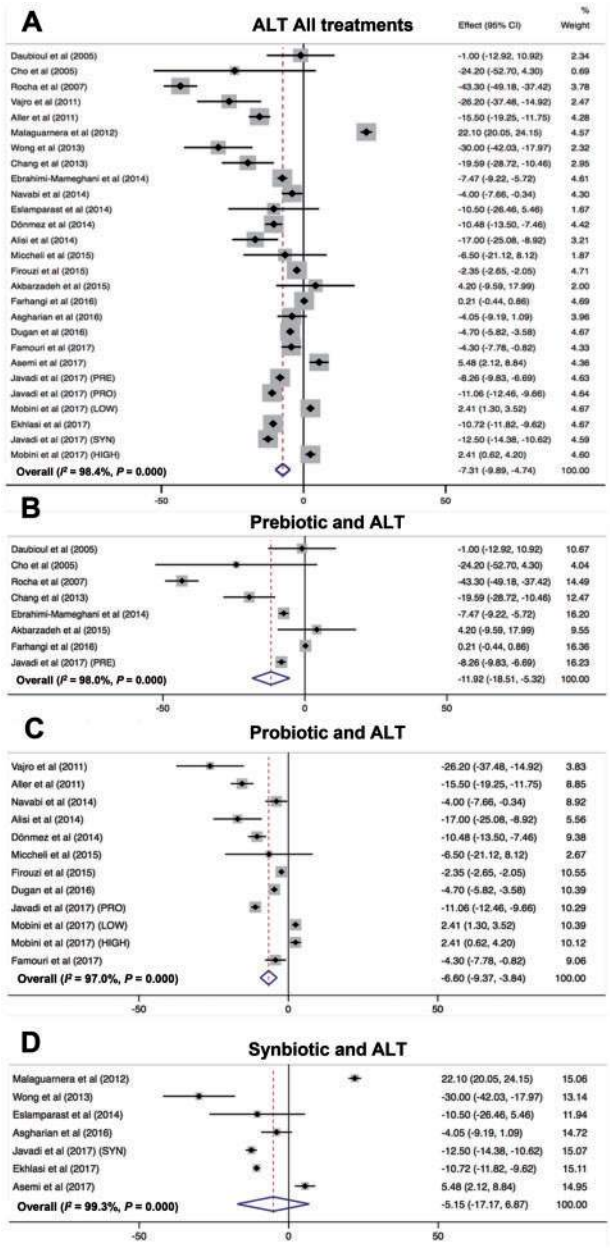

Figure 3 **Effect of prebiotic, probiotic, and synbiotic therapies on alanine aminotransferase (ALT).** *A*, Forest plot for meta-analysis of ALT for all treatments. Subgrouping of serum ALT levels with prebiotic only (*B*), probiotic only (*C*), and synbiotic (*D*). Overall estimate is pooled estimate of serum ALT values. A dashed line indicates an average of the control group, and a solid line indicates an average of the overall pooled estimate. Weights are from random effects; DerSimonian-Laird estimator. *Abbreviations:* ALT, alanine aminotransferase; CI, confidence interval.

(95%CI, −9.37 to −3.84; $P < 0.001$) (Figure 3C), but synbiotics did not affect ALT (Figure 3D). Subgroup analysis by confirmed NAFLD diagnosis (excluding Malaguarnera et al[55]) demonstrated that microbial therapies reduced ALT by 11.74 U/L (95%CI, −14.55 to −8.93; $P < 0.001$), whereas studies without NAFLD confirmation showed no change in ALT (−2.56 U/L; 95%CI, −5.41 to 0.28).

Likewise, all 3 treatment types combined reduced AST by 4.64 U/L (95%CI, −6.56 to −2.71; $P < 0.001$) (Figure 4A). In contrast with ALT, each treatment type individually reduced AST; prebiotics reduced AST by 5.73 U/L (95%CI, −8.05 to −3.41; $P < 0.001$) (Figure 4B), probiotics reduced AST by 3.50 U/L

(95%CI, −5.56 to −1.45; $P < 0.001$) (Figure 4C), and synbiotics reduced AST by −7.73 U/L (95%CI, −13.85 to −1.62; $P < 0.001$) (Figure 4D). Subgroup analysis by confirmed NAFLD diagnosis (excluding Malaguarnera et al[55]) demonstrated that microbial therapies reduced AST by 8.56 U/L (95%CI, −10.65 to −6.47; $P < 0.001$), and once again this effect was absent in the group with no NAFLD confirmation (−1.65 U/L; 95%CI, −3.49 to 0.19). Finally, γ-GT was also decreased by all treatment types combined by −7.86 U/L (95%CI, −11.36 to −4.36; $P < 0.001$) (Figure 5A). Subgroup analysis by NAFLD confirmation (excluding Malaguarnera et al[55]) demonstrated that γ-GT was reduced by microbial therapies both in studies with confirmed NAFLD (−8.69 U/L; 95%CI, −13.46 to −3.92; $P < 0.001$) and, to a lesser extent, in studies with no NAFLD confirmation (−5.56; 95%CI, −9.24 to −1.88; $P < 0.001$).

Figure 5[39,41,46,48,49,55,57,61]displays the results of the meta-analysis on markers of acute inflammation measured in serum. A marginal decrease was observed for both serum TNF-α (−2.04 ng/mL; 95%CI, −4.70 to 0.61) (Figure 5B) and hepatic cytokine CRP (−0.74 mg/L; 95%CI, −1.85 to 0.37) (Figure 5D) for all treatment types combined, but both failed to reach significance. However, subgroup analysis by confirmed NAFLD diagnosis (excluding Malaguarnera et al[55]) revealed that microbial therapies reduced CRP by 1.35 mg/L (95%CI, −2.54 to −0.15; $P < 0.001$), whereas CRP was not reduced in studies with no NAFLD confirmation (−0.11 mg/L; 95%CI, −0.44 to 0.22). Only 1 study that measured TNF-α did not confirm NAFLD diagnosis (Dugan et al[46]), so subgroup analysis could not be performed.

