REVISTA ESPAÑOLA DE
ENFERMEDADES DIGESTIVAS
The Spanish Journal of Gastroenterology

Title:
Probiotics and non-alcoholic fatty liver disease in children and adolescents: a systematic review

Authors:
David Avelar-Rodríguez, Rubén Peña-Vélez, Jelena Popov, Lee Hill, Paul MacDaragh Ryan

DOI: 10.17235/reed.2022.8796/2022
Link: PubMed (Epub ahead of print)

Please cite this article as:
Avelar-Rodríguez David, Peña-Vélez Rubén, Popov Jelena, Hill Lee, Ryan Paul MacDaragh . Probiotics and non-alcoholic fatty liver disease in children and adolescents: a systematic review. Rev Esp Enferm Dig 2022. doi: 10.17235/reed.2022.8796/2022.

REVISTA ESPAÑOLA DE
ENFERMEDADES DIGESTIVAS
The Spanish Journal of Gastroenterology

Probiotics and non-alcoholic fatty liver disease in children and adolescents: a systematic review

David Avelar-Rodriguez[1], Rubén Peña-Vélez[2]*, Jelena Popov[3], Lee Hill[4], Paul MacDaragh Ryan[5]

[1] The Hospital for Sick Children, Toronto, Ontario, Canada

[2] Division of Pediatric Gastroenterology, Hepatology and Nutrition, Hospital General de Puebla Dr Eduardo Vázquez N, Puebla, Mexico.

[3] College of Medicine and Health, University College Cork, Cork, Ireland

[4] Division of Gastroenterology & Nutrition, Department of Pediatrics. McMaster University. Hamilton, Ontario, Canada

[5] Cork University Hospital, Cork, Ireland

David Avelar-Rodriguez davidavelar1@outlook.com

Ruben Peña-Velez rubenpevelez@hotmail.com

Jelena Popov popovj2@mcmaster.ca

Lee Hill hill.lee.devlin@gmail.com

Paul MacDaragh Ryan 108444161@umail.ucc.ie

Corresponding author:

Rubén Peña-Vélez (rubenpevelez@hotmail.com)

Division of Pediatric Gastroenterology, Hepatology and Nutrition, Hospital General de Puebla Dr Eduardo Vázquez N, Puebla, Mexico.

Antiguo Camino a Guadalupe Hidalgo No. 11350, Agua Santa. Puebla de Zaragoza, Puebla , 72490.

REVISTA ESPAÑOLA DE
ENFERMEDADES DIGESTIVAS
The Spanish Journal of Gastroenterology

Abstract

Background: Non-Alcoholic Fatty Liver Disease (NAFLD) in childhood is an increasing public health issue globally with significant long-term consequences. NAFLD management mainly consists of lifestyle modifications, however, adjunct pharmacological therapies are currently lacking. Gut microbiota manipulation via probiotics may alter the course of pediatric NAFLD. The objective of this systematic review was to synthesise all the available literature on the use of probiotics in children and adolescents with NAFLD.

Methods: We systematically searched PubMed, EBSCOhost, Scopus, Web of Science, and Cochrane Library for trials on the use of probiotics in pediatric NAFLD. A quantitative DerSimonian Laird random effects meta-analysis was performed when possible; otherwise, a narrative summary of the study outcomes was presented and discussed. A separate search was completed to include all the ongoing registered trials on probiotics use in pediatric NAFLD.

Results: 5 randomized controlled trials met the inclusion criteria. Of these, 4 trials were included in the final quantitative analysis. Probiotic therapy significantly reduced the levels of ALT (mean difference, -10.39 [-19.85, -0.93]), however significant heterogeneity between studies was identified (I^2, 93%).

Conclusions: There is insufficient evidence to support probiotics in the treatment of pediatric NAFLD given the substantial degree of discordance amongst the available trials. Lifestyle modifications focusing on maintaining a normal BMI and regular exercise continue to be the gold standard approach to treating NAFLD in children.

Keywords: Non-alcoholic fatty liver disease. NAFLD. Probiotics. Childhood obesity.

Introduction

Non-Alcoholic Fatty Liver Disease (NAFLD), as a direct result of the escalating childhood obesity epidemic, is a significant public health issue globally (1). NAFLD is the most common cause of chronic liver disease in the pediatric population, affecting a third of children and adolescents with obesity (2). Indeed, the reported pooled prevalence of

NAFLD in the pediatric population is approximately 7.6%, but rises to 34.2% in the presence of obesity (2). Hispanic adolescents (3) and Mexican children (4) are particularly affected by NAFLD, likely due to genetic predisposition and the high prevalence of obesity affecting these populations. NAFLD is associated with features of metabolic syndrome, such as insulin resistance, central adiposity, dyslipidemia characterized by high triglycerides and low high-density lipoprotein (HDL) cholesterol levels (5) and larger neck circumference (6). Indeed, metabolic syndrome is the strongest risk factor for development of NAFLD and non-alcoholic steatohepatitis (NASH) (7).

