Effects of probiotics on nonalcoholic fatty liver disease: a systematic review and meta-analysis

Yao Tang, Juan Huang, Wen yue Zhang, Si Qin, Yi xuan Yang, Hong Ren, Qin-bing Yang and Huaidong Hu 🆔

Abstract

Background: Nonalcoholic fatty liver disease (NAFLD) has become prevalent in recent decades, especially in developed countries, and approaches for the prevention and treatment of NAFLD are not clear. The aim of this research was to analyze and summarize randomized controlled trials that investigated the effects of probiotics on NAFLD.

Methods: Seven databases (PubMed, Embase, the Web of Science, the Cochrane Library, China National Knowledge Infrastructure, Wan Fang Data, and VIP Database) were searched. Then, eligible studies were identified. Finally, proper data extraction, synthesis and analysis were performed by trained researchers.

Results: Anthropometric parameters: with use of probiotics weight was reduced by 2.31 kg, and body mass index (BMI) was reduced by 1.08 kg/m^2. Liver function: probiotic treatment reduced the alanine aminotransferase level by 7.22 U/l, the aspartate aminotransferase level by 7.22 U/l, the alkaline phosphatase level by 25.87 U/l, and the glutamyl transpeptidase level by –5.76 U/l. Lipid profiles: total cholesterol, low-density lipoprotein cholesterol, and triglycerides were significantly decreased after probiotic treatment. Their overall effects (shown as standard mean difference) were –0.73, –0.54, and –0.36, respectively. Plasma glucose: probiotics reduced the plasma glucose level by 4.45 mg/dl and the insulin level by 0.63. Cytokines: probiotic treatment decreased tumor necrosis factor alpha by 0.62 and leptin by 1.14. Degree of liver fat infiltration (DFI): the related risk of probiotics for restoring DFI was 2.47 (95% confidence interval, 1.61–3.81, $p < 0.001$).

Conclusion: Probiotic treatment or supplementation is a promising therapeutic method for NAFLD.

Keywords: NAFLD, Probiotics, Anthropometric parameters, Liver function, Lipid profiles, Plasma glucose, Cytokines, Degree of liver fat infiltration, Meta-analysis

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is a hepatic disease in which an abnormal amount of fat accumulates in hepatic cells. It occurs when lipid metabolism disorders are present. Hence, it usually occurs together with obesity, which is accompanied by dyslipidemia. However, not all obese patients develop NAFLD, and many lean patients exhibit dyslipidemia and NAFLD.[1–3] As

there is increasing prevalence of fried food in the diet and overnutrition, and as physical labor has become less frequent, the morbidity of dyslipidemia has risen in recent decades, as has that of NAFLD, especially in developed countries. Short-term (3 weeks) hypercaloric diet could induce tenfold greater relative fat accumulation in the liver (27%) than in body weight (2%) and *de novo* lipogenesis is seen to play a role in the pathogenesis of

NAFLD.[4] The current consensus is that hypercaloric/western diet is associated with onset of NAFLD.[5,6] Many NAFLD models were constituted by hypercaloric/western diet.[7–9] Gabbia and colleagues[10] found western diet changed hepatic lipid metabolism and the circulation bile acids in rats, which could represent an early marker of NAFLD development. The global prevalence of NAFLD is approximately 25.2%[11] and NAFLD causes huge social and economic burdens. It affects approximately 64 million people in the USA and 52 million in Europe and costs US$1613 per patient in the USA and 1163€ per patient in Europe. Moreover, the expected 10-year burden of NAFLD is estimated to increase tenfold in the USA and Europe.[2] In China, the prevalence of NAFLD is lower than that in developed countries, but has still reached epidemic proportions in 2013.[12] Its prevalence has continued to increase over the last several years. While hepatic cirrhosis, led by chronic viral hepatitis, accounts for the main proportion of liver transplantations, nonalcoholic steatohepatitis (NASH) is the second leading etiology of liver transplantation.[13] Given the high prevalence and very large burden of NAFLD, many studies have investigated its prevention and treatment, but the exact pathogenesis and efficient treatment of NAFLD are not fully elucidated. NAFLD is a spectrum disease with multifactorial etiology. The so-called 'two-hit' and 'multiple-hit' hypotheses both emphasize its multifactorial etiology. The 'two-hit' hypothesis was first raised by Day and colleagues[14] in 1998 and evolved over time.[15] The 'two-hit' hypothesis assumes that the 'second hit' activates inflammatory cascades and fibrogenesis in the context of obesity, insulin resistance (IR), hepatic steatosis and oxidative stress. NAFLD is advanced by chronic inflammation, caused by complex factors such as inflammatory activity, lipotoxicity, and the recruitment of Kupffer cells. Elena Buzzetti and colleagues[16] deemed NASH a 'multiple-hit' disease and determined that multiple parallel insults and processes contribute to NAFLD. The multiple-hit hypothesis can more reasonably explain the results from clinical practice, such as 'lean NAFLD'. 'Lean NAFLD' patients have different clinical characteristics (no obesity and no IR) from most NAFLD patients and are not in the setting of obesity or IR. Furthermore, Yilmaz[17] performed a review to discuss simple hepatic steatosis and NASH. He believed simple steatosis and NASH to be two independent diseases rather than two stages of NAFLD. NASH can be the initial liver lesion. However, the conclusion requires more clinical trials, animal trials, and liver histological biopsies. It will be very difficult to distinguish whether steatosis causes hepatitis or whether hepatitis causes steatosis because steatosis and hepatitis exist in the same liver, and the early profile of the patient is usually unavailable. The clinician always tries to seek a series of treatments to intervene and cure NAFLD.[18–25] Sridharan and colleagues[26] summarized the most common treatment for NAFLD and performed a meta-analysis that concluded that elafibranor, gemfibrozil, metadoxine, obeticholic acid, pentoxifylline, pioglitazone, probiotics, telmisartan, vildagliptin, and vitamin E significantly increased the response rate compared with that of standard care. Probiotics are defined as live microbial dietary supplements that benefit the host animal by improving its intestinal microbial balance. In recent years, the effect of probiotics on NAFLD has raised concern, and many clinical randomized controlled trials (RCTs) have been performed to verify the treatment effect. These trials have focused on aminotransferase, alkaline phosphatase (ALP), anthropometric indices, lipid profiles, glycemic profiles, and inflammatory factors. For NAFLD patients, the therapeutic endpoint is not only the improvement of biochemical markers but also the alleviation of fat accumulation in the liver which was not mentioned in recent research. In addition, results and conclusions have not been consistent. Most trials have concluded that probiotics and body mass index (BMI) are efficient and, at least in part, beneficial biochemical criteria. Considering the uncompleted and contradictory results from clinical RCTs, we performed this systematic review and meta-analysis to further discuss the effect of probiotics on NAFLD. We analyzed the changes in anthropometric parameters, liver function, lipid profiles, plasma glucose profiles, degree of liver fat infiltration (DFI) and cytokines to evaluate the treatment efficiency of probiotics for NAFLD. This systematic review with meta-analysis was registered on PROSPERO [www.crd.york.ac.uk, ID: CRD42019128193]. The Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) flow chart is presented in Figure 1.

Methods

This systematic review and meta-analysis was conducted according to the PRISMA guidelines[27]
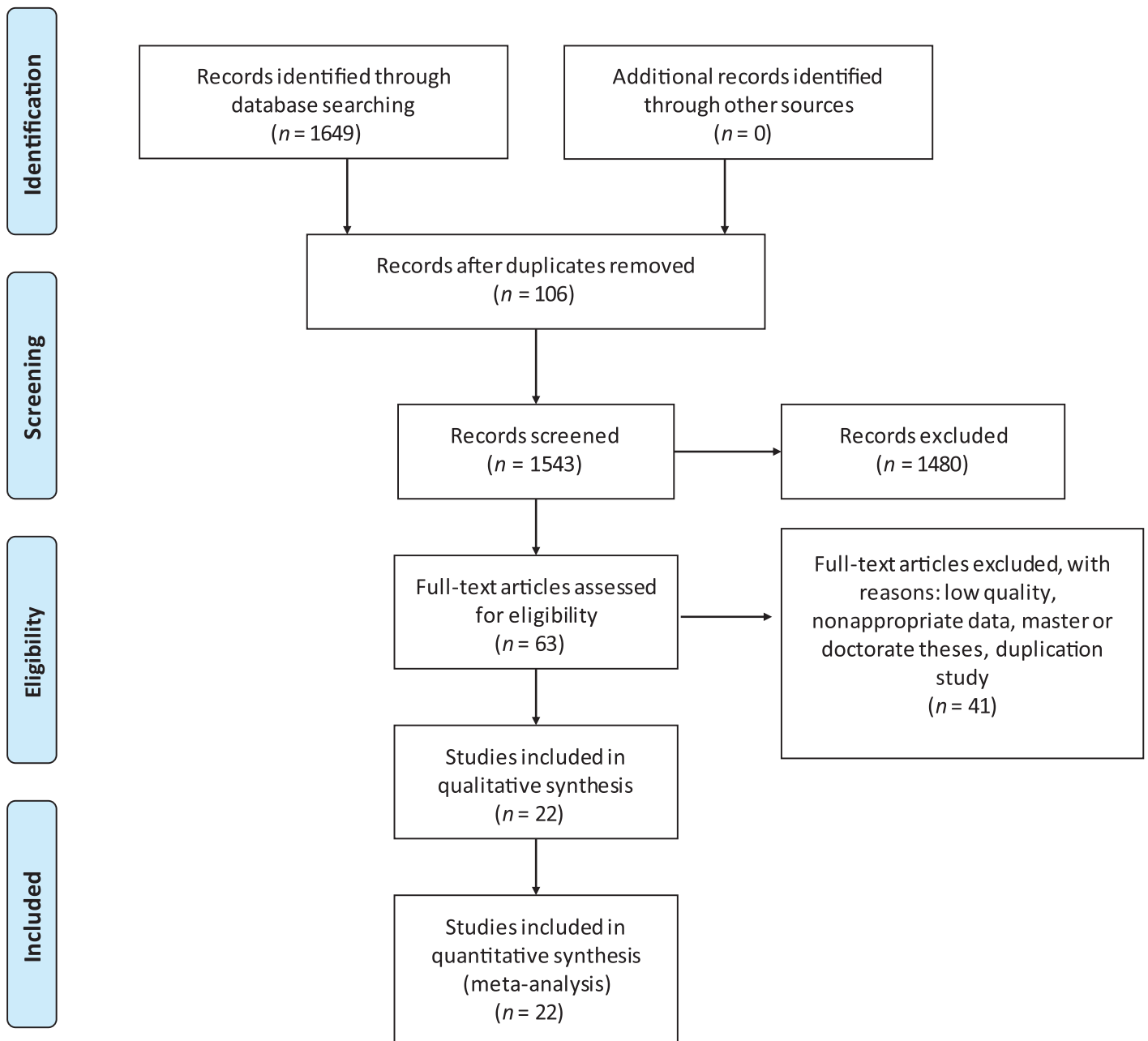
Figure 1. PRISMA flow diagram.
PRISMA, Preferred Reporting Items for Systematic Review and Meta-Analysis.

to guarantee the reliability and integrality of the data and conclusion.

