Gut microbiome–targeted therapies in nonalcoholic fatty liver disease: a systematic review, meta-analysis, and meta-regression

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ABSTRACT

Background: Preclinical evidence suggests that modulation of the gut microbiome could represent a new therapeutic target in nonalcoholic fatty liver disease (NAFLD).

Objectives: The aim of this study was to evaluate the most current evidence for liver-specific and metabolic effects of microbiome-targeted therapies (MTTs) in persons with NAFLD.

Methods: We searched multiple electronic databases for randomized controlled trials (RCTs) published from January 1, 2005 to December 1, 2018 that enrolled persons with NAFLD who received MTT rather than placebo or usual care. MTT was defined as antibiotics, probiotics, synbiotics, or fecal microbiota transplantation (FMT). Clinical outcomes were pooled with the use of random-effects models and heterogeneity was assessed with the I^2 statistic. A random-effects meta-regression was performed to determine sources of heterogeneity in prevalence estimates between studies.

Results: Twenty-one RCTs (1252 participants) were included; 9 evaluated probiotics and 12 evaluated synbiotics, with treatment duration ranging from 8 to 28 wk. No RCTs examined the efficacy of antibiotics or FMT. Probiotics/synbiotics were associated with a significant reduction in alanine aminotransferase activity [ALT, weighted mean difference (WMD): −11.23 IU/L; 95% CI: −15.02, −7.44 IU/L] and liver stiffness measurement (LSM) by elastography (reflecting inflammation and fibrosis) (WMD: −0.70 kPa; 95% CI: −1.00, −0.40 kPa), although analyses showed heterogeneity ($I^2 = 90.6\%$ and $I^2 = 93.4\%$, respectively). Probiotics/synbiotics were also associated with increased odds of improvement in hepatic steatosis, as graded by ultrasound (OR: 2.40; 95% CI: 1.50, 3.84; $I^2 = 22.4\%$). No RCTs examined sequential liver biopsy findings. Probiotics (WMD: −1.84; 95% CI: −3.30, −0.38; $I^2 = 23.6\%$), but not synbiotics (WMD: −0.85; 95% CI: −2.17, 0.47; $I^2 = 96.6\%$), were associated with a significant reduction in body mass index.

Conclusions: The use of probiotics/synbiotics was associated with improvement in liver-specific markers of hepatic inflammation, LSM, and steatosis in persons with NAFLD. Although promising, given the heterogeneity in pooled analyses, additional well-designed RCTs are needed to define the efficacy of probiotics/synbiotics for treatment of NAFLD. This study was registered with PROSPERO as CRD42018091455. *Am J Clin Nutr* 2019;110:139–149.

Introduction

As a result of the obesity pandemic, nonalcoholic fatty liver disease (NAFLD) has emerged as the leading cause of chronic liver disease in the developed world, and its prevalence is rapidly increasing (1). NAFLD, which affects both children and adults, encompasses a spectrum of histopathologic abnormalities ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), a more severe phenotype of disease. NASH is associated with cirrhosis, hepatocellular carcinoma, and liver-related mortality, highlighting the serious consequences of overnutrition and the metabolic syndrome on liver health (2). Despite the overwhelming burden of NAFLD, there are no approved pharmacologic therapies for NAFLD in the United States (3). Lifestyle modification, involving exercise and a change in diet, remains the mainstay of therapy, but achievement of sustained weight loss is difficult for most patients (4).

An ever-increasing body of literature suggests that perturbation to the gut microbiome, a complex and diverse community of microbes residing in the human intestine, is implicated in

the pathogenesis of NAFLD (5). Numerous clinical studies have demonstrated that NAFLD is associated with a decrease in overall bacterial diversity and enrichment in certain Gram-negative bacterial taxa of the gut microbiome (6). Preclinical studies have demonstrated that therapies targeting the gut microbiome, including probiotics and antibiotics, inhibit the development of diet-induced obesity and hepatic steatosis and improve insulin resistance in murine models (7–11). As such, there is great interest in the potential for the human gut microbiome to serve as a target for therapeutic intervention in NAFLD.

Microbiome-targeted therapies (MTTs) have been proposed as a way to manipulate the gut microbiome and can be considered in several categories, namely probiotics, synbiotics, antibiotics, and fecal microbiota transplantation (FMT). Probiotics are defined as a culture of living microorganisms which could have health benefits for the human host if consumed in adequate amounts (12). Synbiotics are a combination of probiotics and prebiotics, in which prebiotics are composed of fermentable dietary fibers (e.g., inulin, fructo-oligosaccharides) that stimulate the growth and survival of probiotics (12). FMT is a procedure in which stool is collected from a healthy donor and transferred to a patient, via a range of delivery routes including colonoscopy, nasogastric tube, and enema (13).

In 2007, a Cochrane Review did not identify any randomized clinical trials with probiotics in NAFLD and determined that there was no evidence to support or refute the use of probiotics in the NAFLD patient population (14). Over the past decade, there has been an increase in the number of clinical trials examining the role of probiotics in NAFLD. A few recent meta-analyses have examined the role of probiotics in NAFLD, albeit with conflicting results and limitations. These limitations include limiting analysis to a single type of MTT (e.g., only probiotics), focusing on outcomes of insulin resistance and lipid profiles (and not liver-specific outcomes), and inclusion of studies that enrolled obese adults with metabolic risk factors but without definite NAFLD (15–18). Given the limitations in the current literature, we aimed to conduct a systematic review, meta-analysis, and meta-regression in order to evaluate the most current evidence for both liver-specific effects and metabolic effects of all MTTs in adults and children with NAFLD.

Methods

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses Statement was used to guide the conduct and reporting of the study (**Supplemental Methods**). We established a protocol for the review, which was registered with PROSPERO prior to commencing the study (https://www.crd.york.ac.uk/prospero/, CRD42018091455, and Supplemental Methods).