Effect on lipid profile

A meta-analysis of serum lipid profile was performed among studies that reported CHOL (Figure 6),[39,42–47,50,55–60,62] LDL-c (Figure 7),[39,42–47,50,55–60,62] HDL-c (Figure 8),[39,42–47,50,55–59,62] and TAG (Figure 9).[38,39,42–47,50,55–60,62] Microbial therapies reduced total serum CHOL by 10.10 mg/dL (95%CI, −13.56 to −6.64; $P < 0.001$) (Figure 6A). Conversely, individually prebiotic and probiotic treatment types did not decrease CHOL (Figure 6B and 6C) but synbiotics did (Figure 6D); synbiotics reduced CHOL by 14.89 mg/dL (95%CI, −17.34 to −12.44; $P < 0.001$), although only 2 synbiotic studies reported CHOL. Subgroup analysis by confirmed NAFLD diagnosis (excluding Malaguarnera et al[55]) also failed to identify changes in CHOL (−6.42 mg/dL; 95%CI, −19.34 to 6.50). However, microbial therapy did decrease CHOL in the studies with

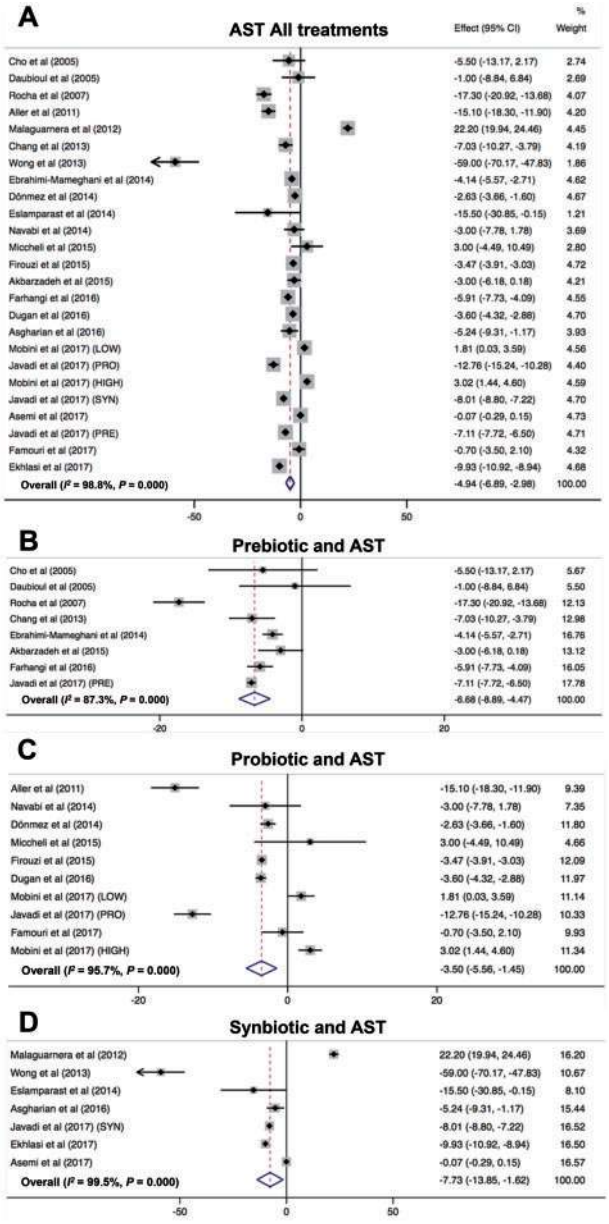

Figure 4 **Effect of prebiotic, probiotic, and synbiotic therapies on aspartate aminotransferase (AST).** *A*, Forest plot for meta-analysis of AST for all treatments. Subgrouping of serum AST levels with prebiotic only (*B*), probiotic only (*C*), and synbiotic (*D*). Overall estimate is pooled estimate of serum AST values. A dashed line indicates an average of the control group, and a solid line indicates an average of the overall pooled estimate. Weights are from random effects; DerSimonian-Laird estimator. *Abbreviations:* AST, aspartate aminotransferase; CI, confidence interval.

no NAFLD confirmation (−11.15 mg/dL; 95%CI, −19.33 to −2.97; $P < 0.001$).

All 3 treatment types combined reduced serum LDL-c by −4.52 mg/dL (95%CI, −8.87 to −0.17; $P < 0.001$) (Figure 7A). Analysis by treatment type demonstrated that prebiotics reduced LDL-c (−6.67 mg/dL; 95%CI, −12.03 to −1.30; $P < 0.001$) (Figure 7B), but

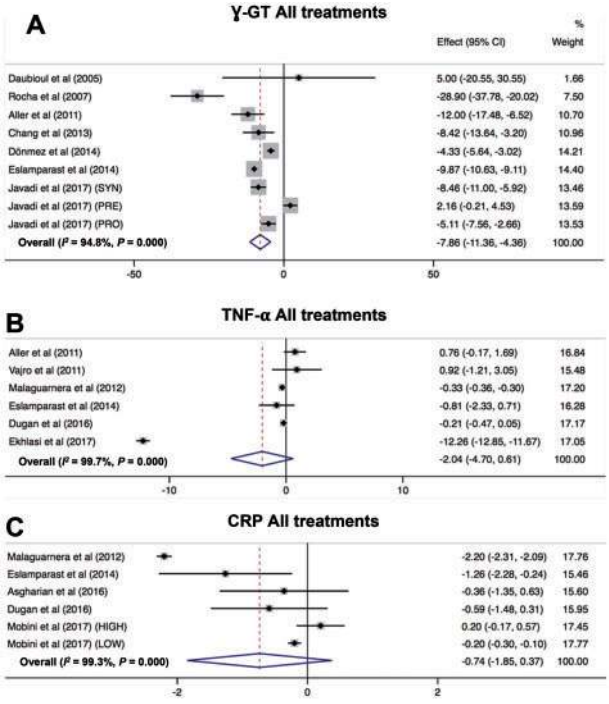

Figure 5 **Effect of prebiotic, probiotic, and synbiotic therapies on gamma-glutamyl transferase (γ-GT) and inflammation.** Forest plot for meta-analysis of γ-GT (*A*), tumor necrosis factor alpha (TNF-α) (*B*), and C-reactive protein (CRP) (*C*) for all treatments. Overall estimate is pooled estimate of serum γ-GT and inflammatory cytokines. A dashed line indicates an average of the control group, and a solid line indicates an average of the overall pooled estimate. Weights are from random effects; DerSimonian-Laird estimator. *Abbreviations:* CI, confidence interval; CRP, C-reactive protein; TNF-α, tumor necrosis factor alpha; γ-GT, gamma-glutamyl transferase.

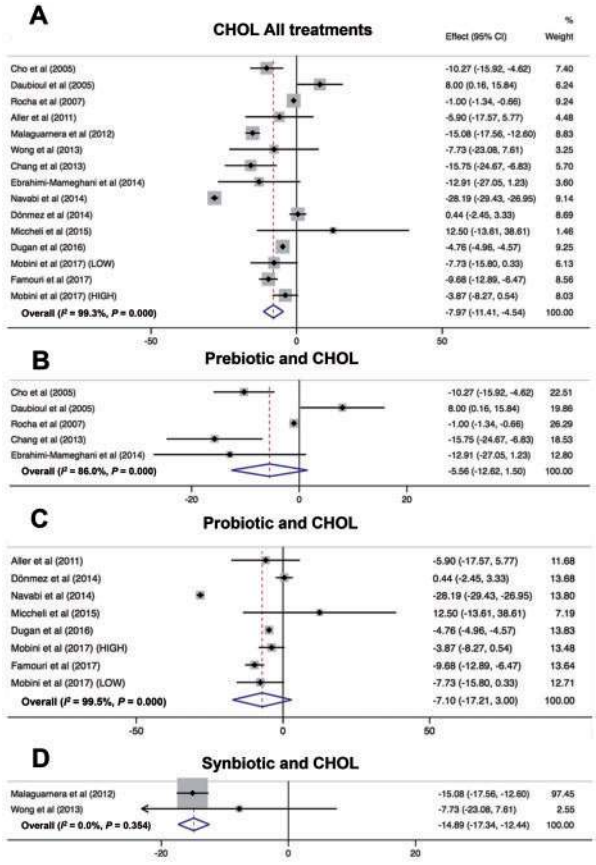

Figure 6 **Effect of prebiotic, probiotic, and synbiotic therapies on total cholesterol (CHOL).** *A*, Forest plot for meta-analysis of CHOL for all treatments. Subgrouping of serum CHOL levels with prebiotic only (*B*), probiotic only (*C*), and synbiotic (*D*). Overall estimate is pooled estimate of serum CHOL values. A dashed line indicates an average of the control group, and a solid line indicates an average of the overall pooled estimate. Weights are from random effects; DerSimonian-Laird estimator. *Abbreviations:* CHOL, cholesterol; CI, confidence interval.