Diagnosis of NAFLD is complicated by high rates of asymptomatic presentation and a lack of appropriately sensitive and specific screening tools in asymptomatic populations (8). Even simple steatosis, which is reversible, can progress to more severe and irreparable stages such as NASH, fibrosis, and ultimately liver cirrhosis and liver failure (9,10). The treatment of NAFLD in children is limited due to lack of effective pharmacological interventions. Recommendations from the Expert Committee on NAFLD and the North American Society of Pediatric Gastroenterology, Hepatology and Nutrition (9), emphasize lifestyle modifications as the only acceptable method to prevent and treat NAFLD/NASH in children. These include avoidance of sugar-sweetened beverages, consumption of a well-balanced diet, daily moderate to high intensity exercise, and less than 2h/day of screen time. Therefore, in addition to effective childhood overweight/obesity prevention strategies and lifestyle modifications, effective adjunctive pharmacological interventions are needed to prevent disease progression.

The pathogenesis and progression of NAFLD are complex and poorly understood processes. The multiple-hit theory has been the most accepted hypothesis; however, it should be noted that the pathogenesis of NAFLD is more complex than previously appreciated, encompassing alterations in metabolic pathways, genetics, and more recently, the gut microbiota (7,11). The gut microbiota represents a complex and diverse ecosystem, in which both the host and commensal microbes appear to benefit from a synbiotic relationship (12,13). Recent evidence suggests that perturbations of the gut microbiota may be involved in several disease states and that therapeutic manipulation of

these microbial communities may ameliorate various gastrointestinal and extraintestinal conditions (14). In line with this, several studies have demonstrated that the gut microbiota may affect the development and progression of NAFLD (15), largely through the alterations to the gut-liver axis (16). In this respect, the liver may act as mediator, ensuring mutualism between the commensal gut microbiota and the host (17). As such, it has been demonstrated that modulation of the gut microbiota through the use of prebiotics and probiotics may be beneficial in the treatment of obesity and obesity-associated NAFLD (13,18-22).

Therefore, considering the increasing prevalence of NAFLD in children in parallel with the childhood obesity epidemic in recent years, as well as the lack of effective pharmacological interventions to treat the disease, we set out to synthesise all the available literature on the use of probiotics in children with NAFLD.

Methods

We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (23,24) in the reporting of the present systematic review. The research question was formulated in accordance with the PICOS (Population, Intervention, Comparison, Outcome, and Type of Study) approach, as follows: *Does treatment with probiotics influence the biochemical, imaging, or anthropometric parameters of children and adolescents with NAFLD when compared to placebo?*

Eligibility criteria

Studies were eligible for the systematic review if they were randomized controlled trials (RCTs; study) that were conducted on individuals younger than 18 years with NAFLD of any ethnicity or sex (population). We considered studies that used any type of probiotic (intervention) versus placebo or no treatment (comparison). In addition, studies had to report the primary and secondary outcome measures (outcome) for assessing the effect of the probiotic intervention; that is, changes in liver biochemical (e.g., liver enzymes, triglycerides) and/or imaging (ultrasound or magnetic resonance), and/or anthropometric

parameters (body mass index). Animal and preclinical studies, as well as case reports and review articles were excluded.

Information sources, search strategy and selection criteria

Two investigators (DAR and PMR) independently searched the PubMed, EBSCOhost, Scopus, Web of Science, and Cochrane Library databases to identify all potentially relevant articles that fit the pre-established eligibility criteria. The search was conducted from database inception to December 2021. Search terms used, alone or in combination, included: "non-alcoholic fatty liver disease", "fatty liver", "nafld", "steatohepatitis", "liver disease", "liver enzymes", "probiotic", "prebiotic", "child", "paediatric", "pediatric" and "adolescent". The search was limited to articles published in English and no time frame restriction was applied. The Covidence systematic review management software (Veritas Health Innovation, Melbourne, Australia. Available at https://www.covidence.org/) was used to conduct the systematic review and manage the retrieved articles. Disagreements between investigators were resolved by consensus with a third investigator. We also searched for ongoing clinical trials registered through Clinicaltrials.gov (https://www.clinicaltrials.gov/) and the International Clinical Trials Registry Platform (ICTRP; https://apps.who.int/trialsearch/), using the terms "non-alcoholic fatty liver disease", "probiotics", "child", "children" and "pediatrics" (DAR and JP). In addition, reference lists from selected papers were screened for relevant articles and a PubMed email alert service was activated in case a study was published after our search was completed.

Data collection process

Three investigators (RPV, LH and JP) extracted and inputted the following data into the collection form: name of first author; publication year; country of study; study population; study design; type of probiotic; duration of intervention; primary outcome measures of biochemical and/or imaging and/or anthropometric parameters; and *p*-values. After the data extraction was completed, all investigators independently reviewed the data to

ensure their accuracy.

Risk of bias in individual studies

To ascertain the validity of the eligible studies, three investigators (DAR, PMR and RPV) working independently determined the risk of bias by using the Cochrane Collaboration's tool for assessing randomised trials.

Meta-analysis

A quantitative DerSimonian Laird random effects meta-analysis was planned to assess the combined effect of probiotic therapy on any NAFLD-related parameters in children with obesity reported in uncovered trials. Additionally, the Cochran's Q test and I^2 test were used in order to assess heterogeneity amongst combined outcomes. All data analysis was performed in RevMan v5.4 (Review Manager; The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). Where meta-analysis was not possible or inappropriate (discordant outcomes or reporting), a narrative summary of the study outcomes was presented and discussed. (25-27).