Study selection

Randomized controlled trials (RCTs) that compared MTT with placebo, usual care, or no intervention in patients with NAFLD were eligible for inclusion. MTT was defined as interventions in any of the following 4 categories: antibiotics, probiotics, synbiotics, or FMT. Prebiotics were not included as MTT for

the purpose of this review. To be eligible, RCTs needed to meet the following criteria: *1*) NAFLD was defined by either liver histology or noninvasive imaging modality (MRI, ultrasound, or elastography); *2*) duration of therapy was ≥4 wk (excluding FMT trials), and *3*) one of the following outcomes was assessed: serum alanine aminotransferase (ALT) concentration, BMI, insulin resistance (HOMA-IR or glycated hemoglobin), triglycerides (TGs), or assessment of change in liver disease severity (steatosis or fibrosis, or a combination of the two) by any of the following modalities: MRI, elastography, or liver biopsy. Specifically, MRI outcomes could include estimated fibrosis stage and MRI-determined proton density fat fraction. Elastography outcomes could include liver stiffness measurement (LSM), reflecting the combined effects of hepatic inflammation and fibrosis, or the controlled attenuation parameter, which estimates hepatic steatosis (19). Liver biopsy outcomes could include scoring of fibrosis stage, ballooning degeneration, steatosis, or lobular inflammation. No restrictions were placed on the setting or context of the included studies. Multiarm trials were eligible for inclusion if there were MTT and placebo groups. Only studies performed in human subjects and published in English were considered. Unpublished studies and studies published only in abstract format were excluded.

Identification and selection of studies

The electronic databases PubMed/MEDLINE, EMBASE, and the Cochrane Library were searched from January 1, 2005 to December 1, 2018. A secondary search was performed where reference lists of all relevant reviews and trials were screened for additional studies. The search strategy was peer-reviewed by an information specialist (Supplemental Methods). During the first screening, 2 reviewers (SRS and BM) evaluated the titles and abstracts of each citation and excluded clearly irrelevant studies. For each potentially eligible study, 2 reviewers (SRS and BM) independently examined the full-text article and assessed whether the study fulfilled the inclusion criteria. Disagreements were resolved through consensus or referral to a third reviewer (NAT). The number of studies identified by the search and excluded at various stages was recorded in a Preferred Reporting Items for Systematic Reviews and Meta-Analyses study flow diagram.

Data extraction and study appraisal

Two independent reviewers (SRS and EH-T) extracted data from each trial with the use of a predesigned, standardized data-extraction form. The data sought included: *1*) study characteristics (year of publication, dates of enrollment, inclusion and exclusion criteria, total number of patients enrolled, country where study performed); *2*) baseline patient characteristics (age, liver disease, obesity); *3*) intervention characteristics (type of MTT, duration of therapy); and *4*) clinical outcomes, as previously defined.

All studies were assessed for methodologic quality with the use of the Cochrane Risk of Bias tool (20). If ≥1 of the domains was rated as high, the trial was considered at high risk of bias. If all domains were judged as low, the trial was considered at low risk of bias. Otherwise, the trial was considered as having an unclear risk of bias.
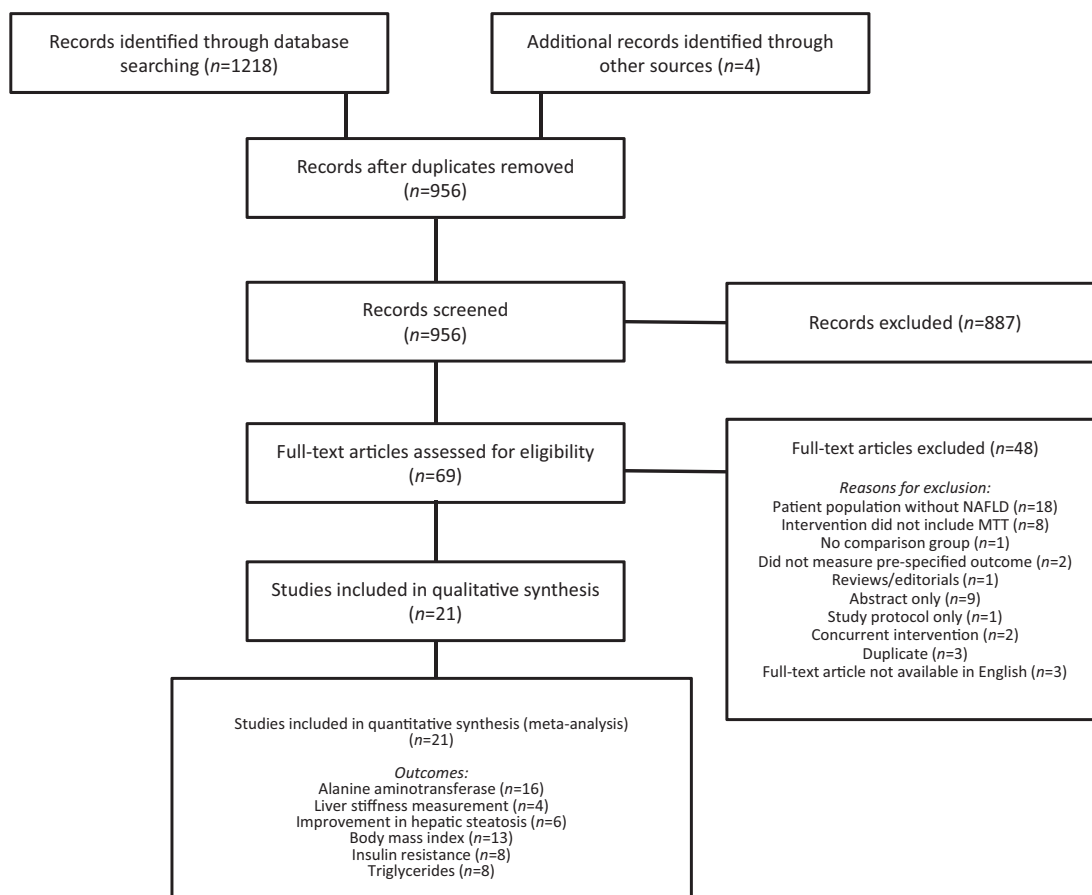
FIGURE 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses diagram: flow of studies through the review process. MTT, microbiome-targeted therapy; NAFLD, nonalcoholic fatty liver disease.

Synthesis and statistical analysis

Clinical heterogeneity was assessed by grouping studies by intervention and outcome. If there were ≥ 3 trials within a single grouping, data were pooled through the use of random-effects meta-analysis. Analyses were stratified by type of MTT. Primary outcomes were liver-related outcomes, specifically serum ALT level, change in hepatic fibrosis via elastography (LSM), and change in hepatic steatosis via ultrasound. Secondary outcomes included metabolic outcomes, specifically change in BMI, TG, and insulin resistance (as measured by HOMA-IR). The following continuous outcomes were assessed: serum ALT, LSM, BMI, TG, and HOMA-IR. For continuous outcomes, we analyzed the difference in means and 95% CIs. Among studies that assessed change in hepatic steatosis by ultrasound grading and categorization (none, mild, moderate/severe), the number of patients with moderate/severe steatosis at baseline who experienced improvement by ≥ 1 grade at the end of the trial was analyzed. For this dichotomous outcome, we calculated the OR and 95% CI. For categoric data, we extracted details about each category assessed and the numbers of patients with an outcome in each category. Heterogeneity was investigated through the use of forest plots and the I^2 statistic. Where data were considered too heterogeneous to pool or not reported in a format suitable for pooling (e.g., data reported as medians or in different units of measurement), we used a narrative synthesis.