Inclusion and exclusion criteria

The included clinical studies met the following criteria: the clinical trials were RCTs; the participants were NAFLD patients without a limitation in age, sex, or race; the intervention was probiotics; the treatment of the controlled group was the same as that of the intervention group except for the intervention method; the outcomes, including DFI, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transpeptidase (γ-GT), albumin (ALB), ALP, BMI, waist circumference (WC), waist-to-hip ratio (WHR), weight, fat mass (FM), total cholesterol (TC), low-density lipoprotein

cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), glucose (Glu), homeostatic model assessment of IR (HOMA-IR), insulin, C-reactive protein (CRP), leptin, adiponectin (Ad), interleukin 6 (IL-6), and tumor necrosis factor alpha (TNF-α), were associated with NAFLD; and the article was published in a peer-reviewed journal or magazine without language or date limitations. The exclusion criteria included the following: patients with alcoholic fatty liver disease; liver transplantation patients; probiotic transplant patients; conference or abstract; dissertation for a master's or doctorate degree; outcomes were presented as the mean difference or interquartile range and the original data could not be acquired; the risk of bias of all papers was assessed by the Cochrane Collaboration's tool;[28] and if more than three unclear risks or at least one high risk existed.

Database resource
The English databases searched included PubMed, Embase, the Cochrane Library, and the Web of Science; the Chinese databases searched included the China National Knowledge Infrastructure (CNKI), Wan Fang Data, and VIP Database. The full texts of the searched papers can be acquired by Chongqing Medical University Library or upon request from the authors.

Search strategy
We searched the databases with free terms, subject heading terms and key words. Probiotics were searched with 'probiotics', 'yogurt', 'yoghurt', 'lactic acid bacterium', 'lactobacillus', 'Bifidobacterium', 'enterococcus', 'streptococcus', 'saccharomyces', 'Lactococcus', 'microbiome', 'microbiota', 'synbiotics', and 'prebiotics'; fatty liver was searched with 'Liver, Fatty', 'Steatohepatitis', 'Steatohepatitides', 'Steatosis of Liver', 'Visceral Steatosis', 'Steatoses, Visceral', 'Steatosis, Visceral', 'Visceral Steatoses', 'Liver Steatosis', 'Liver Steatoses', 'Steatoses, Liver', 'Steatosis, Liver', and 'fatty liver'; and the search strategy for RCTs was guided by the Cochrane Handbook for Systematic Reviews of Interventions (2009) and published strategy studies.[29–33] All synonymous words were united with the logic operator 'OR'; probiotics, fatty liver and RCTs were united with the logic operator 'AND'. The search fields were limited to title or abstract. A reference search was not performed.

Study selection
Three researchers (Y Tang, WY Zhang, and J Huang) performed the study search and selection. All three researchers were trained before the database search to ensure that they shared the same opinions and were informed of the inclusion and exclusion criteria. WY Zhang and J Huang searched the databases and screened the studies independently. They first screened the studies by title and abstract, and the full texts of the screened studies were then scrutinized for further assessment according to the inclusion and exclusion criteria. Finally, the screened full-texts were compared by Y Tang. Divergence and discrepancies were resolved by negotiation and voting among the three researchers.

Data extraction
The extracted tables were prepared and consisted of six sublists: the liver function table included ALT, AST, ALP, ALB, and γ-GT; the anthropometric parameters table included WC, WHR, BMI, weight, and FM; the plasma Glu table included Glu, insulin, HOMA-IR, and glycated hemoglobin A1c (HbA1c); the plasma lipid table included HDL-C, LDL-C, TG, and TC; the cytokines table included leptin, adiponectin, CRP, TNF-α, and IL-6; and the DFI was used to count the number of patients whose liver fat infiltration was relieved after treatment with probiotics. Before data extraction, the meaning of every parameter was fully explained to the three researchers (Y Tang, WY Zhang, and J Huang) to maintain the same criteria in the process of data extraction. WY Zhang and J Huang extracted the data independently. The extracted data were assessed by Y Tang, and any divergence or discrepancy was discussed among the three researchers. In addition, data extraction was conducted according by each study, not by article (i.e. the data obtained from different articles on the same study were extracted only once; if the data between the two articles differed, the data from the most recent article were adopted).

Data synthesis and risk-of-bias assessment
DFI is expressed as the risk ratio, whereas the other parameters are expressed as the mean difference. I^2 statistics were used to assess the magnitude of heterogeneity, and the significance level was set as 0.1. The fixed-effects model was used only when the p value exceeded 0.1 and when the

I^2 value was less than 50%; otherwise, the random-effects model was used. The sensitivity analysis and subgroup analysis would be performed to define the source of heterogeneity. The risk of bias of each study was assessed by the same three researchers who performed data extraction with the Cochrane Collaboration's tool. The study was excluded if one high-risk bias or more than three unclear biases existed. Publication bias was assessed by Egger's test. The statistical procedures were performed by Stata/SE 15.1 (StataCorp LLC Texas, USA). Any referenced statistic adopted a two-sided test, and the significance level was set as 0.05, except for that of the heterogeneity test, which was set as 0.1.

## Results

Study selection

We searched the databases until 8 April 2019. A total of 1649 articles were retrieved, including 261 Chinese articles, 1387 English articles and 1 Persian article. The Persian article[34] was the same study performed by Nabavi and colleagues.[35,36] A total of 1585 articles were excluded after reading the title and abstract. Reasons for exclusion included duplications, reviews, animal trials, conferences and abstracts, alcoholic fatty liver patients, and simple obesity patients (non-NAFLD patients). There were 64 papers whose full texts were assessed. The 64 papers consisted of 34 Chinese papers, 28 English papers and 1 Persian paper.[34] After scrutinizing the full texts, 22 papers were ultimately included in the meta-analysis. The excluded papers included three Chinese master or doctorate theses, 29 low-quality Chinese papers that were assessed by the Cochrane Collaboration's tool in which a high-risk bias or more than three unclear risk biases existed, seven English papers that reported outcomes as the mean difference,[1,37–42] and one English paper[43] that reported outcomes as the interquartile range. Asgharian and colleagues,[44,45] Ekhlasi and colleagues,[46,47] and Javadi and colleagues[48,49] published two articles on one study. Nabavi and colleagues[34–36] published three articles on one study, and the Persian article[34] did not report any different parameters; thus, the Persian article[34] was regarded as a duplication. The studies of Javadi and colleagues[48] and Ekhlasi and colleagues[46,47] consisted of four groups. Every set of two groups was used to investigate the efficiency of probiotics independently; hence, both studies generated two groups of independent data. Finally, the meta-analysis included 22 papers from 18 studies that generated 20 groups of data. The flow chart is displayed in Figure 2.

Study characteristics and risk-of-bias assessment

Table 1[35,36,44–63] summarizes the characteristics of the included studies. All included clinical studies were designed according to the Declaration of Helsinki. They were all approved by the Ethics Committees or Institutional Review Boards they belonged to. The written informed consent was obtained from all participants which was stated in each included study. Each study is noted by the author and publication year. If multiple articles existed for one study, the study is noted by the author and each publication year (e.g. 'Nabavi_2014_15' indicates that Nabavi and colleagues published two articles, with one study each in 2014 and 2015). All the included studies were performed between 2011 and 2019 and involved a total of 1356 NAFLD patients. Two studies[52,55] investigated children (age 11.8 ± 2.2 years, M/F = 56/52, total 108 NAFLD patients), and 16 studies investigated adults (age 43.9 ± 12.4 years, M/F = 749/499, total 1248 NAFLD patients). The treatment period ranged from 4 weeks to 24 weeks, and the interquartile range of the treatment period was 12 weeks (8–16). The mean treatment period was 13.1 weeks. Multistrain probiotic intervention accounted for 77.8% (14 studies). The efficiency of single-strain probiotics was observed only in 4 studies.[56,57,62,63] All the included subjects came from three continents (Europe: 213, Asia: 1093, and South America: 50, Figure 3). All the included studies concluded that probiotics are somewhat beneficial in treating NAFLD, but different investigators had different opinions on the exact benefit of probiotic treatment. The different studies usually observed at least one beneficial parameter, but some studies reported the benefits of probiotics within, but not between, groups.