Results

Our initial search yielded 416 potentially eligible articles, of which six full-text articles were reviewed (Figure 1). Of those six articles, 5 RCTs published between 2011-2019 were included in this systematic review. One article was excluded as it was the poster version of a published article (28). The trials were conducted in Italy, Iran, India, and the United States of America. The most commonly studied probiotic was VSL#3 (*Streptococcus thermophilus,* bifidobacteria *[B. breve, B. infantis, B. longum], Lactobacillus acidophilus, L. plantarum, L. paracasei, and L. delbrueckii* subsp. *bulgaricus*). Other studies trialed combinations of *Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium lactis, and Bifidobacterium bifidum*. Only one study assessed the effect of combined probiotics and lifestyle modifications compared to placebo (29). Three other studies recommended lifestyle modifications for all participants, and thus did not assess the effect of lifestyle

modifications independently of probiotics (30,31). Abdominal ultrasound was used to assess the degree of liver fat in all of the studies but one, which utilized abdominal MRI (32). All seven studies included anthropometric measurements (weight, BMI) and biochemical profiles (lipid panel, liver enzymes ± inflammatory mediators). One study assessed gut microbial abundance pre-treatment and post-treatment using 16S rRNA sequencing on participant stool samples (32). Participants were age-matched in all studies.

Risk of bias and quality assessment

According to the Cochrane Collaboration's tool, the risk of bias in the included studies ranged from low to high risk overall (Figure 2). Funnel plot is presented in Figure 3.

As for the assessment of study quality, the average score for included publications was 17.6 ± 3.1 out of a possible score of 20.0. The criterion-specific ratings varied between 1.1 and 2.0 out of a possible score of 2.0. The lowest criterion rating was sample size calculation and power analysis with only 3 of the 7 studies providing adequate justification and analysis (29,30). Both quality assessment scores are congruent with a similar meta-analysis investigating pre and probiotic interventions in NAFLD (27). The results indicate fairly good quality studies, consistent with the Consolidated Standards of Reporting Trials (CONSORT) requirements of reporting RCTs.

Probiotics on liver enzymes, liver fat and BMI

Ultimately, four placebo-controlled trials including a total of 202 children and adolescents reported ALT levels in an appropriate manner and were included for quantitative meta-analysis (28,29,30,31). Probiotic therapy significantly reduced the levels of the liver enzyme when compared to placebo (mean difference, -10.39 [-19.85, -0.93]). Although the directionality of the effect was conserved across studies, this result is tempered by significant heterogeneity (I^2, 93%). Figure 4 shows the Forrest plot of the effects of probiotics therapy on ALT compared to placebo.

Three RCTs reported variable effects of VSL#3 on reducing the degree of liver fat in children with NAFLD. The first double-blind RCT reported significant improvement in fatty liver scores [OR 0.001 (95% CI 0.0001–0.02)] and BMI [p<0.001] compared to placebo

controls with daily VSL#3 supplementation for 4 months in Italian children with obesity (31). A second RCT demonstrated similar beneficial effects of VSL#3 on children with obesity in India, particularly in combination with a low calorie, low trans-fat, high fruit and vegetable diet, and a moderate exercise regimen (29). After four months of treatment, the greatest number of children presenting with fatty liver grade 0 and greatest reduction in BMI was reported in those treated with VSL#3 combined with lifestyle interventions (38.5%, p<0.001; baseline 27.2±3.74 kg/m2 vs 4-months 24.7±3.83 kg/m2, p<0.001, respectively), compared to VSL#3 alone (33.3%, p<0.001; baseline 27.1±4.07 kg/m2 vs 4-months 26.0±4.06 kg/m2, p<0.001, respectively), lifestyle alone (30.8%, p<0.001; baseline 27±3.57 kg/m2 vs 4-months 25.6±3.46 kg/m2, p<0.001), or placebo (0% p=.317; baseline 27±3.23 kg/m2 vs 4-months 26.9±3.17 kg/m2, NS, respectively). The positive effects of VSL#3 were contradicted by a double-blind RCT in an obese Hispanic adolescent population at risk of developing NAFLD, which utilized a three times daily VSL#3 supplementation for 16 weeks and reported no improvements in degree of liver fat were achieved with probiotic supplementation (32). In fact, total fat mass, total adiposity, and trunk fat mass significantly increased in VSL#3-treated patients compared to placebo controls despite no reported differences in macronutrient or caloric intake.

Studies assessing variable combinations of probiotics also reported conflicting findings. A RCT study in Iranian children diagnosed with obesity and NAFLD achieved a higher frequency of normal liver grades after daily probiotic supplementation (*Lactobacillus acidophilus*, *Bifidobacterium lactis*, *Bifidobacterium bifidum*, and *Lactobacillus rhamnosus*) for 12 weeks compared to the placebo controls (0(0%) vs. 17(53.1%), p<0.001; 0(0%) vs. 5(16.5%), p=0.008, respectively) (30). This therapy also failed to significantly reduce participant weight, BMI, and BMI z scores. Finally, another double-blind RCT on Italian children failed to show improvement in fatty liver or BMI scores after 8 weeks of daily supplementation with *Lactobacillus rhamnosus* (28). A summary of the included trials can be found in Table 1.