Analyses were stratified based on type of MTT. Sensitivity analyses were performed to account for methodologic quality differences, in which trials with high risk of bias were excluded. To explain heterogeneity between studies and to examine the influence of patient-level and intervention-level factors on clinical outcomes, meta-regression was performed. The following factors were studied when feasible: duration of MTT (≤ 8 wk, >8 wk), type of MTT, age of enrolled patient population [children and adolescents (<18 y), adults (≥ 18 y)], and liver disease (NASH as defined by liver histology). When feasible, subgroup analyses were performed with exclusion of RCTs that enrolled pediatric patients (<18 y). Publication bias was assessed through the use of Begg's rank correlation test and Egger's regression test. All reported results of publication bias are Begg's rank correlation test unless otherwise specified.

Statistical significance was assessed at an α level of 0.05. All reported P values are 2-sided. All statistical analyses were performed with Stata version 14.2 (College Station, TX).

Results

Included studies

The study selection process is summarized in **Figure 1**. The search identified 1,218 records, of which 266 were duplicates (identified by >1 electronic database). Based on title and abstract

screening, 69 records were considered potentially relevant and were obtained as full-text studies. After full-text review, a total of 21 RCTs (1,252 participants) that evaluated MTT in NAFLD were included (21–41). The characteristics of included trials are summarized in **Table 1**. Nine RCTs evaluated probiotics in the management of NAFLD, among which 3 enrolled only pediatric patients (<18 y) (21–29). Twelve RCTs evaluated synbiotics in the management of NAFLD, all of which were performed in adults (29–38, 40, 41). No studies that evaluated antibiotics or FMT for treatment of NAFLD fulfilled the inclusion criteria. Probiotic formulations, including the probiotic component of synbiotics, predominantly comprised multiple bacterial species. The duration of probiotic or synbiotic intervention ranged from 8 to 28 weeks. The majority of studies (16 of 21, 67%) utilized placebo as a comparator. One study evaluated a synbiotic as an adjunctive treatment to metformin, where both the intervention and control groups received metformin (36). Another study evaluated a synbiotic as an adjunctive treatment to sitagliptin, where both the intervention and control groups received sitagliptin (40).

Additional study characteristics are listed in **Supplemental Table 1**. Studies were conducted in a wide range of countries, including Iran, Italy, Germany, Spain, Ukraine, Egypt, Brazil, and China (Supplemental Table 1). Among the 21 included studies, 7 (33%) utilized histologic criteria (i.e., performance of liver biopsy) to define NAFLD. All included RCTs utilized a parallel group design; 10 of 21 (47%) studies utilized a BMI cutoff as part of the study inclusion criteria.

Overall, 11 (52%) trials were judged at low risk of bias; 4 (19%) were judged at high risk of bias; and 6 (29%) were judged at unclear risk of bias (**Supplemental Table 2**). A common potential source of bias in trials was blinding of participants. All studies ($n = 47$) that were excluded after full-text review, along with the primary reason for exclusion, are listed in **Supplemental Table 3**.

ALT activity

The effect of probiotics/synbiotics on ALT activity was assessed in 16 studies (889 participants), of which 7 evaluated probiotics (21, 22, 24–27, 29) and 9 evaluated synbiotics (30, 31, 33–37, 39, 40). Altogether, probiotics/synbiotics were associated with a significant reduction in ALT [weighted mean difference (WMD): −11.23 U/L; 95% CI: −15.02, −7.44 U/L; **Figure 2**]. Both probiotics (WMD: −13.53 U/L; 95% CI: −22.79, −4.26 U/L) and synbiotics (WMD: −10.09 U/L; 95% CI: −14.44, −5.74 U/L) were associated with a greater reduction in ALT compared with control.

Significant heterogeneity among the studies was identified ($I^2 = 90.6\%$, $P < 0.001$). In a sensitivity analysis that excluded RCTs with high risk of bias, probiotics/synbiotics remained significantly associated with a reduction in ALT compared with control (WMD: −11.32 U/L; 95% CI: −16.22, −6.34 U/L). A subgroup analysis including only trials that enrolled adult patients revealed that probiotics/synbiotics remained significantly associated with reduction in ALT (WMD: −11.16 U/L; 95% CI: −15.44, −6.87 U/L). Meta-regression analysis revealed no significant relation of MTT type (probiotics compared synbiotics, $P = 0.82$), MTT duration >8 wk ($P = 0.75$), and patient age >18 y ($P = 0.90$) on heterogeneity. However, there was

a significant effect of NASH on heterogeneity ($P = 0.01$). A subgroup analysis limited only to studies that enrolled patients with NASH revealed that probiotics/synbiotics were associated with a significant reduction in ALT (WMD: −35.81 U/L; 95% CI: −56.64, −14.93 U/L). There was no evidence of publication bias ($P = 0.28$).

LSM

The effect of probiotics/synbiotics on LSM, as measured by elastography, was assessed in 4 studies (235 participants), of which 1 evaluated probiotics (25) and 3 evaluated synbiotics (35, 38, 39). The combined WMD in LSM was −0.70 kPa (95% CI: −1.00, −0.40 kPa), favoring benefit with probiotics/synbiotics as compared with control (**Figure 3**). There was significant heterogeneity between studies ($I^2 = 93.4\%$, $P < 0.001$), and meta-regression analysis was not feasible due to the limited number of studies. A sensitivity analysis excluding 1 study deemed at high risk of bias did not change the results (WMD: −2.12 kPa; 95% CI: −3.96, −0.28 kPa) and there was no evidence of publication bias ($P = 0.95$).

Hepatic steatosis

The effect of probiotics/synbiotics on improvement in hepatic steatosis, as graded by liver ultrasound, was assessed in 6 studies (384 participants), of which 2 evaluated probiotics (21, 24) and 4 evaluated synbiotics (30, 36, 37, 41). Together, probiotics/synbiotics were associated with increased odds of having improvement from moderate/severe hepatic steatosis (OR: 2.40; 95% CI: 1.50, 3.84) compared with control (**Figure 4**). This association remained evident in a sensitivity analysis in which 1 study with high risk of bias was excluded (OR: 2.42; 95% CI: 1.21, 4.82), and there was no significant heterogeneity between studies ($I^2 = 22.4\%$, $P = 0.27$).