The risk-of-bias assessment is displayed in Figures 4 and 5. According to the Cochrane Collaboration's tool,[28] there were 8 studies (44.5%) in which a bias did not exist; two studies (11.1%) had three unclear biases; four studies (22.2%) had two unclear biases; and four studies (22.2%) had one
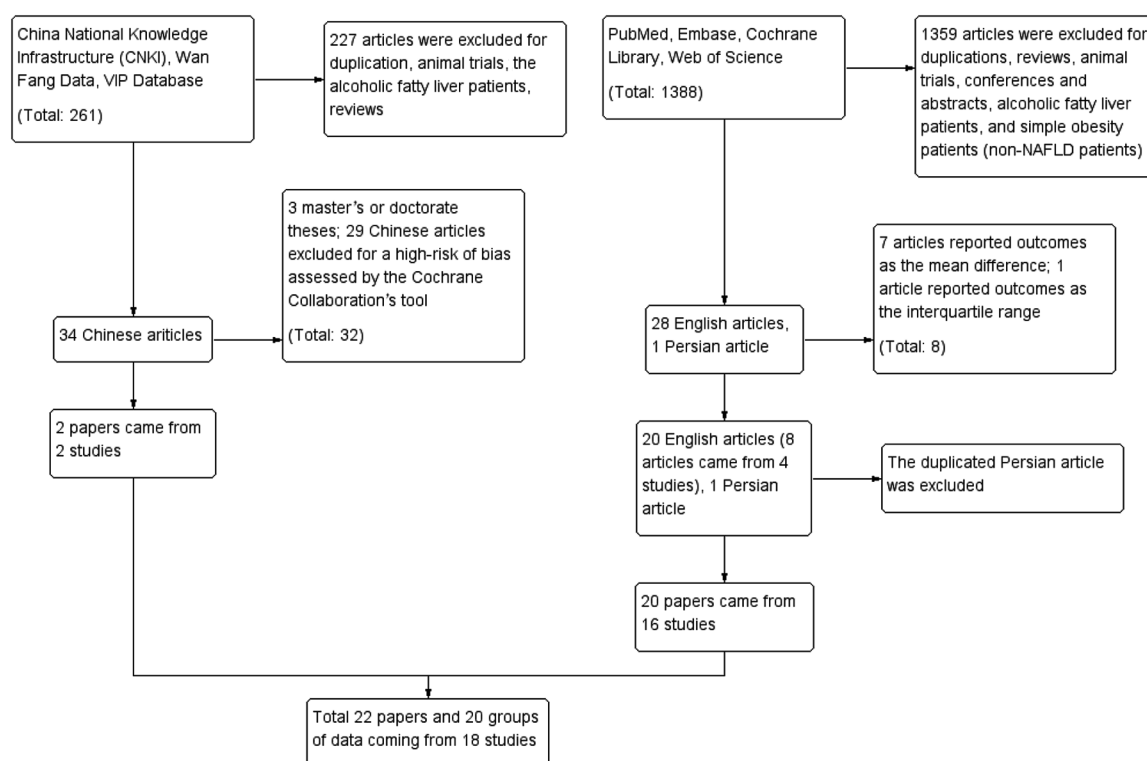
Figure 2. Study selection flow.
NAFLD, nonalcoholic fattly liver disease.

unclear bias. Nonspecific allocation concealment was the main risk of bias, followed by nonspecific random sequence generation. Nearly all studies reported lost data or patients lost to follow up when necessary.

The effect of probiotics on anthropometric parameters

The analyzed anthropometric parameters consisted of WC, WHR, weight (Figure 6), BMI (Figure 7), and FM. FM was analyzed in only two studies. Except for BMI ($I^2 = 89.7\%$, $p = 0.000$), heterogeneity was not detected between studies. BMI was summarized by a random-effects model, and WC, WHR, weight, and FM were summarized by a fixed-effects model. Probiotic treatment reduced BMI by 1.08 [95% confidence interval (CI), −2.05 to −0.11, $p = 0.030$] and weight by 2.31 kg (95% CI, −4.45 to −0.16, $p = 0.035$). There were no significant differences in WC, WHR, FM. A subgroup analysis was performed on BMI. When BMI was evaluated by continent, the heterogeneity did not decrease; when evaluated by treatment duration

time, the heterogeneity decreased only at 16 weeks ($I^2 = 36.3\%$, $p = 0.210$). A sensitivity analysis was used to find heterogeneity between studies, but the omission of any study did not seriously affect the outcomes.

The effect of probiotics on liver function

The analyzed liver function parameters included ALT, AST, ALP, ALB, and γ-GT. Probiotic treatment reduced the ALT level (Figure 8) by 7.22 U/l (95% CI, −11.58 to −2.85, $p = 0.001$), the AST level (Figure 9) by 7.22 U/l (95% CI, 12.10 to −2.35, $p = 0.004$), the ALP level (Figure 10) by 25.87 U/l (95% CI, −37.53 to −14.20, $p < 0.001$), and the γ-GT level (Figure 11) by −5.76 U/l (95% CI, −7.01 to −4.51, $p < 0.001$). It should be noted that ALP was summarized by four groups of data from two studies, and γ-GT was summarized by three groups of data from two studies. There was no significant difference in ALB ($p = 0.109$). Heterogeneity in AST ($I^2 = 88.2\%$, $p < 0.001$) and ALT ($I^2 = 95.3\%$, $p < 0.001$) levels was detected. A sensitivity analysis was performed on ALT and AST levels to determine

Table 1. Characteristics of the included studies.

Study	Trial type	Duration (weeks)	Probiotics	Country	Age (E; C)	M (F) (E; C)	Sample size E (C)	Outcomes
Sepideh *et al.*[61]	RCT-DB	8	*Lactobacillus, Bifidobacterium, Streptococcus*	Iran	42.10 ± 1.99; 47.33 ± 2.53	13 (8); 15 (6)	21 (21)	Glu, insulin, HbA1c, HOMA-IR, TNF-α, IL-6
Shavakhi *et al.*[50]	RCT-DB	24	*Lactobacillus, Bifidobacterium, Streptococcus*	Iran	41.5 ± 12.7; 38.7 ± 11.9	17 (14); 15 (17)	31 (32)	DFI, BMI, TC, TG, Glu, ALT, AST
Ahn *et al.*[51]	RCT-DB	12	*Lactobacillus, Pediococcus, Bifidobacterium*	Korea	41.7 ± 12.5; 44.7 ± 13.3	15 (15); 18 (17)	30 (35)	WHR
Alisi *et al.*[52]	RCT-DB	16	*Streptococcus, Bifidobacterium, Lactobacillus*	Italy	11 (10,12); 10 (9,12)	10 (12); 14 (8)	22 (22)	DFI, BMI, TG, ALT, HOMA-IR
Aller *et al.*[59]	RCT-DB	12	*Lactobacillus, Streptococcus*	Spain	49.4 ± 10.9; 44.3 ± 15.1	10 (4); 10 (4)	14 (14)	WHR, FM, weight, BMI, TC, LDL-C, HDL-C, TG, Glu, insulin, HOMA-IR, TNF-α, IL-6
Asgharian *et al.*[44,45]	RCT-DB	8	*Lactobacillus, Bifidobacterium, Streptococcus*	Iran	46.57 ± 10.5; 47.78 ± 10.2	7 (31); 12 (34)	38 (36)	DFI, WC, WHR, FM, weight, BMI, ALT, AST, CRP
Manzhalii *et al.*[58]	RCT-OL	12	*Lactobacillus, Bifidobacterium, Streptococcus*	Germany	44.3 ± 1.5; 43.5 ± 1.3	11 (27); 16 (21)	38 (37)	BMI, TC, TG, Glu, ALT, AST, γ-GT
Bakhshi-moghaddam *et al.*[53]	RCT-OL	24	*Streptococcus, Lactobacillus, Bifidobacterium*	Iran	39.4 ± 9.88; 41.1 ± 8.5	33 (35) *versus* 17 (17)	68 (34)	DFI
Famouri *et al.*[55]	RCT-TB	12	*Lactobacillus, Bifidobacterium*	Iran	12.7 ± 2.2; 12.6 ± 1.7	14 (18); 18 (14)	32 (32)	DFI, WC, TC, LDL-C, HDL-C, TG, ALT, AST
Ekhlasi *et al.*[46,47]	RCT-DB	8	*Streptococcus, Lactobacillus, Bifidobacterium*	Iran	44 ± 20	48 (12)	15 (15); 15 (15)	WC, weight, BMI, TC, LDL-C, HDL-C, TG, Glu, insulin, AST, ALT, ALP, HOMA-IR, TNF-α, leptin
Javadi *et al.*[48,49]	RCT-DB	12	*Bifidobacterium, Lactobacillus*	Iran	43.90 ± 9.0; 42.21 ± 9.1	17 (3) *versus* 13 (6); 14 (3) *versus* 16 (3)	20 (19); 17 (19)	DFI, WHR, weight, BMI, TC, LDL-C, HDL-C, TG, Glu, insulin, ALT, AST, ALP, γ-GT, ALB, HOMA-IR
Dengyi *et al.*[62]	RCT	12	*Bacillus*	China	45.76 ± 4.25	40 (38)	39 (39)	DFI, TC, HDL-C, LDL-C, TG

(Continued)

Table 1. (Continued)

Study	Trial type	Duration (weeks)	Probiotics	Country	Age (E; C)	M (F) (E; C)	Sample size E (C)	Outcomes
Malaguarnera et al.[57]	RCT-DB	24	*Bifidobacterium*	Italy	46.9 ± 5.4; 46.7 ± 5.7	18 (16); 15 (17)	34 (32)	BMI, TC, LDL-C, HDL-C, TG, Glu, insulin, ALT, AST, ALB, HOMA-IR, CRP, TNF-α
Nabavi et al.[35,36]	RCT-DB	8	*Bifidobacterium, Lactobacillus*	Iran	42.75 ± 8.72; 44.05 ± 8.14	19 (17); 18 (18)	36 (36)	DFI, WC, weight, BMI, TC, LDL-C, HDL-C, TG, Glu, insulin
Sayari et al.[60]	RCT	16	*Lactobacillus, Bifidobacterium, Streptococcus*	Iran	42.48 ± 11.41; 43.42 ± 11.65	45 (25); 38 (30)	70 (68)	weight, BMI, TC, LDL-C, HDL-C, TG, Glu, ALT, AST
Ferolla et al.[56]	RCT	12	*Lactobacillus*	Brazil	57.3 (25,74)	12 (38)	27 (23)	DFI, WC, weight, BMI, TC, LDL-C, HDL-C
Behrouz et al.[54]	RCT-DB	12	*Lactobacillus, Bifidobacterium*	Iran	38.46 ± 7.11; 38.43 ± 10.09	22 (8) *versus* 21 (9)	30 (30)	Weight, BMI, Glu, insulin, HOMA-IR, leptin, Ad
Wang et al.[63]	RCT	4	*Bifidobacterium, Bacillus, Enterococcus*	China	42.9 ± 5; 44.3 ± 4.7	94 (56); 34 (16)	150 (50)	DFI, TC, LDL-C, HDL-C, TG, Glu, ALT, AST, TNF-α, Ad