Probiotics on lipid panel, hormones, and systemic inflammation

Daily supplementation with VSL#3 for 4 months in Italian children significantly increased active/total gut hormone glucagon-like peptide 1 (GLP-1) (31). Incidentally, four weeks of daily *Lactobacillus* in Italian children showed significant decreases in PG-PS IgA, a surrogate marker for SIBO, compared to placebo (28). However, this was contradicted by findings from a Hispanic adolescent population, which showed no changes in active/total GLP-1 and failed to demonstrate alterations in gut microbial abundance through 16S rRNA sequencing of stool samples after 16 weeks of VSL#3 supplementation (32).

A study in Indian children reported reduced leptin levels and increased ghrelin levels after four months of VSL#3 supplementation and lifestyle modifications (29). Again, this was contradicted by findings from a Hispanic adolescent population which investigated 16 weeks of VSL#3 supplementation but in the absence of lifestyle modifications, and reported no alterations in leptin and ghrelin levels (32).

One study reported improvements in additional biochemical parameters, including reductions in AST, ALT, GGT, hs-CRP, LDL-c, cholesterol, triglyceride, FBG, and uric acid, and increases in HDL-c after VSL#3 treatment (29). Similar improvements in biochemical parameters including cholesterol (30), LDL-c (30), TGs (30), AST (30), and ALT (28, 30), were replicated in studies using other probiotic combinations. However, two other studies assessing VSL#3 provided contradictory evidence, with no changes in triglyceride levels, peptide YY, or fasting glucose and insulin post-treatment (31,32). A study found no changes in serum TNF-α after probiotic treatment alone (28).

Registered clinical trials on probiotics and NAFLD in children

We identified six registered clinical trials investigating the effect of probiotics on NAFLD in children and adolescents in ICTRP and one in Clinicaltrials.gov (Table 2). Two studies have been included in the present systematic review (29,30) one study has been completed but not published (Brazil, RBR-9n7kfw), and two trials are currently active (NCT04671186; SLCTR/2016/021). The first trial is a triple-blind, randomized, placebo-controlled trial in New York, United States, assessing the effects of *Lactobacillus rhamnosus* strain GG (Culturelle®) in children and adolescents with NAFLD. The target sample size is 100 participants, and the primary outcome measure includes effects of probiotic in liver

steatosis and fibrosis as assessed by FibroScan® (CAP score and TE staging, respectively). The second trial is a double-blinded randomized placebo-controlled study in Colombo, Sri Lanka assessing the effects of 16-weeks of supplementation with Bio-Kult 14® probiotic on obese children diagnosed with NAFLD/NASH. The anticipated sample size is 170, evenly distributed into the two study arms. The primary objectives of the study include improvements in AST, ALT, AST:ALT ratio, liver fat, and NAFLD/NASH grade. Secondary objectives include improvements in GGT, lipid profile, glucose, metabolic syndrome, body fat, and anthropometric measures. If completed, this will be the largest study assessing the effect of probiotics on pediatric NAFLD to date.

Discussion

The purpose of this systematic review was to assess the quality of evidence currently available for the use microbial therapies (i.e, prebiotics, probiotics, and synbiotics) in the treatment of NAFLD in obese children. A total of five trials were included in this review. Ultimately, while a range of promising effects on both clinical and biochemical parameters were noted, significant interstudy discrepancies reduce reliability and generalisability of these results.

The development and progression of NAFLD is dependent on interactions between genetic, epigenetic, and environmental factors (33). Environmental factors that have been identified in causing phenotypic changes leading to the accumulation of fat in the liver include an unhealthy diet consisting of highly processed foods and inadequate physical activity levels (34). Due to the limited available pharmacological therapies for pediatric NAFLD, lifestyle interventions remain the first-line treatment. However, in recent years, it has been shown that gut microbiota composition may be influenced by environmental factors and as a consequence, gut microbiota dysbiosis has been implicated in intra-hepatic fat accumulation (34). As such, interventions that modulate interactions between gut microbiota and the liver have become a potential target for the management of pediatric NAFLD (35). One such method that has been gaining interest is microbial therapy in the form of probiotics and synbiotics, either alone or as adjuncts to behavioural lifestyle

interventions.