probiotics and synbiotics did not (Figure 7C and 7D). Subgroup analysis (excluding Malaguarnera et al[55]) failed to identify changes in LDL-c for studies with NAFLD confirmation (−4.78; 95%CI, −12.63 to 3.07), as well as for studies with no NAFLD confirmation (−2.99; 95%CI, −7.40 to 1.42).

Given the great inconsistencies in the values for HDL-c reported in Sheu et al,[60] this study was removed for the analysis of overall and subgroup analysis of HDL-c. All 3 treatment types combined did not affect serum HDL-c (0.56 mg/dL; 95%CI, −1.55 to 2.67) (Figure 8A). Prebiotic treatement increased HDL-c by 2.25 mg/dL (95%CI, 0.68 to 3.81; $P < 0.001$) (Figure 8B), probiotics reduced HDL-c by 1.32 mg/dL (95%CI, −2.00 to −0.65; $P < 0.001$) (Figure 8C), and synbiotics had no effect on HDL-c (1.08 mg/dL; 95%CI, −6.69 to 8.84) (Figure 8D). Subgroup analysis (excluding Malaguarnera et al[55] and Sheu et al[60]) failed to identify an effect on HDL-c for studies with NAFLD confirmation (0.66; 95%CI, −1.97 to 3.39), as well as for studies with no NAFLD confirmation (0.36 mg/dL; 95%CI, −1.81 to 2.53, respectively).

Finally, microbial therapies reduced circulating TAG by 10.14 mg/dL (95%CI, −18.02 to −2.22; $P < 0.001$) (Figure 9A). No significant effect was observed for any individual treatment type (Figure 9B–D). Subgroup analysis by confirmed NAFLD diagnosis (excluding Malaguarnera et al[55]) failed to identify an effect on TAG (−9.46; 95%CI, −21.60 to 2.67), but in studies without NAFLD confirmation serum, TAG decreased by 10.86 mg/dL (95%CI, −20.40 to −1.31; $P < 0.001$).

Meta-analysis summary

Overall, these results highlight important effects of microbial therapies on BMI, hepatic enzymes, inflammatory markers, serum cholesterol, and triglycerides. A

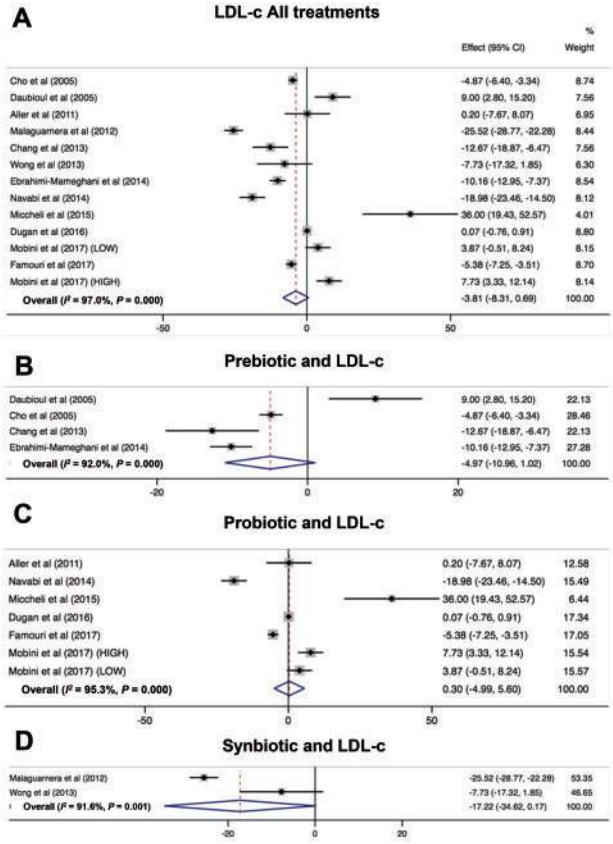

Figure 7 **Effect of prebiotic, probiotic, and synbiotic therapies on low-density lipoprotein cholesterol (LDL-c).** *A*, Forest plot for meta-analysis of serum LDL-c for all treatments. Subgrouping of serum LDL-c levels with prebiotic only (*B*), probiotic only (*C*), and synbiotic (*D*). Overall estimate is pooled estimate of serum LDL-c values. A dashed line indicates an average of the control group, and a solid line indicates an average of the overall pooled estimate. Weights are from random effects; DerSimonian-Laird estimator. *Abbreviations:* CI, confidence interval, LDL-c, low-density lipoprotein cholesterol.

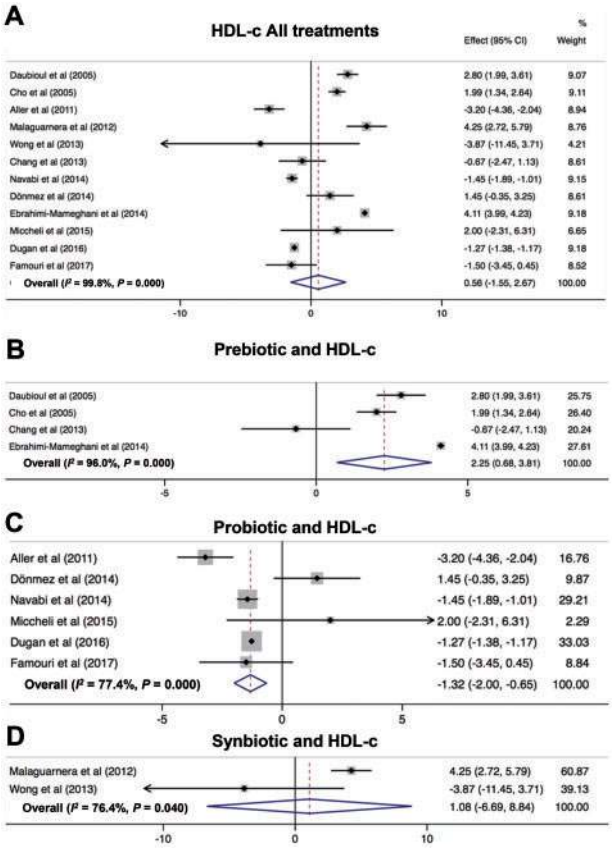

Figure 8 **Effect of prebiotic, probiotic, and synbiotic therapies on high-density lipoprotein cholesterol (HDL-c).** *A*, Forest plot for meta-analysis of HDL-c for all treatments. Subgrouping of serum HDL-c levels with prebiotic only (*B*), probiotic only (*C*), and synbiotic (*D*). Overall estimate is pooled estimate of serum HDL-c values. A dashed line indicates an average of the control group, and a solid line indicates an average of the overall pooled estimate. Weights are from random effects; DerSimonian-Laird estimator. *Abbreviations:* CI, confidence interval; HDL-c, high-density lipoprotein cholesterol.

pictorial summary of these results is displayed in Figure 10.