BMI

The effect of probiotics/synbiotics on BMI was assessed in 12 studies (778 participants), of which 3 evaluated probiotics (21–23, 27) and 9 evaluated synbiotics (30–37, 40). Pediatric studies were excluded from analyses examining changes in BMI. Probiotics (WMD: −1.84 kg/m^2; 95% CI: −3.30, −0.38 kg/m^2), but not synbiotics (WMD: −0.85 kg/m^2; 95% CI: −2.17, 0.47 kg/m^2), were associated with a significantly greater reduction in BMI compared with control (**Figure 5**). There was significant heterogeneity between synbiotic studies ($I^2 = 96.6\%$, $P < 0.001$) but not amongst probiotic studies ($I^2 = 23.6\%$, $P = 0.12$). In a sensitivity analysis that excluded RCTs with high risk of bias, the overall effect of probiotics/synbiotics on BMI was attenuated but remained significant (WMD: −0.99 kg/m^2: 95% CI: −1.96, −0.01 kg/m^2). Meta-regression analysis revealed no significant effect of MTT type ($P = 0.65$), MTT duration >8 wk ($P = 0.73$), patient age >18 y ($P = 0.66$), or NASH ($P = 0.84$) on heterogeneity. There was no evidence of publication bias ($P = 0.92$).

TABLE 1 Characteristics of included trials, stratified by probiotics and synbiotics[1]

First author (publication year)	Patient population	Total sample size	Intervention of experimental group	Duration, wk	Comparison	Outcome[1]	Risk of bias[2]
Probiotic intervention							
Alisi (21) (2014)	Pediatric	48	VSL#3 (multispecies)	16	Placebo	Steatosis on US*, ALT, TGs, HOMA-IR, BMI	Low
Aller (22) (2011)	Adult	30	Lactobacillus bulgaricus and Streptococcus thermophilus	12	Placebo	ALT*, TB, BMI, weight, TC, TGs, FPG, HOMA-IR, IL-6, TNFα	Low
Behrouz (23) (2017)	Adult	111	Webber Naturals (multispecies)	16	Placebo	BMI, weight, FPG, insulin, HOMA-IR, leptin, % body fat, adiponectin	Low
Famouri (24) (2016)	Pediatric	64	Prokid (multispecies)	12	Placebo	Steatosis on US*, ALT, AST, BMI, weight, TC, WC	Low
Kobyliak (25) (2018)	Adult	58	Symbiter (multispecies)	8	Placebo	LSM*, FLI*, AST, ALT, TC, TNFα, IL-6, IL-8, IFNγ	Low
Monem (26) (2017)	Adult	30	Lactobacillus acidophilus	4	Usual care	ALT, AST, albumin, TB	High
Nabavi (27) (2014)	Adult	72	Probiotic yogurt (multispecies)	8	Placebo (conventional yogurt)	ALT, AST, TC, steatosis on US	Unclear
Sepideh (28) (2016)	Adult	50	Lactocare (multispecies)	8	Placebo	FPG, insulin, HOMA-IR, TNFα, IL-6, HbA1c	Unclear
Vajro (29) (2011)	Pediatric	20	Lactobacillus rhamnosus	8	Placebo	ALT*, steatosis on US, TNFα	Unclear
Synbiotic intervention							
Asgharian (30) (2016)	Adult	80	Familact (multispecies + FOS)	8	Placebo	ALT*, AST*, steatosis on US, BMI, weight, CRP	Low
Bakhshimoghaddam (41) (2018)	Adult	102	Synbiotic yogurt (Bifidobacterium animalis + inulin)	24	Usual care or conventional yogurt	Steatosis on US*, ALT, AST, GGT, TC, FPG, insulin, GLP-2	Unclear
Ekhlasi (31) (2016)	Adult	30	Protexin (multispecies + FOS)	8	Placebo	ALT*, AST, TC, TG, BMI, FPG, HOMA-IR, leptin, Apo A-1	Low
Eslamparast (39) (2014)	Adult	52	Protexin (multispecies + FOS)	28	Placebo	ALT*, AST, TB, GGT, ALP, LSM, BMI, CRP, TNFα	Low
Ferolla (32) (2016)	Adult	50	Lactobacillus reuteri + inulin	12	Usual care	MRI PDFF*, ALT, AST, BMI, TC, TG, WC, uric acid	High
Javadi (33) (2017)	Adult	36	Bifidobacterium longum + Lactobacillus acidophilus + inulin	12	Placebo	ALT*, BMI, weight, TB, ALP	Low
Malaguarnera (34) (2011)	Adult	66	Bifidobacterium longum + FOS	24	Placebo	NAS*, ALT, AST, GGT, TB, TC, TG, FPG, insulin, CRP, TNFα	Low
Manzhalii (35) (2017)	Adult	75	Multispecies probiotic + FOS	12	Usual care	ALT*, AST, LSM, TC, TG, BMI	High
Mofidi (38) (2016)	Adult	50	Protexin (multispecies + FOS)	28	Placebo	CAP*, LSM, ALT, AST, FPG, HOMA-IR, TC, TG	Unclear
Sayari (40) (2018)	Adult	138	Familact (multispecies + FOS)	16	Placebo (sitagliptin in both groups)	BMI, FPG, ALT, AST, TC, TG	Low
Shavakhi (36) (2013)	Adult	70	Protexin (multispecies + FOS)	24	Placebo (metformin in both groups)	ALT*, AST*, steatosis on US*, BMI, FPG, TC, TG	Unclear
Wong (37) (2013)	Adult	20	Lepicol (multispecies + FOS)	24	Usual care	IHTG*, ALT, AST, BMI, FPG, LSM, TC, TG	High

[1] The prespecified primary outcome of each study is labeled with an asterisk (*). ALP, alkaline phosphatase; ALT, alanine aminotransferase; Apo, apolipoprotein; AST, aspartate aminotransferase; CAP, controlled attenuation parameter; CRP, C-reactive protein; FPG, fasting plasma glucose; FLI, fatty liver index; FOS, fructo-oligosaccharides; GGT, γ-glutamyltransferase; GLP-2, glucagon-like peptide 2; HbA1c, glycated hemoglobin; IFNγ, interferon γ; IHTG, intrahepatic triglyceride content; LSM, liver stiffness measurement; NAS, NAFLD activity score; PDFF, proton density fat fraction; TB, total bilirubin; TC, total cholesterol; TG, triglyceride; US, abdominal ultrasound; WC, waist circumference.