VSL#3 was the most commonly used microbial therapy in the trials uncovered by this review. VSL#3 is a commercial probiotic mixture that consists of eight bacterial strains (36) and is suggested to have a protective effect on intestinal barrier function. VSL#3 has been shown to have a biological barrier function through colonisation of the intestinal mucosa by co-inhabiting gut microbiota (37). However, the purported beneficial effects of the formulation on lipid metabolism and NAFLD are perhaps the most convincing, given the mechanistic preclinical evidence currently available. The importance of bile acids as signalling molecules in the gut microbiome-host interaction is established for a range of cardiometabolic disorders (38) and VSL#3 is known to contain highly active bile salt hydrolase (BSH) producing strains. The function of BSH is the deconjugation of tauro or glyco-conjugated bile acids, thereby altering their hydrophobicity and reabsorptive capacity, ultimately impacting the composition of the host circulating and cholecystic bile pool (39). This process, in turn, impacts upon enterohepatic circulating and synthesis of bile acids through the farnesoid X receptor-fibroblast growth factor 15 (FXR-FGF15) axis in a manner which reduces circulating lipid profile (40) and could potentially improve features of NAFLD. However, VSL#3 showed variable effects in the current review. Two studies (29,31) observed a reduction in liver fat when supplemented with VSL#3, however the greatest improvements were observed when VSL#3 was combined with lifestyle modifications. In contrast, no improvement in liver fat content was noted by Jones *et al (*32) in obese Hispanic adolescents. In fact, total fat mass, total adiposity, and trunk fat mass significantly increased in VSL#3-treated patients compared to placebo controls despite no reported differences in macronutrient or caloric intake.

Three other studies (30,32) using various combinations of microbial therapy showed significant improvements in fatty liver scores but were unable to show improvements in BMI, while one study (28) showed no improvements in either fatty liver scores or BMI. The studies we assessed also showed considerable variation in their effects on liver enzymes, lipid and hormone levels, and systemic inflammation. We were unable to determine any consistencies in these findings according to probiotic type given the scarcity of data and

high study heterogeneity in reporting within the included studies.

An alternate pathway through which NAFLD is theorised to respond to VSL#3 supplementation is by promoting GLP-1 production and secretion by intestinal L-cells (31,41). GLP-1 has been shown to stimulate the beta-cell insulin production and target tissue insulin sensitivity, while simultaneously slowing gastric emptying and promoting satiety. Ultimately, the increased concentration of this short-lived gut hormone leads to improved glucose and fat metabolism. Upregulation of GLP-1 has also been associated with increased regulation of metabolic flux of muscle mitochondria and production of succinyl-coenzyme A, all of which are essential components of energy metabolism (41). In murine models, GLP-1 is suggested to improve hepatic insulin resistance by reducing the TNF-α-IκB kinase β-NF-κB pathway, the activity of TNF-regulated kinase Jun N-terminal kinase, and uncoupling protein-2 (42,43). Nevertheless, despite promising literature supporting the beneficial effects of VSL#3 on GLP-1 and energy metabolism, the two studies assessed in this review displayed contradictory effects (31,32).

Discrepancies between studies raise important questions about variable response to VSL#3 supplementation across different ethnicities (31,32), and concerns attributable to flaws in study design which rely on dietary recall and low power. Similarly, as with all probiotic intervention studies, there is the propensity for a significant degree of inter-strain specificity in terms of the therapeutic effect, thereby making a neat synthesis of such trials challenging. Although the majority of studies were deemed to be of acceptable quality, inadequate justification of sample size and power analysis may limit the interpretation. Moreover, an important intrinsic limitation of the present systematic review is the fact that we were unable to conduct a meta-analysis for the majority of the outcomes of interest due to significant inconsistencies in clinical assessment and reporting. It should be considered that the results of the meta-analysis are very difficult to interpret due to the heterogeneity of the intervention between the different studies: different probiotic, different dose, and different treatment time (between 8 and 16 weeks). On the other hand, the funnel plot suggests the possibility of publication bias (Figure 3), so the results of the meta-analysis should be interpreted with caution. NAFLD is

a complex and multisystec disease, where alterations in the gut microbiota may play a role in a spectrum of multiple metabolic disorders. Further studies should consider evaluating the administration of probiotics in conjunction with other effective interventions and assess the long-term effect, as well as evaluating the impact on and interaction with the pathophysiological processes of the disease (cytokine injury, hyperinsulinemia, hepatic iron and/or lipid peroxidation, variation of the extracellular matrix, energy homeostasis, and change in the immune system function). Ultimately, this review highlights the shortcomings of the current literature and emphases the importance of a large-scale, high-quality RCT to clarify the role of probiotic formulations in prevention or mitigation of NAFLD. Finally, the major strengths of the present systematic review lie in the number of databases explored by our search and the inclusion of ongoing trials registered in official trial registries.

At present, there is insufficient evidence to support a beneficial role of probiotics and synbiotics in the treatment of pediatric NAFLD given the substantial degree of discordance amongst the available trials. However, with some promising signals and several large-scale, prospectively registered RCTs currently underway, the outlook is promising. Lifestyle modifications focusing on maintaining a normal BMI and regular exercise continue to be the gold standard approach to treating NAFLD in children with obesity.

Funding: No funding.

Conflicts of interest/Competing interests: The authors declare no conflict of interest.

Ethics approval: No ethics approval required given nature of the paper.

References

1. Berardis S, Sokal E. Pediatric non-alcoholic fatty liver disease: An increasing public health issue. Eur J Pediatr. 2014;173(2):131–9.
2. Anderson EL, Howe LD, Jones HE, et al. The prevalence of non-alcoholic fatty liver

disease in children and adolescents: A systematic review and meta-analysis. PLoS One. 2015;10(10).