Study quality assessment

Table 3 reports criterion-specific and global ratings from the study quality assessment. The average score for the publications included in the study was 16.24 ± 2.29 out of 20. Most studies included the criteria, scoring between 1.15 and 1.85 out of 2.0 points. In contrast, only about half of the studies provided justification for sample size selection.[38,39,50,52,53,56,58,59,62]

Risk-of-bias assessment

No publication bias was identified, as neither Egger's tests nor Begg's tests were statistically significant. The Cochrane bias assessment tool was used to evaluate overall bias and within-study bias of the included studies. The general and within-study risks of bias are shown in Figure S1A in the Supporting Information online. Individual study bias can be found in Figure S1B in the Supporting Information online. Most studies (84.0%) were randomized; for 4 studies, the randomization process was not clearly described or was omitted. Methods of allocation concealment were extensively described in 68.0% (n = 17) of the studies, and most of them described a blinding method in the study design (n = 18). Much of the studies provided sufficient (80.0%) but not extensive information regarding the blinding outcome assessment, but given the quantitative nature of the outcome variables, it was unlikely that the outcome measurement would be influenced by the blinding of participants. Incomplete outcome data or attrition bias was considered low if the studies had no

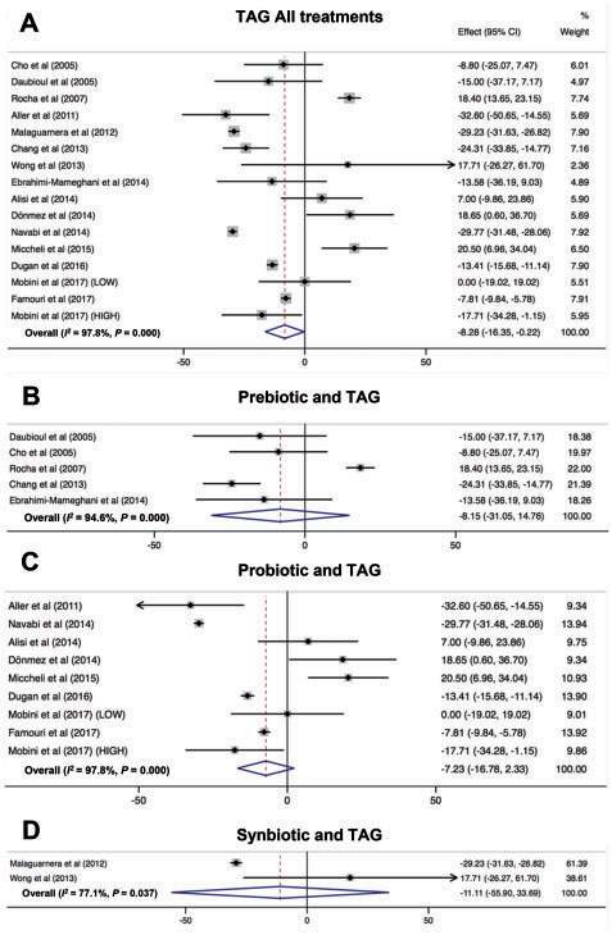

Figure 9 **Effect of prebiotic, probiotic, and synbiotic therapies on serum triglycerides (TAG).** *A*, Forest plot for meta-analysis of TAG for all treatments. Subgrouping of serum TAG levels with prebiotic only (*B*), probiotic only (*C*), and synbiotic (*D*). Overall estimate is pooled estimate of serum TAG values. A dashed line indicates an average of the control group, and a solid line indicates an average of the overall pooled estimate. Weights are from random effects; DerSimonian-Laird estimator. *Abbreviations:* CI, confidence interval; TAG, triglycerides.

dropouts, if an intention-to-treat data analysis was conducted and provided adequate explanation for exclusion, and there were even numbers of dropouts between treatments and controls (as applicable). Most studies (88.0%) included in the analysis had low attrition bias, as well as low selective reporting (96.0%). Individual within-study bias assessment can be found in Figure S1B in the Supporting Information online.

DISCUSSION

This study systematically reviewed and quantitatively synthesized scientific evidence regarding microbial therapy (ie, prebiotics, probiotics, and synbiotics) in the treatment of NAFLD. A total of 1309 patients from 25 different studies were included in the review. The meta-analysis found important reductions in BMI, hepatic enzymes (ALT, AST, and γ-GT), serum cholesterol, and triglycerides following treatment (summarized in Figure 10). Subgroup analyses by treatment type (ie, prebiotics, probiotics, and synbiotics) indicated similar effects of prebiotics and probiotics on BMI, liver enzymes, and HDL-c but differential effects on LDL-c.

Improvement of hepatic function in NAFLD patients is clinically assessed by quantifying the standard clinical diagnostic markers of liver dysfunction (systemic liver enzymes ALT, AST, and sometimes γ-GT), and the presence of elevated enzymes in circulation is regarded as a reliable indicator of liver damage. Probiotic interventions are found to be effective at reducing serum hepatic enzymes in NAFLD and other clinical populations, including those who are pregnant,[63] those who have hepatitis,[64] and those who have alcoholic fatty liver disease.[65] Prebiotic interventions remain an understudied area, although NAFLD is the primary patient population in whom hepatic enzymes have been investigated in association with these treatments. In this review, both prebiotics and probiotics were associated with a decrease in AST, ALT, and γ-GT, indicating a protective effect through potential alteration of intestinal microbial composition and metabolism in patients with NAFLD. Aspartate aminotransferase, ALT, and γ-GT are markers of liver health but only serve as general markers of liver damage (ie, hepatic cell death) rather than markers of specific liver function. Therefore, the mechanisms contributing to their decreased concentration in response to microbial therapies are likely to be multifactorial. In NAFLD patients, inflammation of the intestine is thought to promote the translocation of bacteria and their products, which, in turn, stimulate liver resident Kupffer cells[66] and stellate cells[67] to promote a highly inflammatory and fibrotic state that leads to liver cell death and the concomitant leakage of hepatic enzymes. Despite the great amount of evidence in animals[68–71] and humans[72] that demonstrates the efficacy of microbial therapies in the treatment of liver diseases,[73] the mechanisms that link prebiotics or probiotics with decreased serum hepatic enzymes are not fully elucidated. A major weakness of these studies is the complete lack of any investigation into the intestinal microbiota, which assumedly would change in composition and/or function in response to microbial therapy. In a review, Wieland et al[74] investigated composition of intestinal microbiota in both animals and humans with NAFLD. Although the methodologies and findings from the 5 human studies were heterogeneous, at least 2 of the human studies reported relationships between increased *Proteobacteria* taxa (notable for its Gram-negative, pathogenic