Ad, adinopectin; ALB, albumin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; BMI, body mass index; C, control group; CRP, C-reactive protein; DB, double-blind; DFI, degree of liver fat infiltration; E, experimental group; F, female; FM, fat mass; γ-GT, gamma glutamyl transpeptidase; Glu, glucose; HbA1c, glycated hemoglobin A1c; HDL-C, ; HOMA-IR, homeostatic model assessment of insulin resistance; IL-6, interleukin 6; LDL-C, ; M, male; OL, open label; RCT, randomized controlled trial; TB, triple blind; TC, total cholesterol; TG, triglycerides; TNF-α, tumor necrosis factor alpha; WC, waist circumference; WHR, waist-to-hip ratio.
Age is expressed as the mean ± SD or the median (interquartile range).
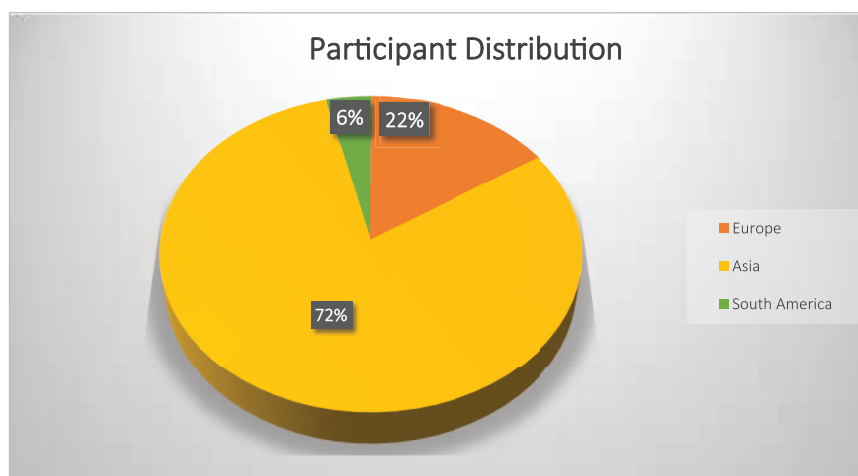

Figure 3. Participant distribution.

heterogeneity between studies. The overall effect did not change significantly after omitting any study. When ALT data were sorted by treatment duration time, heterogeneity ($I^2 = 0.0\%$, $p = 0.968$) was nearly eliminated in the 8-week group, but significant heterogeneity still existed in the other groups. When evaluated by continent, there was no heterogeneity in the Europe group ($I^2 = 0.0\%$, $p = 0.551$), but heterogeneity still existed in the Asia group ($I^2 = 79\%$, $p < 0.001$). The heterogeneity analysis of AST yielded similar results.

The effect of probiotics on lipid profiles

TC, LDL-C, HDL-C and TG were analyzed. Considering that four studies[57,58,62,63] reported results in different dimensions and with different measurement methods from other studies, we adopted the standard mean difference (SMD) to summarize the data. TC, LDL-C and TG (Figures 12–14) significantly decreased after probiotic treatment. Their overall effects (shown as the SMD) were -0.73 (-1.28 to -0.18), $p = 0.009$; -0.54 (-0.99 to -0.09), $p = 0.018$; and -0.36 (-0.63 to -0.08), $p = 0.011$, respectively. Although HDL-C showed an increasing trend (0.43, -0.03 to 0.89, $p = 0.0.69$), the statistic difference was not significant. Heterogeneity in the four parameters was detected. A sensitivity analysis was conducted to define heterogeneity between studies. There was no significant heterogeneity in TC, HDL-C, or TG. Two studies[62,63] exhibited significant heterogeneity in LDL-C compared with other studies. However, after omitting these two studies, the heterogeneity decreased only slightly from 88.5% to 78.8%, and the overall effects still exhibited significant differences. We performed three subgroup analyses according to the dimension, treatment duration, and continent. In all subgroups analyses, the heterogeneity of TC decreased only in the 8-week and 24-week treatment duration times ($I^2 = 0.0\%$, $p = 0.675$ and $I^2 = 0.0\%$, $p = 0.349$, respectively); the heterogeneity of LDL-C decreased only in the 8-week treatment duration time ($I^2 = 0.0\%$, $p = 0.487$); the heterogeneity of HDL-C decreased in the Europe group ($I^2 = 0.0\%$, $p = 0.710$) and in the 8-week treatment duration group ($I^2 = 10.5\%$, $p = 0.327$); and the heterogeneity of TG decreased in the Europe group ($I^2 = 10.2\%$, $p = 0.342$) and in the 8-, 16-, and 24-week treatment duration groups ($I^2 = 24.8\%$, $p = 0.265$; $I^2 = 53.1\%$, $p = 0.144$; and $I^2 = 0.0\%$, $p = 0.492$, respectively). According to the results of the subgroup analyses,

Figure 4. Risk-of-bias graph.

the partial heterogeneity came from race and treatment duration. However, these results could not explain all sources of heterogeneity. The dimension or measurement method did not generate heterogeneity in this meta-analysis.
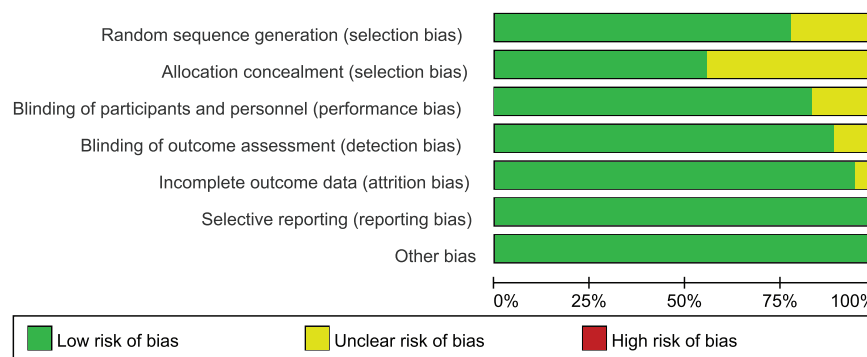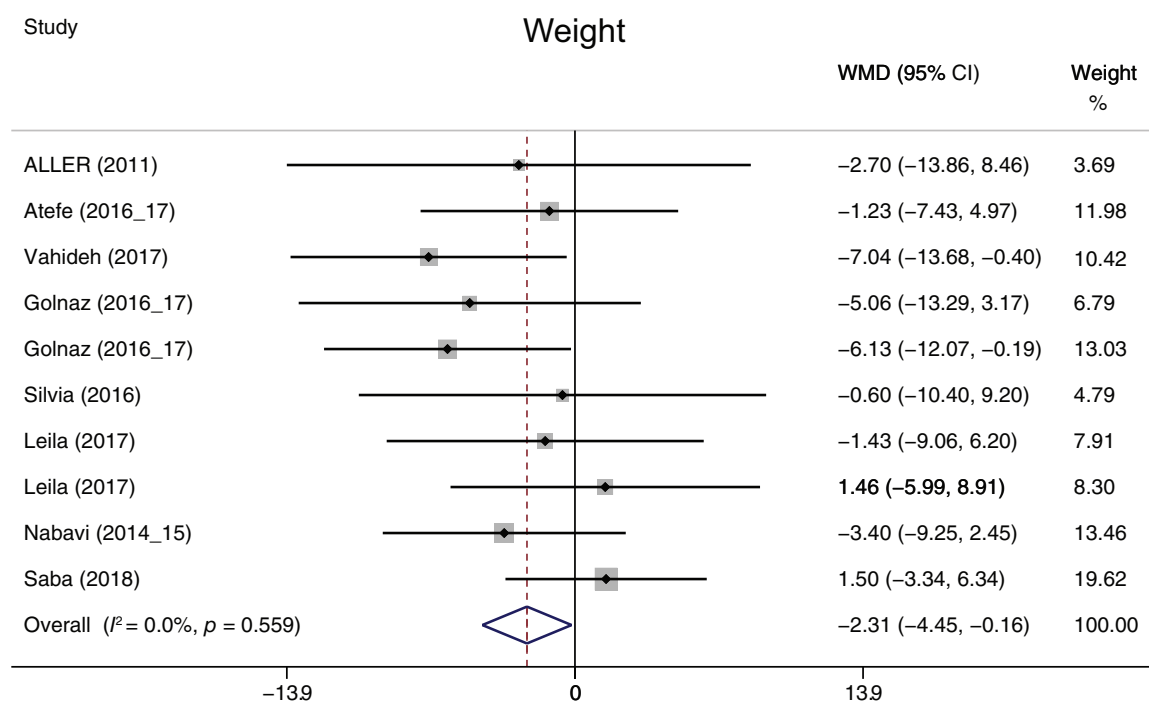
Figure 5. Risk-of-bias summary.