3. Rehm JL, Connor EL, Wolfgram PM, et al. Predicting hepatic steatosis in a racially and ethnically diverse cohort of adolescent girls. J Pediatr. 2014;165(2):319-325.e1.
4. Betancourt-Garcia MM, Arguelles A, Montes J, et al. Pediatric Nonalcoholic Fatty Liver Disease: the Rise of a Lethal Disease Among Mexican American Hispanic Children. Obes Surg. 2017;27(1):236–44.
5. Alterio A, Alisi A, Liccardo D, et al. Non-alcoholic fatty liver and metabolic syndrome in children: A vicious circle. Horm Res Paediatr. 2014;82(5):283–9.
6. Peña-Vélez R, Garibay-Nieto N, Cal-Y-Mayor-Villalobos M, et al. Association between neck circumference and non-alcoholic fatty liver disease in Mexican children and adolescents with obesity. J Pediatr Endocrinol Metab. 2020;33(2):205–13.
7. Friedman SL, Neuschwander-Tetri BA, Rinella M, et al. Mechanisms of NAFLD development and therapeutic strategies. Nat Med. 2018;24(7):908–22.
8. Draijer L, Benninga M, Koot B. Pediatric NAFLD: an overview and recent developments in diagnostics and treatment. Expert Rev Gastroenterol Hepatol. 2019;13(5):447–61.
9. Vos MB, Abrams SH, Barlow SE, et al. NASPGHAN Clinical Practice Guideline for the Diagnosis and Treatment of Nonalcoholic Fatty Liver Disease in Children: Recommendations from the Expert Committee on NAFLD (ECON) and the North American Society of Pediatric Gastroenterology, Hepatology and Nu. J Pediatr Gastroenterol Nutr. 2017;64(2):319–34.
10. Cobbina E, Akhlaghi F. Non-alcoholic fatty liver disease (NAFLD)–pathogenesis, classification, and effect on drug metabolizing enzymes and transporters. Drug Metab Rev. 2017;49(2):197–211.
11. D'Adamo E, Castorani V, Nobili V. The Liver in Children With Metabolic Syndrome. Front Endocrinol (Lausanne). 2019;10:1–17.
12. Avelar Rodriguez D, Peña Vélez R, Toro Monjaraz EM, et al. The Gut Microbiota: A

Clinically Impactful Factor in Patient Health and Disease. SN Compr Clin Med 2019;1:188-99.

13. Kirpich IA, Marsano LS, McClain CJ. Gut-liver axis, nutrition, and non-alcoholic fatty liver disease. Clin Biochem. 2015;48(13-14):923-30.
14. Putignani L, Del Chierico F, Vernocchi P, et al. Gut Microbiota Dysbiosis as Risk and Premorbid Factors of IBD and IBS Along the Childhood-Adulthood Transition. Inflamm Bowel Dis. 2016;22(2):487–504.
15. Zhu JZ, Dai YN, Wang YM, et al. Prevalence of Nonalcoholic Fatty Liver Disease and Economy. Dig Dis Sci. 2015;60(11):3194–202.
16. Miele L, Marrone G, Lauritano C, et al. Gut-liver axis and microbiota in NAFLD: insight pathophysiology for novel therapeutic target. Curr Pharm Des. 2013;19(29):5314–24.
17. Balmer ML, Slack E, de Gottardi A, et al. The Liver May Act as a Firewall Mediating Mutualism Between the Host and Its Gut Commensal Microbiota. Sci Transl Med. 2014;6(237):237ra66--237ra66.
18. Ma YY, Li L, Yu CH, et al. Effects of probiotics on nonalcoholic fatty liver disease: A meta-analysis. World J Gastroenterol. 2013;19(40):6911–8.
19. Paolella G, Mandato C, Pierri L, et al. Gut-liver axis and probiotics: Their role in non-alcoholic fatty liver disease. World J Gastroenterol. 2014;20(42):15518–31.
20. Gao X, Zhu Y, Wen Y, et al. Efficacy of probiotics in non-alcoholic fatty liver disease in adult and children: A meta-analysis of randomized controlled trials. Hepatol Res. 2016;46(12):1226–33.
21. Perumpail B, Li A, John N, et al. The Therapeutic Implications of the Gut Microbiome and Probiotics in Patients with NAFLD. Diseases. 2019;7(1):27.
22. Ferolla SM, De Almeida Armiliato GN, Couto CA, et al. Probiotics as a complementary therapeutic approach in nonalcoholic fatty liver disease. World J Hepatol. 2015;7(3):559–65.
23. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care

interventions: Explanation and elaboration. PLoS Med. 2009;6(7).