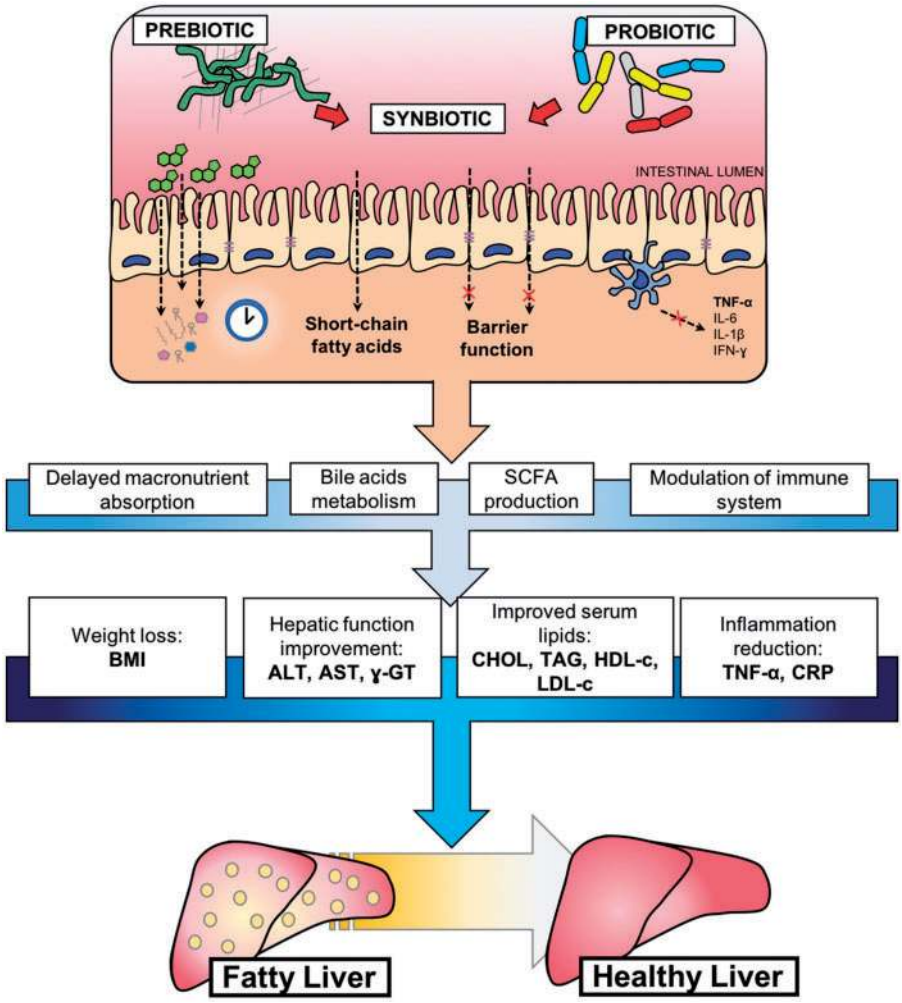

Figure 10 **Summary of the effect of prebiotic, probiotic, and synbiotic treatments that lead to nonalcoholic fatty liver disease improvement. Intestinal effects of prebiotics, probiotics, and synbiotics have been attributed to a number of mechanisms, such as delayed macronutrient absorption, bile acid interactions, bacterial fermentation byproduct absorption (short-chain fatty acid), improved barrier function to decrease toxic product filtration (trimethylamine, lipopolysaccharide, etc), and enhanced immune surveillance to reduce intestinal inflammation,.** Such effects will act in concert to promote weight loss, improve liver function, and elicit an anti-inflammatory and hypolipidemic effect. These factors will contribute, together with weight reduction, to the improvement of hepatic function and disease prognosis. *Abbreviations*: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CHOL, cholesterol; CRP, C-reactive protein; HDL-c, high-density lipoprotein cholesterol; IFN-γ, interferon γ; IL-1β, interleukin 1β; IL-6, interleukin 6; LDL-c, low-density lipoprotein cholesterol; SCFA, short-chain fatty acid; TAG, triglycerides; TNF-α, tumor necrosis factor alpha; γ-GT, gamma-glutamyl transferase.

members) and decreased butyrate-producing genera (namely *Roseburia* and *Faecalibacterium*) and NAFLD status. However, these studies did not apply microbial therapy and/or measure selected markers of hepatic function longitudinally. Future studies should strive to collect such data so that these alterations can be associated with changes in markers of liver function and inflammation, supporting or rebutting proposed mechanisms.

Unlike previous reviews and meta-analyses regarding NAFLD,[31,32] this meta-analysis addressed markers of weight loss, lipid metabolism, and inflammation for the first time. Extensive evidence exists for the impact of microbial therapies on weight loss and improved lipid profiles in the treatment of obesity.[31,75] Patients with NAFLD and type 2 diabetes show chronic hepatic fat deposition and inflammation, as well as altered hepatic export of lipoproteins, which lead to metabolic dysfunction. The mechanisms by which these therapies improve cholesterol and inflammatory statuses is likely similar, given the considerable overlap between these patient populations. In NAFLD, processes, including bile acid metabolism, alterations in cholesterol and lipid metabolism, altered barrier function and inflammation, and increased satiety signaling, have been proposed, although no direct evidence for these mechanisms currently exists in humans.[76] However, the findings that microbial therapy reduces serum CHOL and TAG lend further plausibility to these mechanisms.

Table 3 **Study quality assessment**

Criterion	Mean (SD)
1. Was the research question clearly stated?	1.80 (0.41)
2. Were the inclusion and exclusion criteria clearly stated?	1.69 (0.46)
3. Were the participants in the study representative of the pathological population?	1.56 (0.66)
4. Were the main findings of the study clearly described?	1.85 (0.36)
5. Did healthy controls' age and sex match those of the pathological group?	1.59 (0.57)
6. Was hepatic function well defined?	1.57 (0.53)
7. Was the assessment assay clearly stated and uniformly applied to all participants?	1.83 (0.38)
8. Was methodology appropriate to measure hepatic function?	1.63 (0.49)
9. Was a sample size justification via power analysis provided?	1.15 (0.88)
10. Were potential confounders properly controlled in the analysis?	1.57 (0.49)
Total score	16.24 (2.29)

Adapted from Moon et al. (2016)[34] and Littell et al. (2018).[36] Scores for each criterion range from 0 to 2, depending on whether the criterion was unmentioned or unmet (0), partially met (1), or completely met (2). The total study score ranges between 0 and 20.
Abbreviation: SD, standard deviation.