Figure 6. Weight.
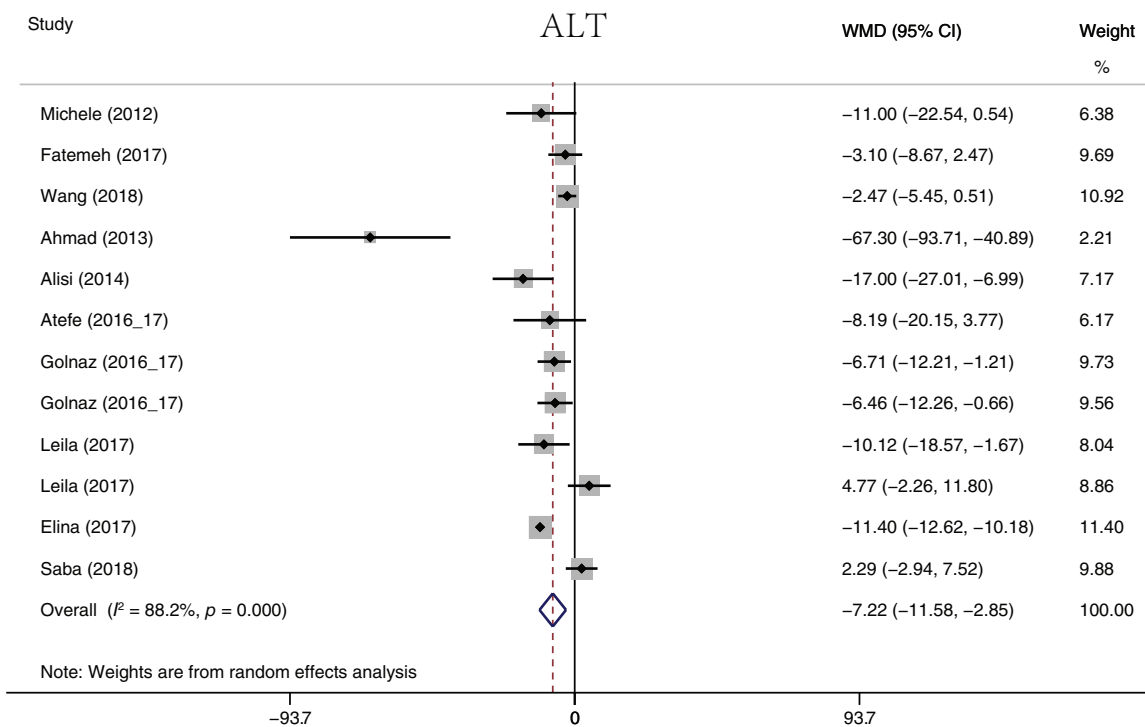WMD, weighted mean difference; CI, confidence interval.

The effects of probiotics on plasma glucose

The parameters of this section included Glu (Figure 15), insulin (Figure 16), and HOMA-IR. Given that the reported insulin and HOMA-IR values exhibit considerable variance, the SMD was used to summarize the data on insulin and HOMA-IR. Probiotics reduced the plasma glucose level by 4.45 mg/dl (95% CI, −6.67 to −2.22, $p = 0.001$) and the insulin level (−0.63, −1.16 to −0.10, $p = 0.02$). There was no significant statistic difference in HOMA-IR ($p = 0.1$). Heterogeneity in Glu, insulin and HOMA-IR was detected. The sensitivity analysis did not reveal significant variation in Glu, but noteworthy heterogeneity existed in the study by Sepideh and colleagues[61] compared with other studies. When the study was omitted, the heterogeneity (I^2 index) of insulin decreased from 84.2% to 46.6% and that of HOMA-IR decreased from 84.9% to 44.5%. Although the I^2 index significantly decreased after omitting the study, the p value in the chi-squared test did not exceed 0.1 (the predefined statistic critical value). Omission of the study did not affect the former statistic conclusion. When the subgroup analysis was performed, the heterogeneity of Glu decreased in the 12-week group
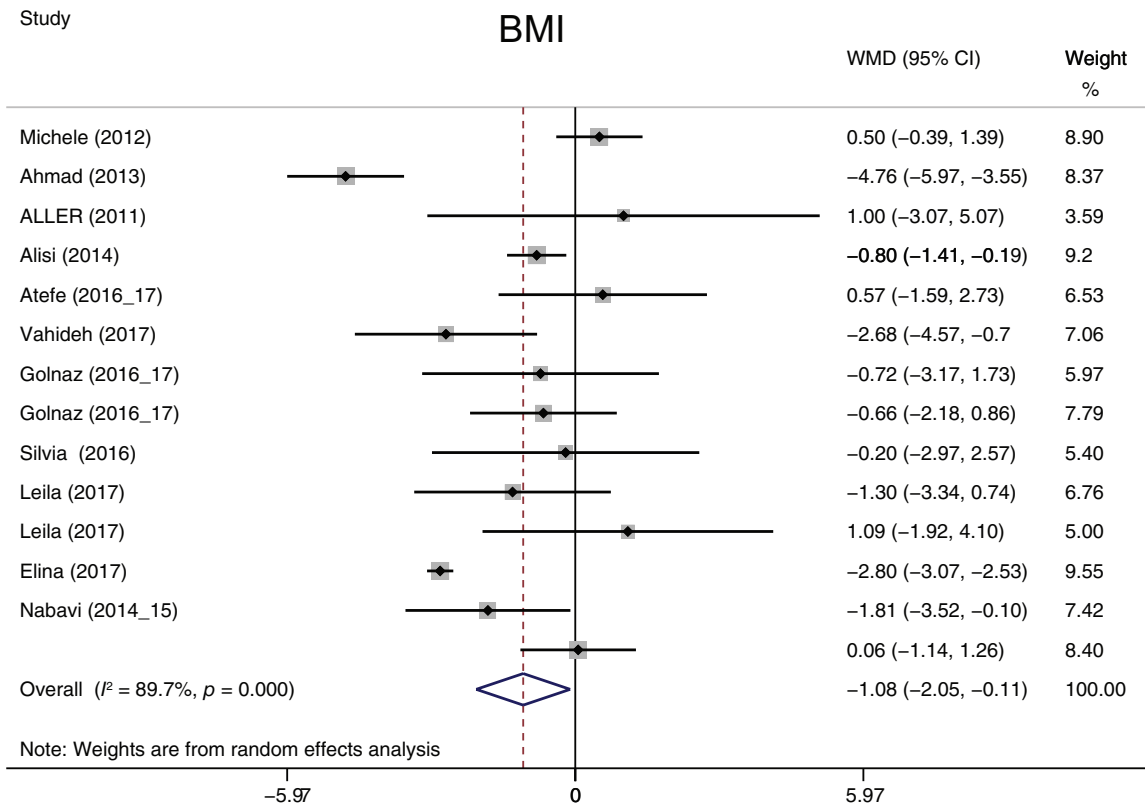
Figure 7. Body mass index.
BMI, body mass index; CI, confidence interval; WMD, weighted mean difference.
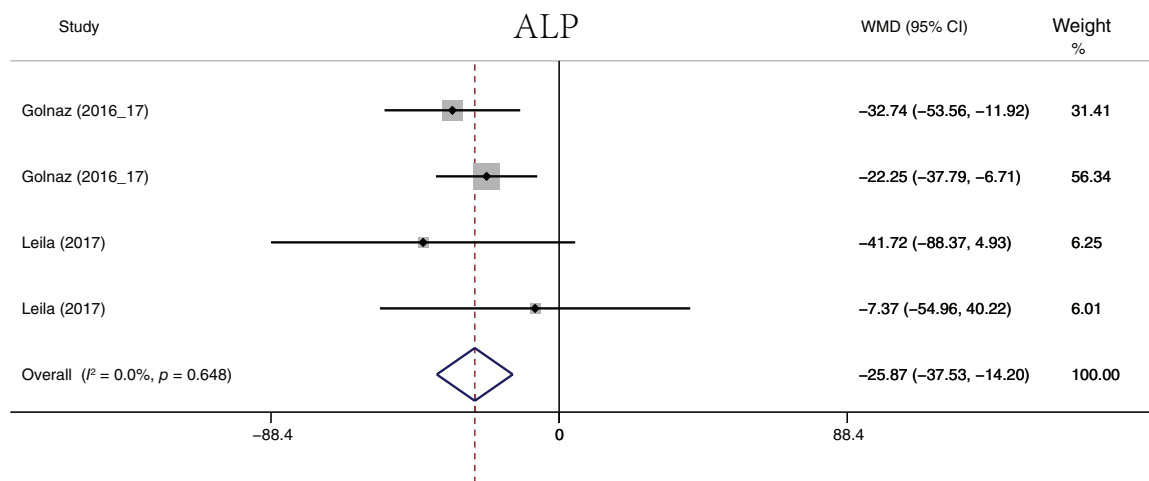

Figure 8. Alanine aminotransferase.
ALT, alanine aminotransferase; CI, confidence interval; WMD, weighted mean difference.
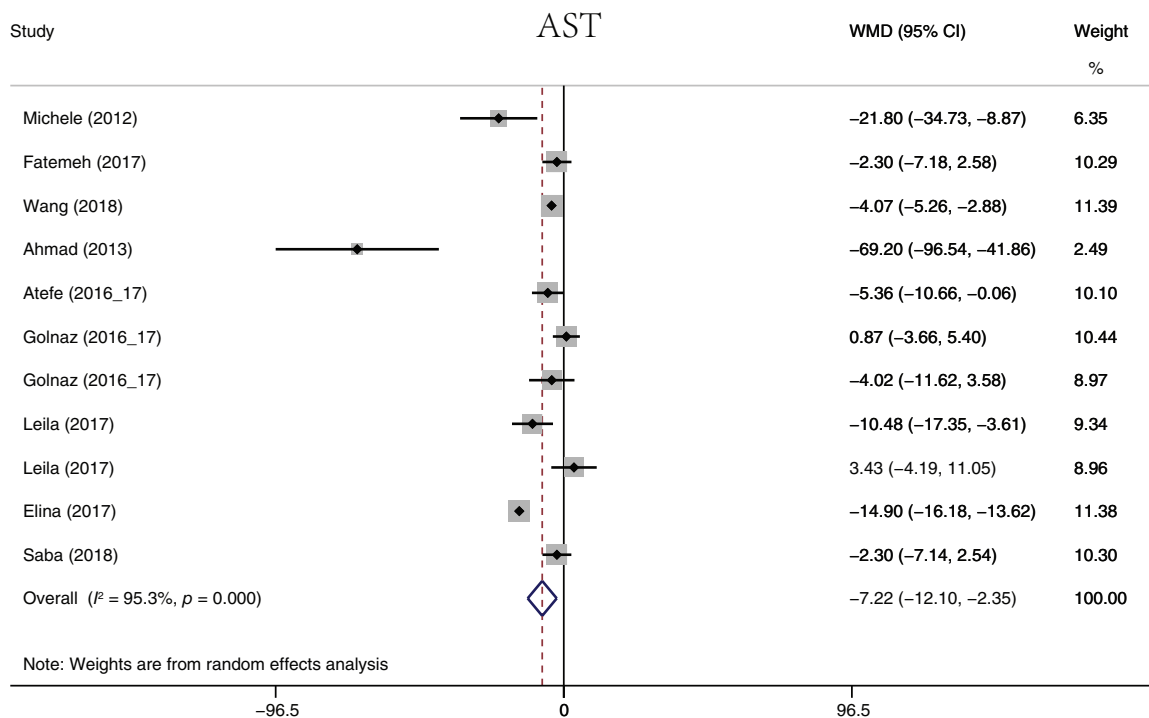
Figure 9. Aspartate aminotransferase.
AST, aspartate aminotransferase; CI, confidence interval; WMD, weighted mean difference.