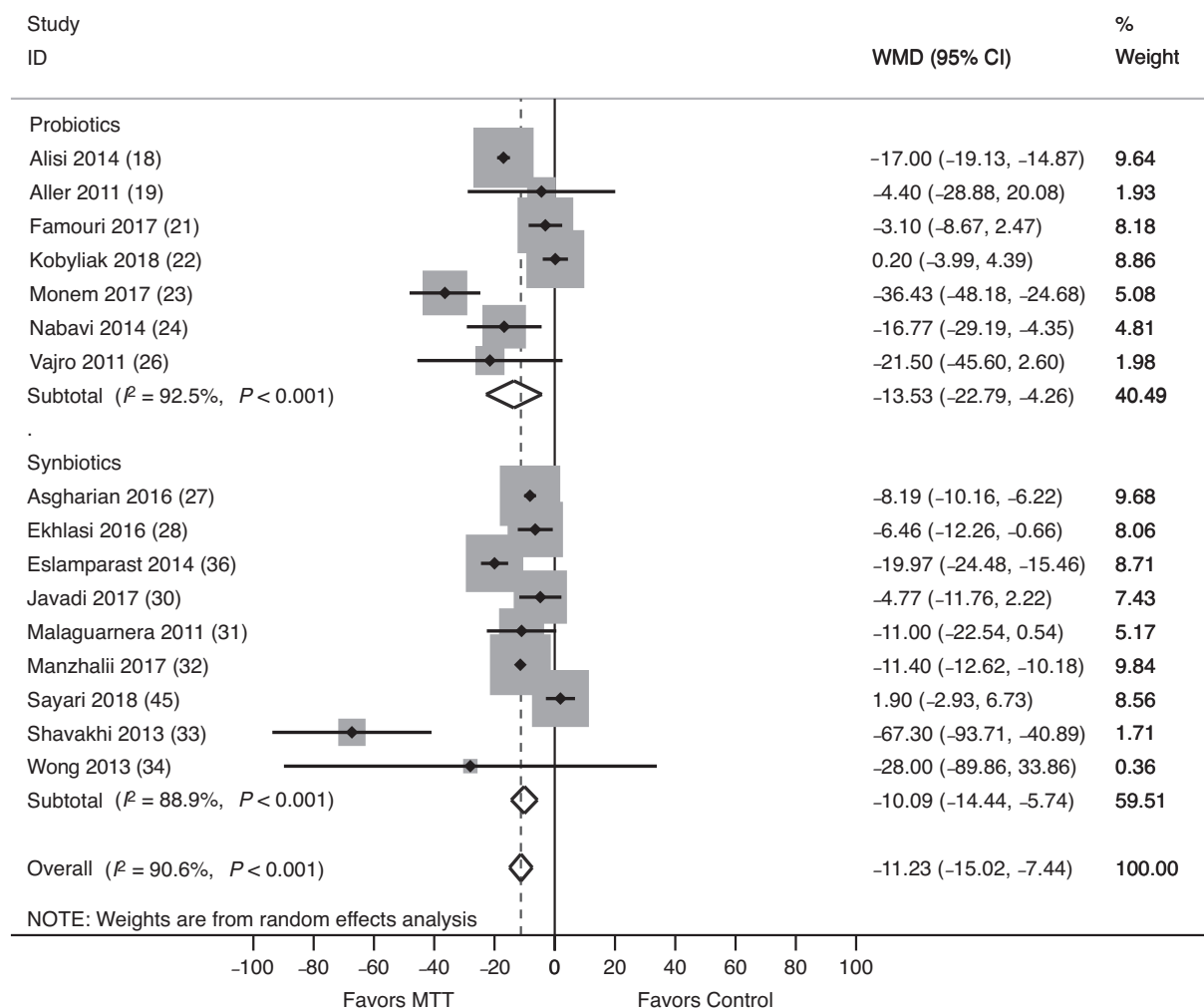[2] Assessed via the Cochrane Risk of Bias Tool.

FIGURE 2 Forest plot of the effect of MTT on serum ALT, stratified by probiotics and synbiotics, and measured by the WMD. Probiotics/synbiotics were associated with a significant reduction in ALT compared with control. ALT, alanine aminotransferase; MTT, microbiome-targeted therapy; WMD, weighted mean difference.

TGs

The effect of probiotics/synbiotics on TG was assessed in 8 studies (504 participants), of which 4 evaluated probiotics (21–23, 28) and 4 evaluated synbiotics (31, 34, 38, 40). Neither probiotics (WMD: 3.30 mg/dL; 95% CI: −9.36, 15.96 mg/dL) nor synbiotics (WMD: −15.78 mg/dL; 95% CI: −33.16, 1.60 mg/dL) were associated with a greater reduction in TG compared with control (**Supplemental Figure 1**). No studies that examined TG were deemed to have a high risk of bias, and therefore a sensitivity analysis was not performed. There was no significant heterogeneity in probiotic analyses ($I^2 = 44.9\%$, $P = 0.14$), but there was significant heterogeneity in synbiotic analyses ($I^2 = 83.9\%$, $P < 0.00$). No publication bias was detected ($P = 0.23$).

Insulin resistance: HOMA-IR

The effect of probiotics/synbiotics on insulin resistance, as measured by HOMA-IR, was assessed in 8 studies (487 participants), of which 4 evaluated probiotics (21–23,

28) and 4 evaluated synbiotics (31, 34, 38, 40). Altogether, probiotics/synbiotics were not associated with a significant improvement in HOMA-IR (WMD: −0.41 mg/dL × μmol/mL/405; 95% CI: −1.37, 0.55 mg/dL × μmol/mL/405) compared with control (**Supplemental Figure 2**), and this did not vary by type of MTT. There was significant heterogeneity between studies ($I^2 = 99.3\%$, $P < 0.001$); however, this was not explained by MTT duration >8 wk ($P = 0.74$) or patient age >18 y ($P = 0.56$). No studies were deemed at high risk of bias, and no evidence of publication bias was found ($P = 0.76$).