The limited amount of clinical evidence exploring prebiotic and probiotic treatments for liver disease speaks to the need to implement large cohort studies to confirm the findings of these trials with small sample sizes. Synbiotic treatments were the least represented (only 7 studies were identified), yet this combinatorial therapy may be associated with the most complex and individualized alterations in microbial and host physiology. Absence of effect in synbiotic subgroup analyses where effects were seen in probiotic and/or prebiotic analyses (eg, decreased ALT and LDL-c and increased HDL-c with prebiotics and decreased HCL-c with probiotics) may reflect an underpowered analysis or that these complex biological interactions cannot simply be considered the sum of the components (eg, prebiotic effect plus probiotic effect) but have a unique synbiotic effect. The high heterogeneity in treatment characteristics (prebiotic, probiotic, synbiotic) and their durations (0.5–7 months) among identified studies should be noted, especially in that no 2 studies implemented the same intervention. This, along with discordant baseline characteristics among studies (age, sex, location, disease severity, etc) that are known to affect intestinal microbiota, may account for the high variation in treatment responses for each outcome variable. Future studies should also strive to appropriately control for each component of their treatments (ie, isolated prebiotic or probiotic interventions to compare with combinatorial treatments). Javadi et al[54] (investigated prebiotic, probiotic, and synbiotic separately) and Ebrahimi-Mameghani et al[47] (provided vitamin E with both placebo and prebiotic interventions) are model studies in this respect. Conversely, the majority of the included studies (72.0%) used nutritional controls, mostly in the forms of 3-day dietary recalls before and after the study and/or "healthy dietary advice" being provided during the study to both treatment and control groups. This analysis accounted for heterogeneity by using random-effect models and running subgroup analyses as appropriate. Despite the high heterogeneity, a consensus was achieved regarding the effect on BMI and liver enzymes. However, unfortunately, given the limited number of studies and the heterogeneity in the study designs and the outcomes assessed, it is not possible to determine risk reduction based on dosage, duration, or type of microbial therapy from the current analyses.

Most NAFLD intervention studies use prescription drugs to improve hepatic lipid metabolism, inflammation, and fibrosis. However, such drugs are costly, have known side effects, and have not been overly successful in the general NAFLD/NASH population. Lombardi et al[77] have summarized the pharmacological efforts to treat NAFLD, and they found limited evidence for the effective treatment of NAFLD or steatohepatitis. Therefore, safer alternatives, including lifestyle and nutritional modifications (including prebiotic and probiotics), are needed to substantially improve overall liver health. Mechanisms of microbe–intestine–liver crosstalk that cannot be readily addressed by conventional therapies include increased energy harvest by the obese microbe phenotype, the capability of short-chain fatty acids reaching circulation to alter hepatic metabolism of lipids and cholesterol (as well as fatty acid oxidation by muscle and brown adipose tissue), alteration of inflammatory cytokines from adipose and intestine via microbial metabolites such as lipopolysaccharide, and in some cases probiotic- or prebiotic-induced weight loss. Furthermore, microbial therapies can be implemented at substantially less cost than traditional pharmacotherapies.

CONCLUSION

This review raises the need for accurate and effective noninvasive biomarkers of NAFLD incidence and progression. This analysis demonstrates high variability in concentration of serum hepatic enzymes, currently the

most commonly used noninvasive clinical criteria for diagnosis of NAFLD. Although other methods, such as ultrasound or biopsy, confirm liver steatosis, these methods are more invasive or prone to subjectivity and do not easily lend themselves to long-term monitoring of the disease. This study demonstrates that cholesterol subfractions improved concomitantly with hepatic enzymes, which may represent promising targets for future investigation. Ensuring accurate and complete reporting of anthropometric parameters in clinical study populations will also help identify relevant clinical markers in future meta-analyses. Altogether, these measurements may help increase the predictive power, target the mechanisms, and facilitate personalization of these interventions. Additional trials are required to evaluate the effect of treatment type on inflammatory markers, investigate the effect of combinatorial synbiotic therapeutic strategies, and elucidate the underlying mechanisms. Moreover, the independent and combinatorial use of microbial therapies and factors such as dietary pattern changes, lifestyle modifications, change in BMI, and micronutrient supplementation[43,47] (ie, vitamins E, C, etc.) that may confound but potentiate the benefits should be dissected in future investigations. Although fecal microbial transplant has been effectively implemented in the treatment of patients with intestinal conditions, including inflammatory bowel disease and especially *Clostridium difficile* infection,[78] the systemic nature of NAFLD, potential for unforeseen side effects, and complete lack of reported attempts to use this therapy in this clinical population may discourage wide implementation of this option at present. Alternative treatments of NAFLD that aim to improve intestinal microbial dysbiosis should consider the limitations of the available biomarkers for the progression of the disease, in addition to the inherent challenges of personalized microbial-based therapies.

Acknowledgments

Author contributions. B.R.L. and D.H.S. contributed equally to this work and are co–first authors. B.R.L., D.H.S., R.A., and R.S.R. conceived and designed research; B.R.L. and D.H.S. performed experiments; B.R.L. and D.H.S. analyzed data; B.R.L., D.H.S., R.A., and R.S.R. interpreted results of experiments; D.H.S. prepared figures; B.R.L. and D.H.S. drafted the manuscript; B.R.L., D.H.S., R.A., and R.S.R. edited and revised the manuscript; and B.R.L., D.H.S., R.A., and R.S.R. approved the final version of manuscript.

Funding. Salary support for R.S.R. was provided by VA-Merit Grant I01BX003271–01.

Declaration of interest. The authors have no relevant interests to declare.

Supporting Information

The following Supporting Information is available through the online version of this article at the publisher's website.

Appendix S1 **PRISMA guidelines checklist**
Table S1 **Search algorithm used in Pubmed**
Table S2 **Search algorithm used in EMBASE**
Figure S1 **Risk-of-bias table and summary**. *A*, Individual study risk of bias for selection, blinding, attrition, and reporting bias. "+" denotes low risk of bias (green), "?" denotes unclear risk of bias (yellow), and "−" indicates high risk of bias (red). *B*, Summary of risk-of-bias assessment.[37–51,53–62]