Figure 10. Alkaline phosphatase.
ALP, alkaline phosphatase; CI, confidence interval; WMD, weighted mean difference.

($I^2 = 0.0\%$, $p = 0.643$), the 24-week group ($I^2 = 17.5\%$, $p = 0.271$), and the Europe group ($I^2 = 0.0\%$, $p = 0.7$); the heterogeneity of insulin decreased in the 12-week group ($I^2 = 42.8\%$, $p = 0.155$) and the Europe group ($I^2 = 0.0\%$, $p = 0.398$); and the heterogeneity of HOMA-IR decreased in the 12-week group ($I^2 = 32\%$, $p = 0.221$). In summary, the heterogeneity of Glu was derived from treatment duration and race; the heterogeneity of insulin and HOMA-IR was mainly derived from the study of Sepideh and colleagues[61] and partially derived from treatment
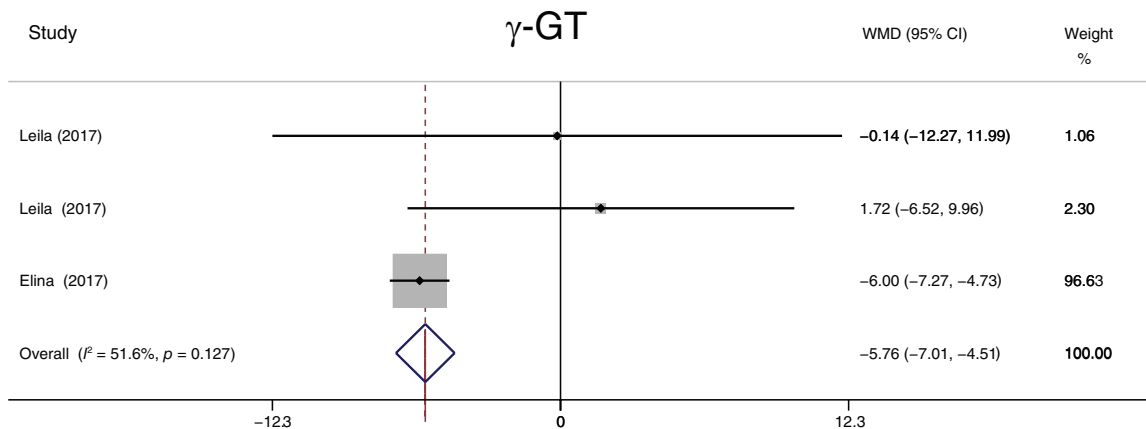
Figure 11. γ-glutamyl transpeptidase.
γ-GT, γ-glutamyl transpeptidase; CI, confidence interval; WMD, weighted mean difference.
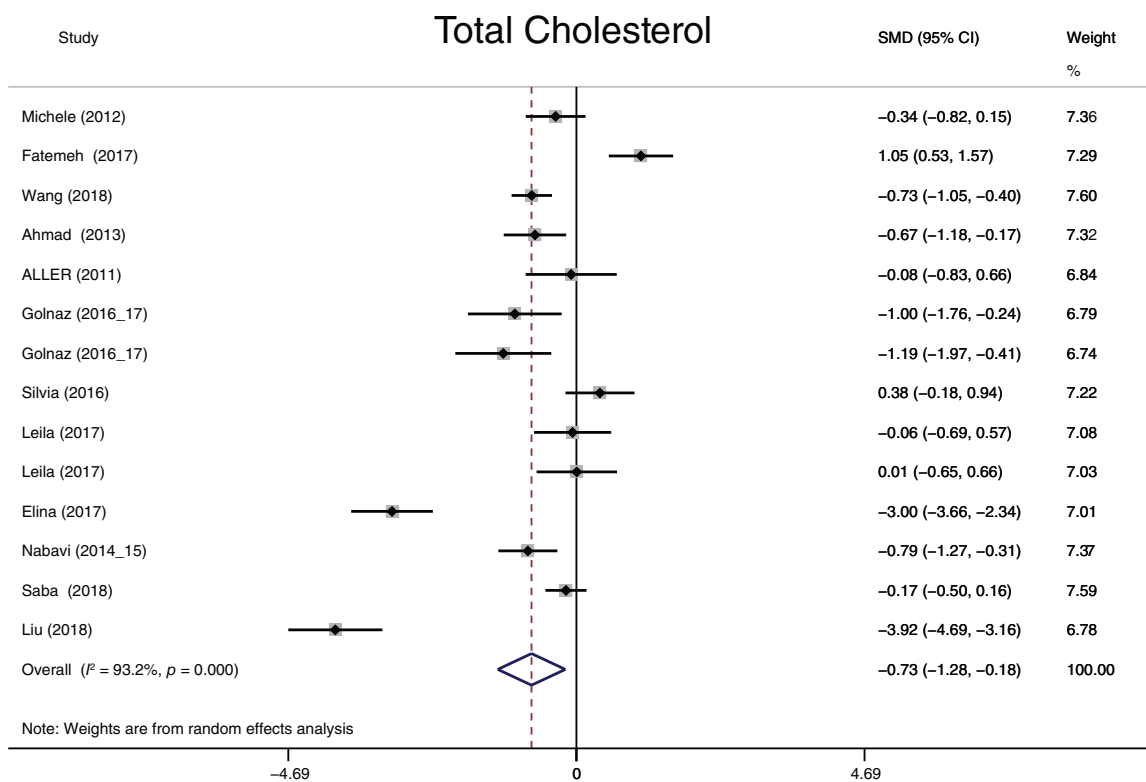
Figure 12. Total cholesterol.
CI, confidence interval; SMD, standard mean difference.

duration and race. We had intended to analyze Peptide C and HbA1c but there were not enough data to perform the summary.

The effect of probiotics on cytokines
This section describes the analysis and summary of five parameters (CRP, TNF-α, IL-6, leptin, and Ad). The data described in this section are lacking; therefore, the results of this section should be interpreted with caution. Probiotic treatment decreased TNF-α (−0.62, −0.84 to −0.40, $p = 0.026$) and leptin (−1.14, −1.54 to −0.75, $p < 0.001$) levels (Figures 17, 18). There was no statistically significant difference in CRP, IL-6, or Ad. Except for leptin, heterogeneity was

Study	LDL-C	SMD (95% CI)	Weight %
Michele (2012)		−0.93 (−1.43, −0.42)	8.61
Fatemeh (2017)		0.18 (−0.31, 0.67)	8.67
Wang (2018)		0.00 (−0.32, 0.32)	9.22
ALLER (2011)		−0.32 (−1.07, 0.42)	7.64
Golnaz (2016_17)		−0.96 (−1.72, −0.20)	7.58
Golnaz (2016_17)		−0.55 (−1.28, 0.18)	7.70
Silvia (2016)		0.70 (0.12, 1.27)	8.36
Leila (2017)		−0.55 (−1.19, 0.09)	8.08
Leila (2017)		−0.03 (−0.68, 0.63)	8.03
Nabavi (2014_15)		−0.42 (−0.88, 0.05)	8.76
Saba (2018)		−1.14 (−1.50, −0.78)	9.11
Liu (2018)		−2.53 (−3.13, −1.93)	8.25
Overall (I^2 = 88.5%, p = 0.000)		−0.54 (−0.99, −0.09)	100.00

Note: Weights are from random effects analysis

−3.13 0 3.13

Figure 13. Low-density lipoprotein.
CI, confidence interval; LDL-C, low-density lipoprotein cholesterol; SMD, standard mean. difference.
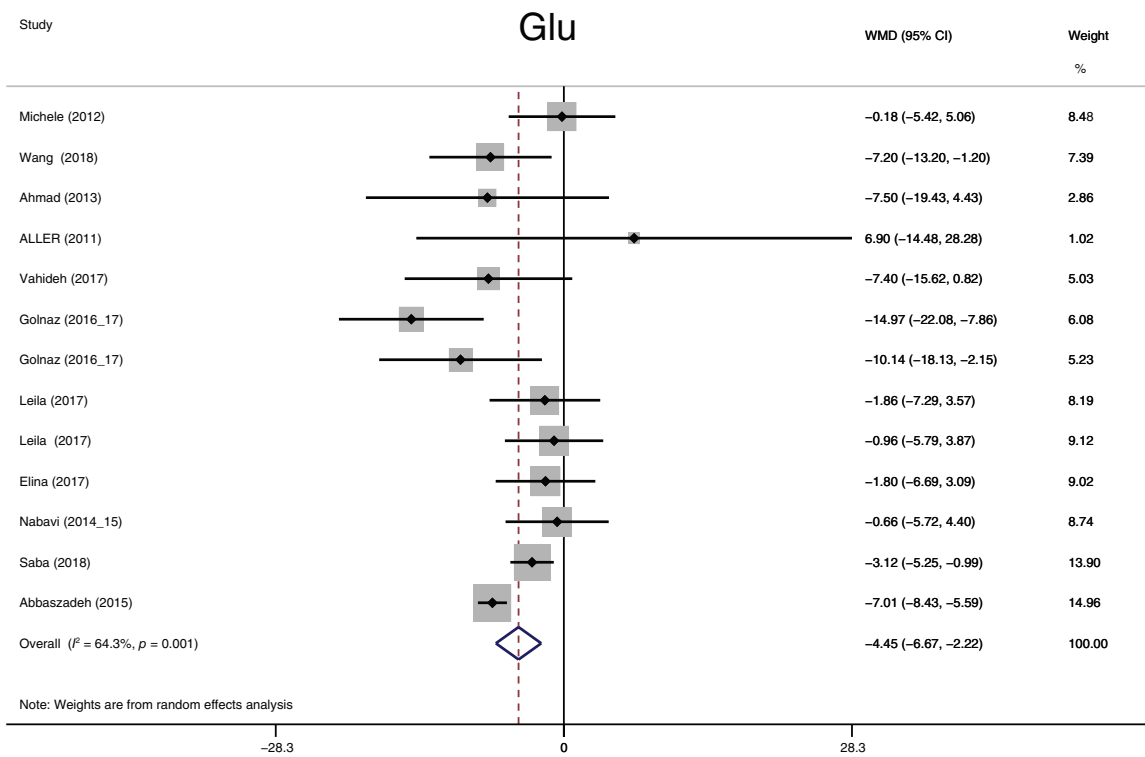
Study	TG	SMD (95% CI)	Weight %
Michele (2012)		−0.37 (−0.86, 0.12)	7.61
Fatemeh (2017)		0.25 (−0.24, 0.74)	7.57
Wang (2018)		−0.62 (−0.95, −0.30)	8.79
Ahmad (2013)		−0.62 (−1.12, −0.11)	7.47
ALLER (2011)		0.07 (−0.67, 0.81)	5.77
Alisi (2014)		0.18 (−0.41, 0.77)	6.82
Golnaz (2016_17)		−1.19 (−1.97, −0.41)	5.52
Golnaz (2016_17)		−0.79 (−1.54, −0.05)	5.75
Leila (2017)		0.05 (−0.57, 0.68)	6.56
Leila (2017)		0.15 (−0.51, 0.80)	6.36
Elina (2017)		0.20 (−0.25, 0.65)	7.86
Nabavi (2014_15)		−0.45 (−0.91, 0.02)	7.76
Saba (2018)		−0.33 (−0.66, 0.01)	8.72
Liu (2018)		−1.57 (−2.08, −1.06)	7.44
Overall (I^2 = 74.6%, p = 0.000)		−0.36 (−0.63, −0.08)	100.00