24. Shamseer L, Moher D, Clarke M, et al. Preferred reporting items for systematic review and meta-analysis protocols (prisma-p) 2015: Elaboration and explanation. BMJ. 2015;349:1–25.
25. Higgins JPT, Altman DG, Gøtzsche PC, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ. 2011;343(7829):1–9.
26. Littell JH, Corcoran J, Pillai V. Systematic reviews and meta-analysis. Oxford University Press; 2008.
27. Loman BR, Hernández-Saavedra D, An R, et al. Prebiotic and probiotic treatment of nonalcoholic fatty liver disease: A systematic review and meta-analysis. Nutr Rev. 2018;76(11):822–39.
28. 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(6):740–3.
29. Goyal P, Thapa BR, Sharma NR, et al. Probiotic and lifestyle modification in obese pediatrics with non-alcoholic fatty liver disease. Indian J Community Heal. 2019;31(1):50–6.
30. 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(3):413–7.
31. 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(11):1276–85.
32. Jones RB, Alderete TL, Martin AA, et al. Probiotic supplementation increases obesity with no detectable effects on liver fat or gut microbiota in obese Hispanic adolescents: a 16-week, randomized, placebo-controlled trial. Pediatr Obes. 2018;13(11):705–14.
33. Alisi A, Cianfarani S, Manco M, et al. Non-alcoholic fatty liver disease and metabolic syndrome in adolescents: Pathogenetic role of genetic background and intrauterine

environment. Ann Med. 2012;44(1):29–40.

34. Quigley EM, Monsour HP. The Gut Microbiota and Nonalcoholic Fatty Liver Disease. Semin Liver Dis. 2015;35(3):262–9.
35. Hemarajata P, Versalovic J. Effects of probiotics on gut microbiota: Mechanisms of intestinal immunomodulation and neuromodulation. Therap Adv Gastroenterol. 2013;6(1):39–51.
36. Cheng FS, Pan D, Chang B,et al. Probiotic mixture VSL#3: An overview of basic and clinical studies in chronic diseases. World J Clin Cases. 2020;8(8):1361–84.
37. Ren Z, Guo C, Yu S, et al. Progress in mycotoxins affecting intestinal mucosal barrier function. Int J Mol Sci. 2019;20(11).
38. Ryan PM, Stanton C, Caplice NM. Bile acids at the cross-roads of gut microbiome-host cardiometabolic interactions. Diabetol Metab Syndr. 2017;9(1):1–12.
39. Degirolamo C, Rainaldi S, Bovenga F, et al. Microbiota modification with probiotics induces hepatic bile acid synthesis via downregulation of the Fxr-Fgf15 axis in mice. Cell Rep. 2014;7(1):12–8.
40. Sanaie S, Ebrahimi-Mameghani M, Mahmoodpoor A, et al. Effect of a Probiotic Preparation (VSL#3) on CardiovascularRisk Parameters in Critically-Ill Patients. J Cardiovasc Thorac Res. 2013;5(2):67–70.
41. 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(7):1118–25.
42. Li Z, Yang S, Lin H, et al. Probiotics and antibodies to TNF inhibit inflammatory activity and improve nonalcoholic fatty liver disease. Hepatology. 2003;37(2):343–50.
43. Stancovski I, Baltimore D. NF-kappaB activation: the I kappaB kinase revealed? Cell. 1997;91(3):299–302.

REVISTA ESPAÑOLA DE
ENFERMEDADES DIGESTIVAS
The Spanish Journal of Gastroenterology

Table 1. Characteristics of selected trials

Author	Year	Country	Population and NAFLD diagnosis	Study design	Type of probiotic ± lifestyle interventions	Duration of intervention	Study highlights	*p*-value	Quality assessment score[†]
Goyal *et al* ***(29)***	2019	India	106 obese children with NAFLD NAFLD diagnosed by ultrasound	Randomized controlled trial Four groups: VSL#3 plus lifestyle intervention (n=26; mean age of 12.06±1.76 years) VSL#3 (n=27; mean age of 11.7±2.21 years) Lifestyle intervention (n=26; mean age of 11.4±1.05 years) Placebo group (n=27l mean age of 11.0±1.20 years)	VSL#3*	16 weeks	VSL#3 plus lifestyle intervention resulted in decreased fatty liver grades, anthropometric and biochemical parameters and beneficially impacted upon obesity hormones compared with single VSL#3 therapy or lifestyle intervention alone.	<0.001	19/20

Jones *et al* ***(32)***	2018	USA	19 obese children with NAFLD (12-18yo) NAFLD diagnosed by abdominal MRI	Randomized, double-blind, placebo-controlled trial Intervention group (n=8; mean age of 14.4 (2.24) years) Placebo group (n=11; mean age of 14.9 (1.81) years)	VSL#3	16 weeks	Total adiposity and trunk adiposity significantly increased in intervention group. No significant effects on liver fat/fibrosis, insulin/glucose, gut microbiome or gut hormones.	<0.01	17/20
Famouri *et al* ***(30)***	2017	Iran	64 children with BMI >85th percentile NAFLD diagnosed by ultrasound	Randomized triple-blind, placebo-controlled trial Intervention group (n=32; mean age of 12.7 (2.2) years) Placebo group (n=32; mean age of 12.6 (1.7) years)	*Lactobacillus acidophilus* ATCC B3208; *Bifidobacterium lactis* DSMZ 32269; *Bifidobacterium bifidum* ATCC SD6576; *Lactobacillus rhamnosus* DSMZ 21690. + Lifestyle recommendations (daily activity,	12 weeks	WC, AST, ALT and cholesterol significantly decreased in the intervention group. Normalization of fatty liver in 53.1% of children in intervention group vs 16.5% in placebo group. No significant changes seen in between-group LDL or triglyceride levels.	<0.05 <0.05	20/20