REFERENCES

1. Lomonaco R, Sunny NE, Bril F, et al. Nonalcoholic fatty liver disease: current issues and novel treatment approaches. Drugs. 2013;73:1–14.
2. Hsu CC, Ness E, Kowdley KV. Nutritional approaches to achieve weight loss in non-alcoholic fatty liver disease. Adv Nutr. 2017;8:253–265.
3. Sookoian S, Pirola CJ. Genetic predisposition in nonalcoholic fatty liver disease. Clin Mol Hepatol. 2017;23:1–12.
4. Mokhtari Z, Gibson DL, Hekmatdoost A. Nonalcoholic fatty liver disease, the gut microbiome, and diet. Adv Nutr. 2017;8:240–252.
5. Neuschwander-Tetri BA, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD single topic conference. Hepatology. 2003;37:1202–1219.
6. Obika M, Noguchi H. Diagnosis and evaluation of nonalcoholic fatty liver disease. Exp Diabetes Res. 2012;2012:145754.
7. Peng L, Wang J, Li F. Weight reduction for non-alcoholic fatty liver disease. Cochrane Database Systematic Rev 2011;6:CD003619.
8. Rahimlou M, Ahmadnia H, Hekmatdoost A. Dietary supplements and pediatric non-alcoholic fatty liver disease: present and the future. World J Hepatol. 2015;7:2597–2602.
9. Roman S, Ojeda-Granados C, Ramos-Lopez O, Panduro A. Genome-based nutrition: an intervention strategy for the prevention and treatment of obesity and nonalcoholic steatohepatitis. World J Gastroenterol. 2015;21:3449–3461.
10. Zelber-Sagi S, Godos J, Salomone F. Lifestyle changes for the treatment of nonalcoholic fatty liver disease: a review of observational studies and intervention trials. Therap Adv Gastroenterol. 2016;9:392–407.
11. Eslami L, Merat S, Malekzadeh R, et al. Statins for non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. Cochrane Database Syst Rev. 2013;12:CD008623.
12. Liu ZL, Xie LZ, Zhu J, et al. Herbal medicines for fatty liver diseases. Cochrane Database System Rev. 2013;8:CD009059.
13. Gouni-Berthold I, Papanas N, Maltezos E. The role of oral antidiabetic agents and incretin mimetics in type 2 diabetic patients with non-alcoholic fatty liver disease. Curr Pharm Des 2014;20:3705–3715.
14. Xiao J, Guo R, Fung ML, et al. Therapeutic approaches to non-alcoholic fatty liver disease: past achievements and future challenges. Hepatobiliary Pancreat Dis Int. 2013;12:125–135.
15. Mazzella N, Ricciardi LM, Mazzotti A, et al. The role of medications for the management of patients with NAFLD. Clin Liver Dis. 2014;18:73–89.
16. Rector RS, Thyfault JP, Wei Y, et al. Non-alcoholic fatty liver disease and the metabolic syndrome: an update. World J Gastroenterol 2008;14:185–192.
17. Boursier J, Diehl AM. Nonalcoholic fatty liver disease and the gut microbiome. Clin Liver Dis. 2016;20:263–275.
18. Henao-Mejia J, Elinav E, Jin C, et al. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. Nature. 2012;482:179–185.
19. Dumas ME, Barton RH, Toye A, et al. Metabolic profiling reveals a contribution of gut microbiota to fatty liver phenotype in insulin-resistant mice. Proc Natl Acad Sci U S A. 2006;103:12511–12516.
20. 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;63:764–775.