Discussion

NAFLD, the hepatic manifestation of the metabolic syndrome, is anticipated to become the leading worldwide cause of end-stage liver disease in the coming decades, but effective treatments are lacking. Given mounting preclinical evidence that supports a strong association between the gut microbiome and NAFLD, there is increasing interest in the use of MTTs in the management of NAFLD. In this meta-analysis, a comprehensive assessment
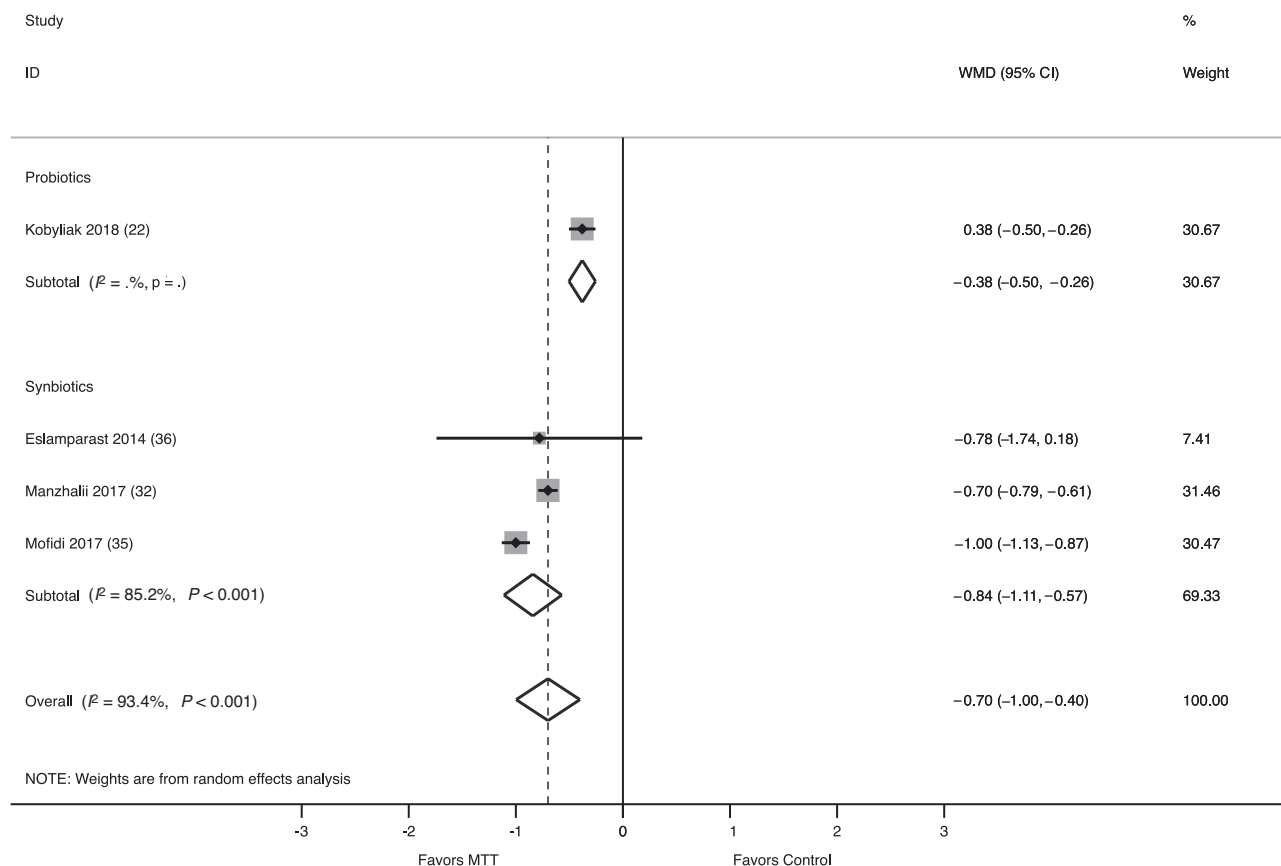
FIGURE 3 Forest plot of the effect of MTT on LSM, as measured by elastography, stratified by probiotics and synbiotics, and measured by the WMD. Probiotics/synbiotics were associated with a significant reduction in LSM compared with control. LSM, liver stiffness measurement; MTT, microbiome-targeted therapy; WMD, weighted mean difference.

of the effect of MTTs on a wide array of liver-specific and metabolic outcomes was conducted. We identified a total of 21 RCTs that evaluated probiotics or synbiotics in NAFLD. Our findings suggest that both probiotics and synbiotics may have beneficial effects on key liver-specific outcomes. Specifically, probiotics/synbiotics were associated with a significant reduction in LSM as well as greater odds of a reduction in hepatic steatosis. Although there was also an association between probiotics/synbiotics and reduction in ALT, persons with definite NASH experienced the greatest improvement in ALT with these MTTs.

The association of probiotics/synbiotics with components of the metabolic syndrome was less consistent. Probiotics/synbiotics were associated with a significant reduction in BMI, although this effect was predominantly driven by probiotic and not synbiotic trials. However, probiotics/synbiotics were not associated with a significant improvement in insulin resistance, as measured by HOMA-IR, and the favorable effect of probiotics/synbiotics on TGs was only seen with synbiotics compared with control. Given the reduced levels of ALT and improvement in hepatic steatosis, the lack of effect of probiotics/synbiotics on insulin resistance and the less-apparent effect on serum TGs was unexpected. This could be reflecting differential effects of probiotics/synbiotics

on hepatic insulin resistance compared with other tissues. Future studies may benefit from additional measures of insulin sensitivity and resistance to better delineate the underpinnings of the probiotic/synbiotic association with glucose homeostasis.