Note: Weights are from random effects analysis
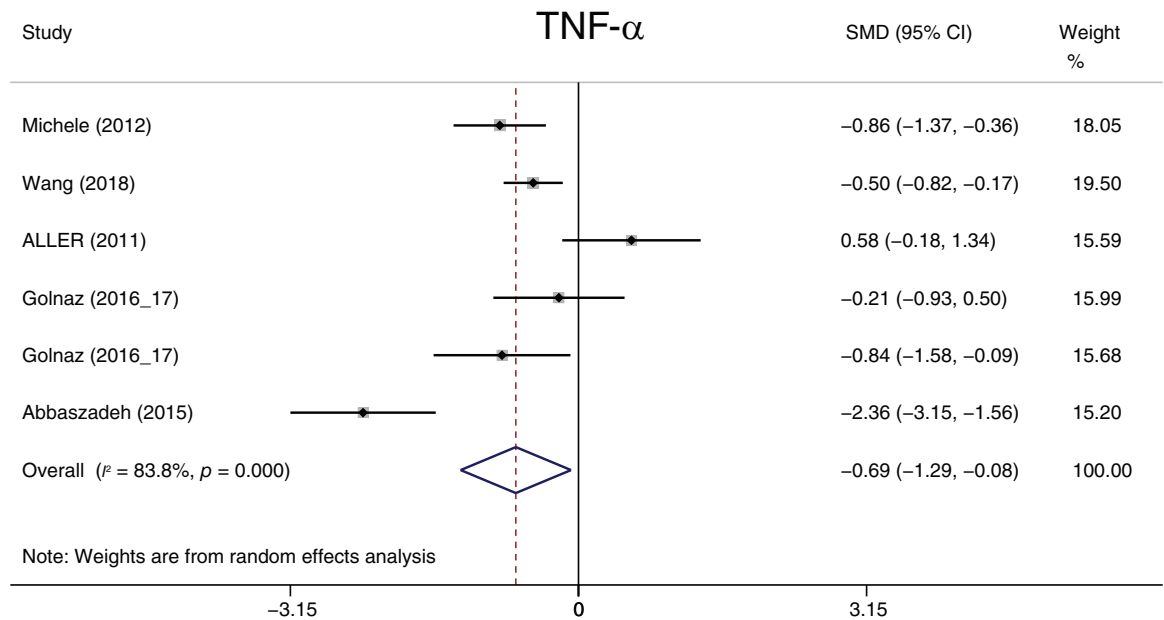
−2.08 0 2.08

Figure 14. Triglycerides.
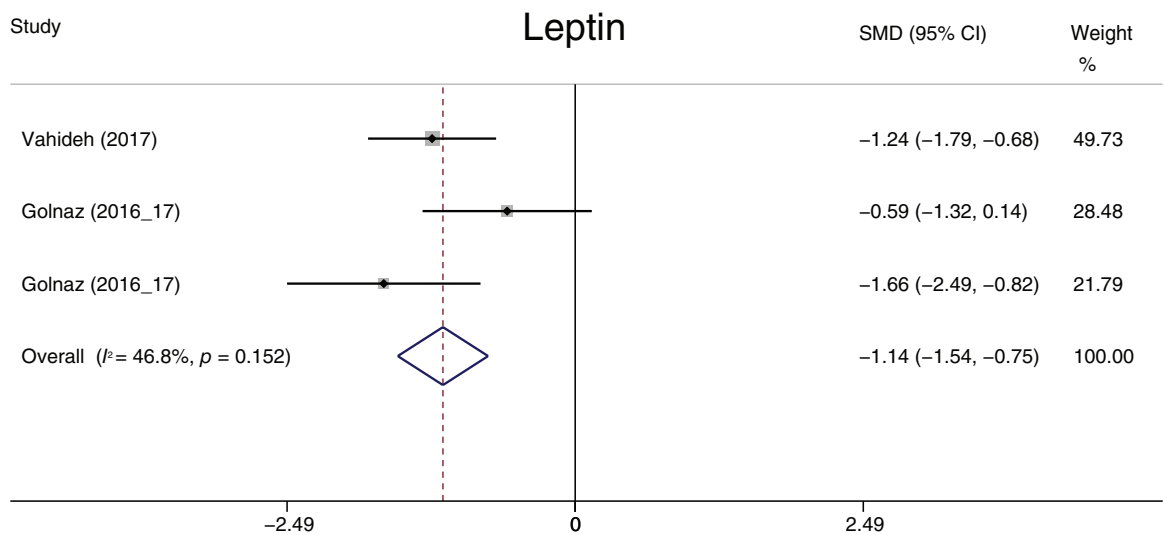CI, confidence interval; SMD, standard mean; TG, triglycerides.

Figure 15. Plasma glucose.
CI, confidence interval; Glu, glucose; WMD, weighted mean difference.
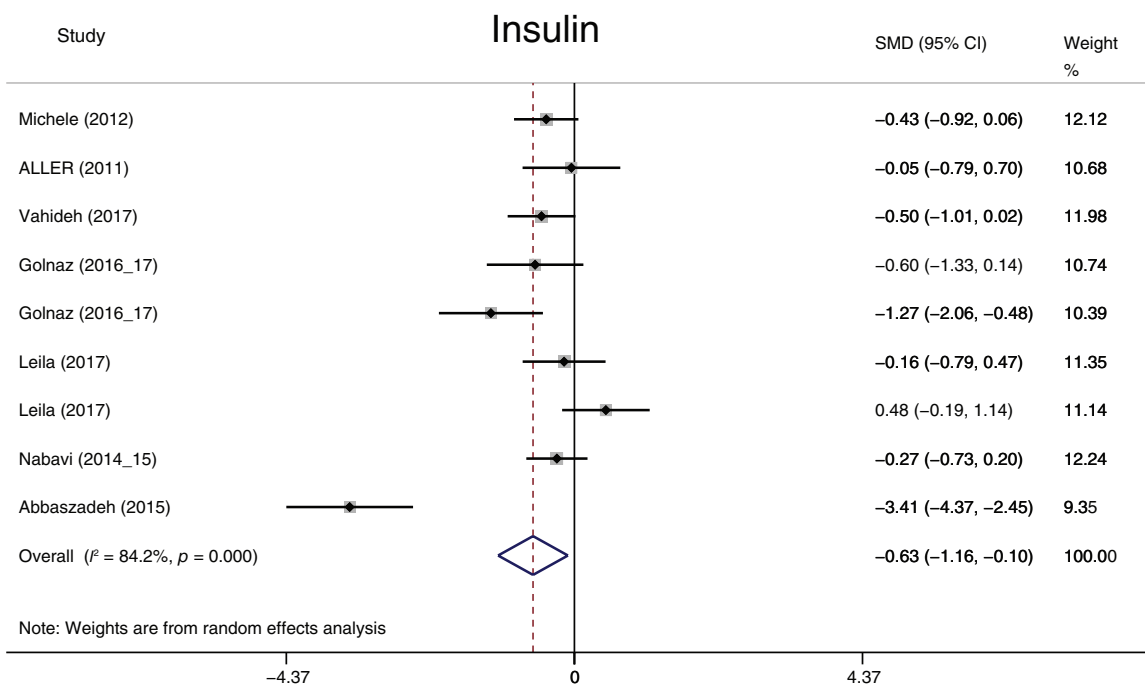
Figure 16. Insulin.
CI, confidence interval; SMD, standard mean.

Figure 17. Tumor necrosis factor alpha.
CI, confidence interval; SMD, standard mean; TNF-α, tumor necrosis factor alpha.

Figure 18. Leptin.
CI, confidence interval; SMD, standard mean.

detected in all parameters. Considering the deficient data, we performed sensitivity and subgroup analyses only on TNF-α. Aller and colleagues[59] and Sepideh and colleagues[61] were the main sources of heterogeneity according to the sensitivity analysis. After omitting these two studies, the heterogeneity decreased from 83.8% to

0.0%. The omission of the two studies did not affect the statistical conclusion.