21. 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;5:8096.
22. Scorletti E, Byrne CD. Extrahepatic diseases and NAFLD: the triangular relationship between NAFLD, type 2-diabetes and dysbiosis. Dig Dis. 2016;34(suppl 1):11–18.
23. Wang B, Jiang X, Cao M, et al. Altered fecal microbiota correlates with liver biochemistry in nonobese patients with non-alcoholic fatty liver disease. Sci Rep. 2016;6:32002.
24. Franzosa EA, Morgan XC, Segata N, et al. Relating the metatranscriptome and metagenome of the human gut. Proc Natl Acad Sci U S A. 2014;111:E2329–E2338.
25. Hutkins RW, Krumbeck JA, Bindels LB, et al. Prebiotics: why definitions matter. Curr Opin Biotechnol. 2016;37:1–7.
26. Donohoe DR, Garge N, Zhang X, et al. The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. Cell Metab. 2011;13:517–526.
27. den Besten G, van Eunen K, Groen AK, et al. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. J Lipid Res. 2013;54:2325–2340.
28. Buffie CG, Pamer EG. Microbiota-mediated colonization resistance against intestinal pathogens. Nat Rev Immunol. 2013;13:790–801.
29. Rooks MG, Garrett WS. Gut microbiota, metabolites and host immunity. Nat Rev Immunol. 2016;16:341–352.
30. Younossi ZM, Reyes MJ, Mishra A, et al. Systematic review with meta-analysis: non-alcoholic steatohepatitis—a case for personalised treatment based on pathogenic targets. Aliment Pharmacol Ther. 2014;39:3–14.
31. Tarantino G, Finelli C. Systematic review on intervention with prebiotics/probiotics in patients with obesity-related nonalcoholic fatty liver disease. Future Microbiol. 2015;10:889–902.
32. Ma YY, Li L, Yu CH, et al. Effects of probiotics on nonalcoholic fatty liver disease: a meta-analysis. World J Gastroenterol. 2013;19:6911–6918.
33. Moher D, Shamseer L, Clarke M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst Rev. 2015;4:1.
34. Moon Y, Sung J, An R, et al. Gait variability in people with neurological disorders: a systematic review and meta-analysis. Hum Mov Sci. 2016;47:197–208.
35. Higgins JP, Altman DG, Gotzsche PC, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ. 2011;343:d5928.
36. Littell JH, Corcoran J, Pillai VK. Systematic Reviews and Meta-Analysis. New York, NY: Oxford University Press; 2008.
37. Akbarzadeh Z, Nourian M, Askari G, et al. The effect of psyllium on anthropometric measurements and liver enzymes in overweight or obese adults with nonalcoholic fatty liver disease (NAFLD). J Isfahan Med School. 2015;33:1771–1783.
38. Alisi A, Bedogni G, Baviera G, et al. Randomised clinical trial: the beneficial effects of VSL#3 in obese children with non-alcoholic steatohepatitis. Aliment Pharmacol Ther. 2014;39:1276–1285.
39. Aller R, De Luis DA, Izaola O, et al. Effect of a probiotic on liver aminotransferases in nonalcoholic fatty liver disease patients: a double blind randomized clinical trial. Eur Rev Med Pharmacol Sci. 2011;15:1090–1095.
40. Asemi Z, Aarabi MH, Hajijafari M, et al. Effects of synbiotic food consumption on serum minerals, liver enzymes, and blood pressure in patients with type 2 diabetes: a double-blind randomized cross-over controlled clinical trial. Int J Prev Med. 2017;8:43.
41. Asgharian A, Askari G, Esmailzade A, et al. The effect of symbiotic supplementation on liver enzymes, C-reactive protein and ultrasound findings in patients with non-alcoholic fatty liver disease: a clinical trial. Int J Prevent Med. 2016;7:59.
42. Chang HC, Huang CN, Yeh DM, et al. Oat prevents obesity and abdominal fat distribution, and improves liver function in humans. Plant Foods Hum Nutr. 2013;68:18–23.
43. Cho SH, Kim TH, Lee NH, et al. Effects of *Cassia tora* fiber supplement on serum lipids in Korean diabetic patients. J Med Food. 2005;8:311–318.
44. Daubioul CA, Horsmans Y, Lambert P, et al. Effects of oligofructose on glucose and lipid metabolism in patients with nonalcoholic steatohepatitis: results of a pilot study. Eur J Clin Nutr. 2005;59:723–726.
45. Dönmez N, Kisadere I, Balaban C, et al. Effects of traditional homemade koumiss on some hematological and biochemical characteristics in sedentary men exposed to exercise. Biotech Histochem. 2014;89:558–563.
46. Dugan CE, Aguilar D, Park YK, et al. Dairy consumption lowers systemic inflammation and liver enzymes in typically low-dairy consumers with clinical characteristics of metabolic syndrome. J Am College Nutr. 2016;35:255–261.
47. Ebrahimi-Mameghani M, Aliashrafi S, Javadzadeh Y, et al. The effect of *Chlorella vulgaris* supplementation on liver enzymes, serum glucose and lipid profile in patients with non-alcoholic fatty liver disease. Health Promot Perspect. 2014;4:107–115.
48. Ekhlasi G, Zarrati M, Agah S, et al. Effects of symbiotic and vitamin E supplementation on blood pressure, nitric oxide and inflammatory factors in non-alcoholic fatty liver disease. EXCLI J. 2017;16:278–290.
49. Eslamparast T, Poustchi H, Zamani F, et al. Synbiotic supplementation in nonalcoholic fatty liver disease: a randomized, double-blind, placebo-controlled pilot study. Am J Clin Nutr. 2014;99:535–542.
50. Famouri F, Shariat Z, Hashemipour M, et al. Effects of probiotics on nonalcoholic fatty liver disease in obese children and adolescents. J Pediatr Gastroenterol Nutr. 2017;64:413–417.
51. Farhangi MA, Javid AZ, Dehghan P. The effect of enriched chicory inulin on liver enzymes, calcium homeostasis and hematological parameters in patients with type 2 diabetes mellitus: a randomized placebo-controlled trial. Primary Care Diabetes. 2016;10:265–271.
52. Ferolla SM, Couto CA, Costa-Silva L, et al. Beneficial effect of synbiotic supplementation on hepatic steatosis and anthropometric parameters, but not on gut permeability in a population with nonalcoholic steatohepatitis. Nutrients. 2016;8:397.
53. Firouzi S, Mohd-Yusof BN, Majid HA, et al. Effect of microbial cell preparation on renal profile and liver function among type 2 diabetics: a randomized controlled trial. BMC Complement Altern Med. 2015;15:433.
54. Javadi L, Ghavami M, Khoshbaten M, et al. The effect of probiotic and/or prebiotic on liver function tests in patients with nonalcoholic fatty liver disease: a double blind randomized clinical trial. Iran Red Crescent Med J. 2017;19:e46017.
55. Malaguarnera M, Vacante M, Antic T, et al. Bifidobacterium longum with fructo-oligosaccharides in patients with non alcoholic steatohepatitis. Dig Dis Sci. 2012;57:545–553.
56. Miccheli A, Capuani G, Marini F, et al. Urinary (1)H-NMR-based metabolic profiling of children with NAFLD undergoing VSL#3 treatment. Int J Obes. 2015;39:1118–1125.
57. Mobini R, Tremaroli V, Ståhlman M, et al. Metabolic effects of *Lactobacillus reuteri* DSM 17938 in people with type 2 diabetes: a randomized controlled trial. Diabetes Obes Metab. 2017;19:579–589.
58. Nabavi S, Rafraf M, Somi MH, et al. Effects of probiotic yogurt consumption on metabolic factors in individuals with nonalcoholic fatty liver disease. J Dairy Sci. 2014;97:7386–7393.
59. Rocha R, Cotrim HP, Siqueira AC, et al. [Non alcoholic fatty liver disease: treatment with soluble fibres]. Arq Gastroenterol. 2007;44:350–352.
60. Sheu WH-H, Lee IT, Chen W, et al. Effects of xylooligosaccharides in type 2 diabetes mellitus. J Nutr Sci Vitaminol. 2008;54:396–401.
61. Vajro P, Mandato C, Licenziati MR, et al. Effects of *Lactobacillus rhamnosus* strain GG in pediatric obesity-related liver disease. J Pediatr Gastroenterol Nutr. 2011;52:740–743.
62. Wong VW, Won GL, Chim AM, et al. Treatment of nonalcoholic steatohepatitis with probiotics. A proof-of-concept study. Ann Hepatol. 2013;12:256–262.
63. Asemi Z, Esmaillzadeh A. Effect of daily consumption of probiotic yoghurt on serum levels of calcium, iron and liver enzymes in pregnant women. Int J Prevent Med. 2013;4:949–955.
64. Gratz SW, Mykkanen H, El-Nezami HS. Probiotics and gut health: a special focus on liver diseases. World J Gastroenterol. 2010;16:403–410.
65. 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. 2008;42:675–682.
66. Uesugi T, Froh M, Arteel GE, et al. Toll-like receptor 4 is involved in the mechanism of early alcohol-induced liver injury in mice. Hepatology. 2001;34:101–108.
67. Aoyama T, Paik YH, Seki E. Toll-like receptor signaling and liver fibrosis. Gastroenterol Res Practice. 2010;2010:192543.
68. Lee HY, Park JH, Seok SH, et al. Human originated bacteria, *Lactobacillus rhamnosus* PL60, produce conjugated linoleic acid and show anti-obesity effects in diet-induced obese mice. Biochim Biophys Acta. 2006;1761:736–744.
69. Ma X, Hua J, Li Z. Probiotics improve high fat diet-induced hepatic steatosis and insulin resistance by increasing hepatic NKT cells. J Hepatol. 2008;49:821–830.
70. Xu RY, Wan YP, Fang QY, et al. Supplementation with probiotics modifies gut flora and attenuates liver fat accumulation in rat nonalcoholic fatty liver disease model. J Clin Biochem Nutr. 2012;50:72–77.
71. Esposito E, Iacono A, Bianco G, et al. Probiotics reduce the inflammatory response induced by a high-fat diet in the liver of young rats. J Nutr. 2009;139:905–911.
72. Malaguarnera M, Gargante MP, Malaguarnera G, et al. Bifidobacterium combined with fructo-oligosaccharide versus lactulose in the treatment of patients with hepatic encephalopathy. Eur J Gastroenterol Hepatol. 2010;22:199–206.
73. Patel R, DuPont HL. New approaches for bacteriotherapy: prebiotics, new-generation probiotics, and synbiotics. Clin Infect Dis. 2015;60(suppl 2):S108–S121.
74. Wieland A, Frank DN, Harnke B, et al. Systematic review: microbial dysbiosis and nonalcoholic fatty liver disease. Aliment Pharmacol Ther. 2015;42:1051–1063.
75. Barengolts E. Gut microbiota, prebiotics, probiotics, and synbiotics in management of obesity and prediabetes: review of randomized controlled trials. Endocrine Practice. 2016;22:1224–1234.
76. Leung C, Rivera L, Furness JB, et al. The role of the gut microbiota in NAFLD. Nat Rev Gastroenterol Hepatol. 2016;13:412–425.
77. Lombardi R, Onali S, Thorburn D, et al. Pharmacological interventions for non-alcohol related fatty liver disease (NAFLD): an attempted network meta-analysis. Cochrane Database Syst Rev. 2017;3:CD011640.
78. Gianotti RJ, Moss AC. Fecal microbiota transplantation: from *Clostridium difficile* to inflammatory bowel disease. Gastroenterol Hepatol (N Y). 2017;13:209–213.