There were no meaningful differences in the efficacy of probiotics in children and adults with NAFLD, although this comparison was limited by the relative paucity of trials examining probiotics in the pediatric population (and no trials examined synbiotics in children/adolescents). Moreover, subgroup analyses including only trials that enrolled adult participants revealed consistent associations with probiotics and liver and metabolic outcomes. Although the composition of the gut microbiota stabilizes in adulthood, the gut microbial community is dynamic during childhood (12). Several studies have found perturbations in the gut microbiota in children with NAFLD, which may differ from gut microbial dysbiosis noted in adults with this disease (42–44). As a result, one would expect that response to probiotics/synbiotics could vary based on patient age, but there were limited data to draw any conclusions about differential response to probiotics/synbiotics in childhood compared with adulthood. Nevertheless, further studies examining the role of MTTs in the management of NAFLD in the pediatric patient population are important.
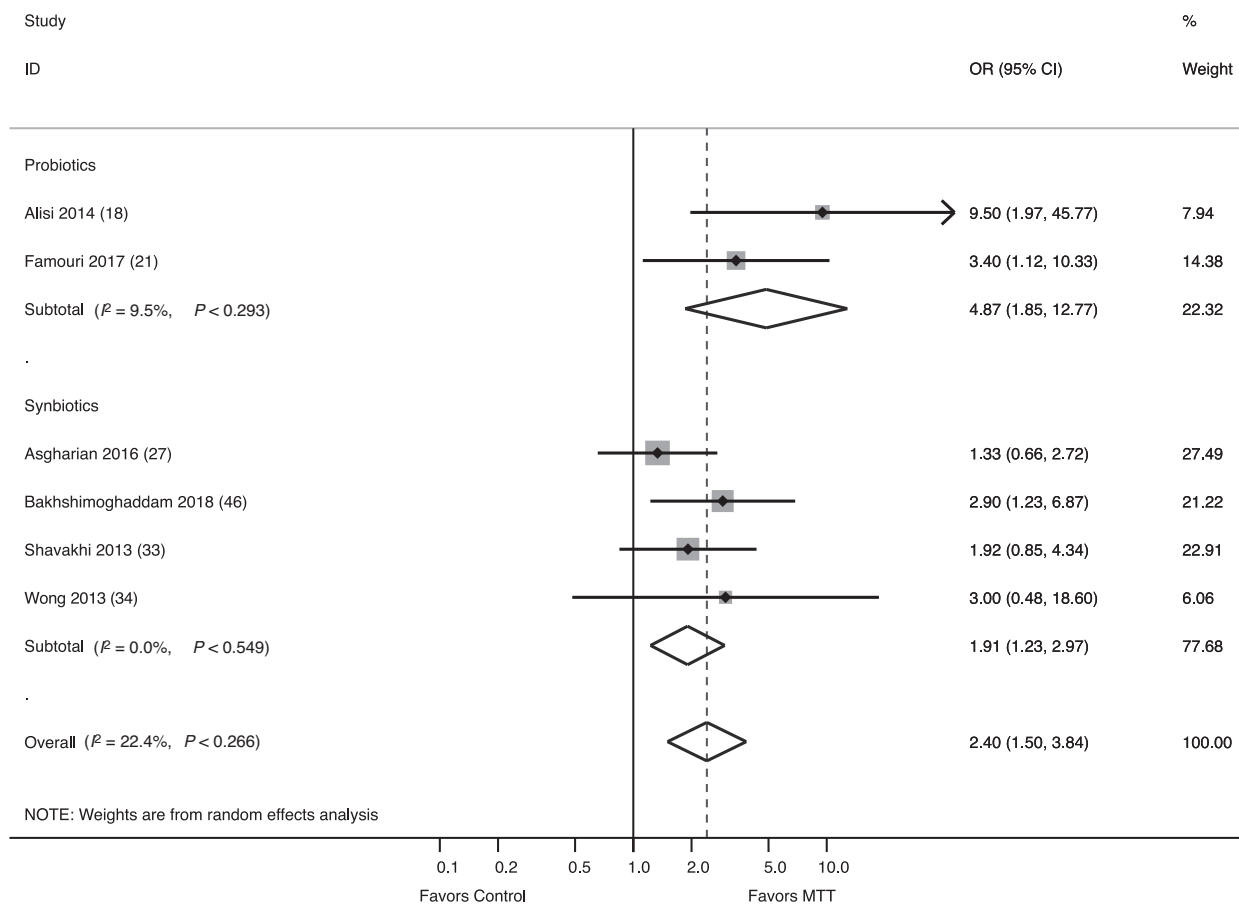
FIGURE 4 Forest plot of the effect of MTT on improvement in hepatic steatosis, as graded by ultrasound, stratified by probiotics and synbiotics. Probiotics/synbiotics were associated with increased odds of having improvement from moderate/severe hepatic steatosis compared with control. MTT, microbiome-targeted therapy.

There was significant overlap in the probiotic and synbiotic formulations utilized in included studies. The majority of probiotics were multispecies formulations predominantly comprising *Lactobacillus* or *Bifidobacteria* strains, or combinations of these. However, it is worth noting that there is a lack of standardization in the formulation and viability of currently marketed probiotic/synbiotic supplements (45), which makes direct comparisons amongst formulations difficult. Although a distinct gut microbial signature has yet to be discovered in NAFLD (5), changes in the relative abundance of *Lactobacillus*, *Bifidobacterium*, and *Streptococcus* genera have been consistently reported in varying clinical phenotypes of NAFLD (46), providing biological plausibility for the positive effects noted with the probiotic/symbiotic formulations utilized in the majority of included RCTs. However, host factors such as diet and genetics can both interact with the gut microbiome composition and elicit varying host responses to MTT (12), and, ultimately, our analyses did not yield specific insights into the ideal composition or duration of MTT for the treatment of NAFLD. Additional mechanistic studies to decipher the exact role of the gut microbiota in the pathogenesis of NAFLD, and how this may differ based on patient-specific factors and environmental exposures, may guide the development of MTTs that are more precisely formulated for the management of NAFLD.