The effect of probiotics on DFI
Finally, we collected the number of patients whose liver fat infiltration was restored at the
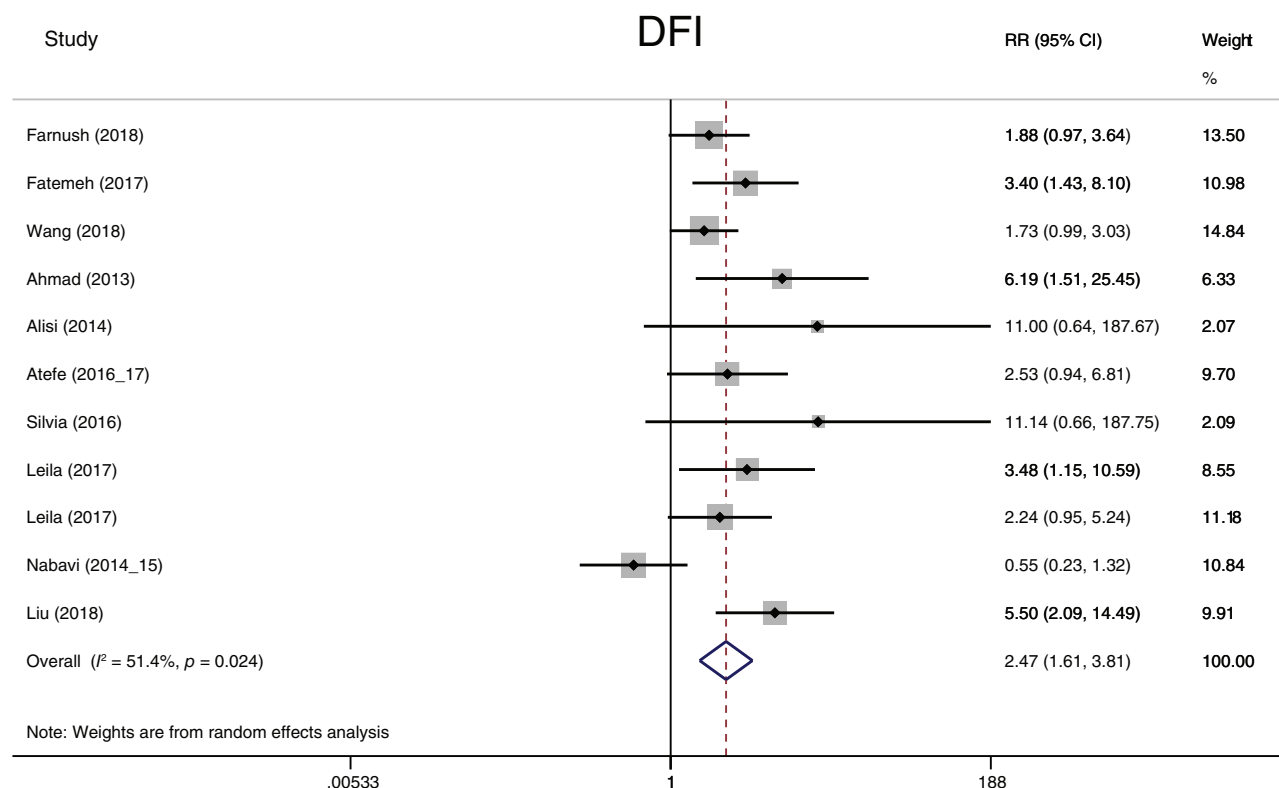
Figure 19. Degree of liver fat infiltration.
CI, confidence interval; DFI, degree of liver fat infiltration; RR, relative risk.

treatment endpoint and summarized the data by relative risk (Figure 19). According to the overall effects (2.47, 1.61–3.81, $p < 0.001$), there existed a significant association between probiotic treatment and a decrease in fat infiltration, and probiotic treatment improved liver fat infiltration. Heterogeneity ($I^2 = 51.4\%$, $p = 0.024$) was detected among the studies. No study showed significant heterogeneity with any other study according to the sensitivity analysis. A subgroup analysis was performed according to race and treatment duration. When evaluated by continent, the heterogeneity of the Europe group decreased ($I^2 = 0.0\%$, $p = 0.995$) and that of the Asia group ($I^2 = 55.2\%$, $p = 0.022$) did not change. When evaluted by treatment duration, heterogeneity decreased in the 12-week group ($I^2 = 0.0\%$, $p = 0.606$), and the p value of the chi-squared test increased in the 24-week group ($I^2 = 57.3\%$, $p = 0.126$).

Publication bias assessment
For each parameter, only when the included groups exceeded 10 was the publication bias assessed by Egger's test. The publication bias of weight ($p = 0.670$), BMI ($p = 0.050$), WC ($p = 0.077$), ALT ($p = 0.415$), AST ($p = 0.761$), Glu ($p = 0.445$), TC ($p = 0.288$), LDL-C ($p = 0.732$), HDL-C ($p = 0.1916$), TG ($p = 0.801$), and DFI ($p = 0.083$) was assessed. No significant publication bias was detected.

Discussion
This systematic review with meta-analysis focused on nearly all known related data sorted by six aspects: anthropometric parameters, liver function, lipid profiles, glucose profiles, cytokines, and DFI. In regard to the anthropometric parameters, probiotics decreased weight ($p = 0.035$) and BMI ($p = 0.030$). Being overweight and having a high BMI are associated with NAFLD;[64–67] however, the details on this association are unclear. It is difficult to determine whether NAFLD is only accompanied by overweight or whether being overweight plays a role in NAFLD pathology. Sarr and colleagues[8] reported that a low birth weight (LBW) is independently associated with hepatic steatosis in guinea pigs by affecting gene

expression and the metabolomic profile in early adulthood. Bugianesi and colleagues[68] reported that an LBW increased the risk of hepatic steatosis in childhood. Moreover, being overweight in childhood will increase the risk of NAFLD in adulthood.[69] Weight seems to play different roles in newborns, fetuses, and children or adults, but the mechanism requires further research. With regards liver function, probiotics improved ALT, AST, ALP, and γ-GT. AST, ALT and γ-GT are signs of hepatic cell injury; elevated γ-GT and ALP levels are related to biliary obstruction (e.g. hepatic cirrhosis induced by NAFLD). ALT indicates the worst metabolic profile and persistent NAFLD according to recent research.[70] Elevated liver enzymes (ALT, AST, and γ-GT) could also be related to liver disease mortality.[71] Therefore, probiotics are beneficial for hepatic cell protection. In regard to the lipid profile, probiotics deceased TC, LDL-C, and TG, all of which are the main risk factors of NAFLD. Lipids were detected in all liver tissues of NAFLD patients. Cholesterol and TG can accumulate in hepatic cells and liver tissue and induce deleterious hypoxic and nitric oxide signal-transduction pathways.[72,73] LDL-C metabolism is associated with steatotic liver tissue, and lipid heterogeneity exists between steatotic and nonsteatotic liver tissue (i.e. the lipid composition or metabolism may be different) which may help us understand the mechanisms of lipid accumulation in NAFLD.[74] Considering the association of hyperlipidemia with NAFLD, statin treatment targeting dyslipidemia seems promising,[75] but its safety is debatable.[76,77] Probiotic treatment could be a promising supplemental therapeutic method targeting dyslipidemia. In regard to plasma glucose, probiotics decreased Glu and insulin levels. Diabetes and NAFLD are like 'twins'. NAFLD patients are usually insulin resistant, similar to diabetic patients, and diabetic patients usually have fatty liver.[78,79] In a high-fat-diet mouse model of obesity, diabetes was shown to worsen NAFLD.[80] The inflammation associated with diabetes also accelerates NASH. Improving IR and hyperglycemia will be beneficial for NAFLD patients. In regard to serum inflammation, although the summarized data indicate that probiotics decreased TNF-α and leptin levels, the results and conclusion of this section must be reviewed carefully, given the deficient data and considerable heterogeneity. TNF-α is an inflammatory factor activated by nuclear factor-kappa B, promotes NASH, and accelerates IR.[81,82] Leptin is a debatable factor in NAFLD and obese patients, and can regulate energy homeostasis.[83,84] Animal trials, human trials, and reviews seem to support the application of leptin or its efficacy in obesity or NAFLD considering its beneficial effect on IR and liver fat accumulation.[85–90] However, Imajo and colleagues[91] reported that obesity-induced leptin plays a crucial role in NASH progression *via* enhanced responsivity to endotoxin, and Polyzos and colleagues[92] concluded that a high circulating leptin level was associated with severity of NAFLD. Above all, the effect of leptin on NAFLD still needs further investigation. Finally, we found that NAFLD patients receiving probiotic treatment were more likely to exhibit reduced DFI. Although liver fat accumulation does not cause clinical symptoms in most instances, liver fat is reported to disrupt glycerol metabolism[93] and is associated with impaired cardiac and autonomic function, inflammation and oxidative stress.[94,95] We believe that DFI should also be a therapeutic goal.

Although probiotics seem to improve NAFLD in many aspects, the specific mechanism is unclear. Microbial products (e.g. the short-chain fatty acid acetate, butyrate and propionate), microbial enzymes (e.g. bile salt hydrolase) and the dysbiosis-induced dysregulation of gut endothelial barrier function seem promising in explaining the mechanism involved.[96–98] Considering the complex and multiple effects of probiotics on NAFLD, we believe it is unlikely that any single bacteria can generate sufficient treatment efficiency. However, we need more animal or human trials to investigate the effect of a single bacteria on NAFLD to understand the effect of every bacterium and then pack the most efficient probiotics for clinical application. Regretfully, most studies have investigated only the effect of multistrain probiotics because this kind of study is more likely to achieve positive results. In addition, the bacteria investigated differed among the studies. Although our study indicates that probiotics are a promising treatment for NAFLD, the subgroup analysis did not explain all sources of heterogeneity. Moreover, the degree of improvement in each parameter was moderate, and the included subjects were mainly from Asia. The conclusion of the current meta-analysis should be cautiously reviewed when considering race.

Conclusion

Probiotics can reduce weight and BMI, improve liver function, decrease plasma lipid and glucose levels, alleviate inflammation and restore liver fat infiltration. Probiotic treatment or supplementation is a promising therapeutic method for NAFLD.

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