The number of trials examining the efficacy of MTTs in NAFLD has increased over the past 10 y, but there are limitations in the current evidence. First, we did not identify RCTs that have examined the efficacy of either antibiotics or FMT for the treatment of NAFLD. To date, FMT trials in human subjects have been limited to obese adults with metabolic syndrome but without defined NAFLD. FMT from lean to obese donors was shown to improve insulin sensitivity, albeit with only short-term improvement (47, 48). These intriguing results suggest that FMT may provide therapeutic benefit in NAFLD, and at least 2 clinical trials examining FMT in adults with biopsy-confirmed NASH are actively recruiting subjects (Clinicaltrials.gov, NCT02469272 and CT02469272). Second, the number of trials including patients with biopsy-proven NAFLD was modest, and even fewer focused only on NASH, the histologic type at highest risk of liver-related complications (3). As such, there was likely significant variation in the severity of baseline liver disease amongst studies and we could not fully compare the efficacy of probiotics/synbiotics in different histologic phenotypes of NAFLD. Although heterogeneity in the analyses could in part be attributed to liver disease phenotype (histologic-confirmed NASH compared with not), this could not be explained by additional patient-level factors such as the age of the patient population (pediatric compared with adult) or by intervention-
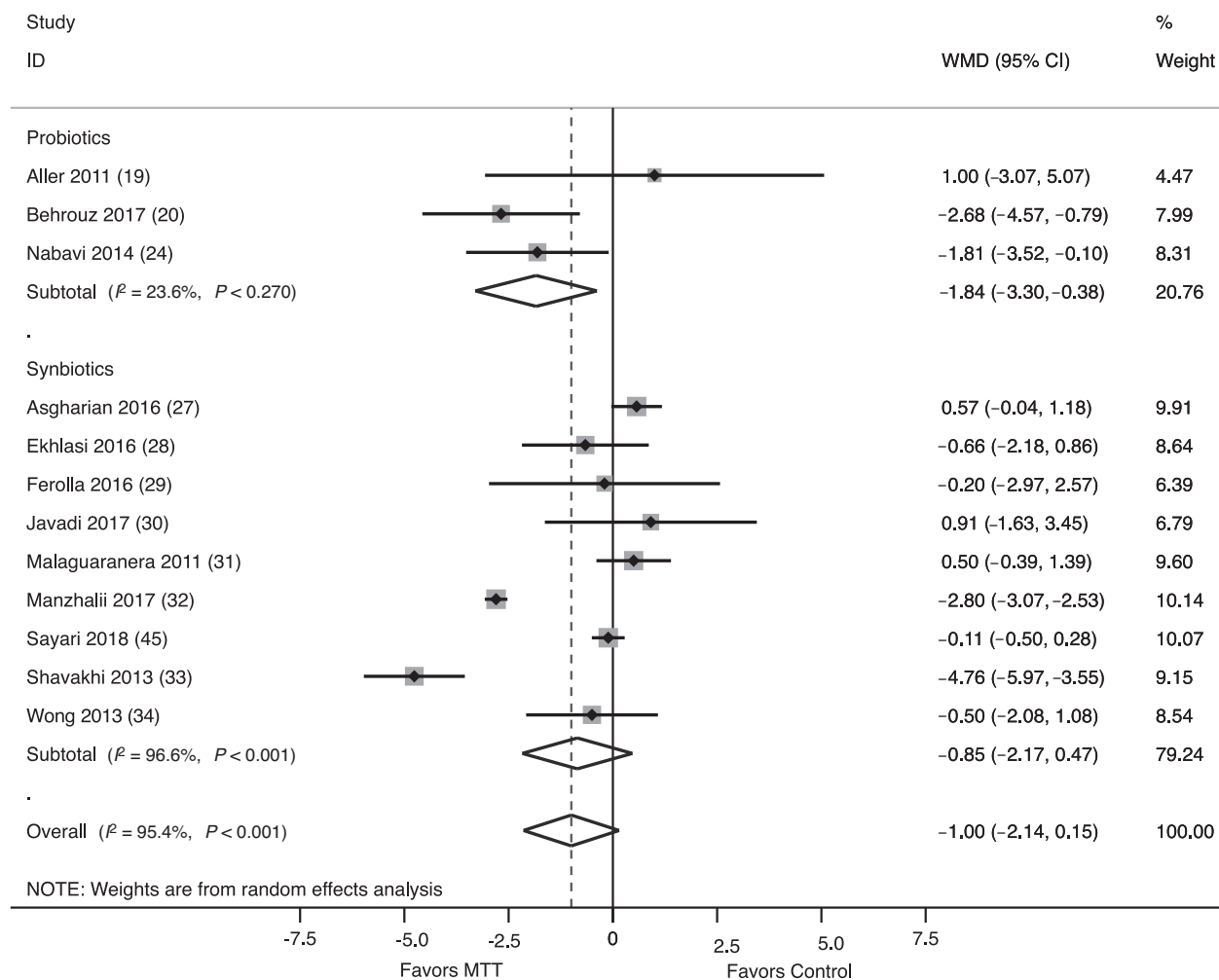
Study		WMD (95% CI)	%
ID			Weight

Probiotics

Aller 2011 (19)	1.00 (−3.07, 5.07)	4.47
Behrouz 2017 (20)	−2.68 (−4.57, −0.79)	7.99
Nabavi 2014 (24)	−1.81 (−3.52, −0.10)	8.31
Subtotal (I^2 = 23.6%, P < 0.270)	−1.84 (−3.30, −0.38)	20.76

Synbiotics

Asgharian 2016 (27)	0.57 (−0.04, 1.18)	9.91
Ekhlasi 2016 (28)	−0.66 (−2.18, 0.86)	8.64
Ferolla 2016 (29)	−0.20 (−2.97, 2.57)	6.39
Javadi 2017 (30)	0.91 (−1.63, 3.45)	6.79
Malaguaranera 2011 (31)	0.50 (−0.39, 1.39)	9.60
Manzhalii 2017 (32)	−2.80 (−3.07, −2.53)	10.14
Sayari 2018 (45)	−0.11 (−0.50, 0.28)	10.07
Shavakhi 2013 (33)	−4.76 (−5.97, −3.55)	9.15
Wong 2013 (34)	−0.50 (−2.08, 1.08)	8.54
Subtotal (I^2 = 96.6%, P < 0.001)	−0.85 (−2.17, 0.47)	79.24

| Overall (I^2 = 95.4%, P < 0.001) | −1.00 (−2.14, 0.15) | 100.00 |

NOTE: Weights are from random effects analysis

-7.5   -5.0   -2.5   0   2.5   5.0   7.5

Favors MTT   Favors Control

FIGURE 5 Forest plot of the effect of MTT on BMI, stratified by probiotics and synbiotics, and measured by the WMD. Probiotics/synbiotics were associated with a significant reduction in BMI compared with control. MTT, microbiome-targeted therapy; WMD, weighted mean difference.

level factors including duration of probiotic/synbiotic therapy. Additionally, the majority of trials did not perform sequential liver biopsies or evaluate other measures of hepatic steatosis, such as the controlled attenuation parameter via transient elastography or proton density fat fraction via MRI, and such measures should be included in future trials (49). Although the liver-specific outcomes measured in our analyses may serve as surrogate markers of heptic necroinflammation and liver fibrosis, longitudinal histologic assessment of NAFLD remains standard, when compared with noninvasive measurements. Change in hepatic steatosis as graded by ultrasound is not a well-validated measure of disease improvement and is subject to interobserver variability. However, improvement in hepatic steatosis by ultrasound was reported on a similar categoric scale in 6 RCTs (with grading performed by blinded evaluators in all 6 studies), and therefore this was the most reliable measure of change in hepatic steatosis that could be pooled for analysis. Despite these limitations, we believe these results are encouraging and support the consideration of larger, well-designed studies to evaluate MTT as treatment for NAFLD. This is especially relevant given the low cost of probiotics and synbiotics and the relative paucity of major adverse effects (50).

In summary, modulation of the gut microbiome through administration of probiotics or synbiotics could represent a promising new therapeutic strategy in NAFLD. Our results corroborate findings from preclinical studies (6–10) and should prompt larger trials in patients with biopsy-proven NAFLD to further delineate the efficacy of MTTs in NAFLD.

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