					increase fruits and vegetables and decreases saturated fats and carbohydrates)				
Alisi *et al* ***(31)***	2014	Italy	44 children with NAFLD NAFLD diagnosed by elevated ALT, histopathology and ultrasound	Randomized double-blind, placebo-controlled trial Intervention group (n=22; median age of 10 (9-12) years) Placebo group (n=22; median age of 11 (10-12) years)	VSL#3 + Low-calorie diet (50–60% carbohydrate) ; 23–30% fat, 15–20% fatty acid: two-thirds saturated, one-third unsaturated protein) + Moderate aerobic exercise (30–45 min/d, ≥3x/week)	16 weeks	BMI significantly decreased while GLP-1 and aGLP-1 significantly increased in intervention group. No significant changes seen in ALT, HOMA or triglycerides. See main text for results on fatty liver changes.	<0.001	18/20
Vajro *et a*	2011	Italy	20 obese children	Double-blind, placebo-	*Lactobacillus rhamnosus GG*	8 weeks	ALT and PG-PS IgA significantly	0.03	18/20

(28)			with NAFLD NAFLD diagnosed by ultrasound and persisting hypertransaminasemia (ALT >40 U/L)	controlled trial Intervention group (n=10) Placebo group (n=10) Mean age of 10.7 ± 2.1 years			decreased in intervention group. No significant changes in liver ultrasound, anthropometric parameters or TNF-α.		

REVISTA ESPAÑOLA DE
ENFERMEDADES DIGESTIVAS
The Spanish Journal of Gastroenterology

Vajro *et a* ***(28)***									

NAFLD: Nonalcoholic fatty liver disease; BMI: Body mass index; MRI: Magnetic resonance imaging; ALT: Alanine aminotransferase; CAP: Controlled attenuation parameter; LSM: Liver stiffness measurement: LDL: Low-density lipoprotein; TNF-α: Tumor necrosis factor-alpha; CRP: C-reactive protein; TAS; Anti-oxidant status; AST: Aspartate aminotransaminase; HDL: high-density lipoprotein; GLP-1: glucagon-like peptide 1; aGLP-1: activated GLP-1; HOMA: Homoeostasis model assessment; antipeptidoglycan-polysaccharide antibodies (PG-PS IgA).

**Streptococcus thermophilus, bifidobacterial (B. breve, B. infantis, B. longum), Lactobacillus acidophilus, L. plantarum, L. paracasei,* and *L. delbrueckii* subsp*. Bulgaricus.*

Table 2. Registered clinical trials of probiotics for NAFLD in obese children (registered through ICTRP and Clinicaltrials.gov)

Location	Trial number	Recruitment status	Age (yr)	Target sample size (n)	Intervention	Protocol
New York, United Stated	NCT04671186	Recruiting	5-18	100	*Lactobacillus rhamnosus* strain GG (Culturelle®) 10 billion CFU/day Randomized, triple-blinded, placebo-controlled trial	One capsule oral daily (10 billion CFU/day) for 6 months
Salvador, Brazil	RBR-9n7kfw	Completed	10-19	28	*Lactobacillus acidophilus* LA-5® + *Bifidobacterium lactis* BB-12® (1 x 10^9 CFU each) vs 1 g of anhydrous cellulose Randomized, double-blinded	Sachets reconstituted in liquid; oral administration; 1x daily for 12 wk
Punjab, India **(29)**	CTRI/2017/12/010997	Completed	5-18	100	VSL#3 vs cornstarch Randomized, double-blinded	<10yo: oral capsule administration; 4x daily for 4 months ≥10yo: 8x daily for 4 months
Bangkok, Thailand	TCTR20170128001	Completed	6-18	40	Chicory inulin (2.24 gram/sachet) + *Lactobacillus*	Sachets reconstituted in

					acidophilus LA5 + *Bifidobacterium lactis* BB12 (≥1.5 Billion CFU) vs Maldextrin 2.3g Randomized, double-blinded	liquid; oral administration; 1x daily for 16 wk
Colombo, Sri Lanka	SLCTR/2016/021	Recruiting	5-15	170	Bio-Kult 14® * + structured diet + 60 mins of daily physical activity vs placebo + structured diet + 60 mins of daily physical activity Randomized, double-blinded	<12yo: oral capsule administration; 1x daily for 6 months ≥12yo: oral capsule administration; 2x daily for 6 months
Isfahan, Iran **(30)**	IRCT2013100414882N1	Completed	10-18	64	Prokids® ** vs placebo Randomized, double-blinded	Oral capsule administration; 1x daily for 12 wk

CFU: colony forming units.

**Bacillus subtilis, Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium longum, Lactobacillus acidophilus, Lactobacillus delbrueckii ssp. bulgaricus, Lactobacillus casei, Lactobacillus plantarum, Lactobacillus rhamnosus, Lactobacillus helveticus, Lactobacillus salivarius, Lactococcus lactis ssp. lactis, Streptococcus thermophilus.*

*** L. plantarum, L. acidophilus, B. infantis, B. lactis, Prebiotic Fructooligosaccharides.*

Figure 1. Prisma flow diagram.

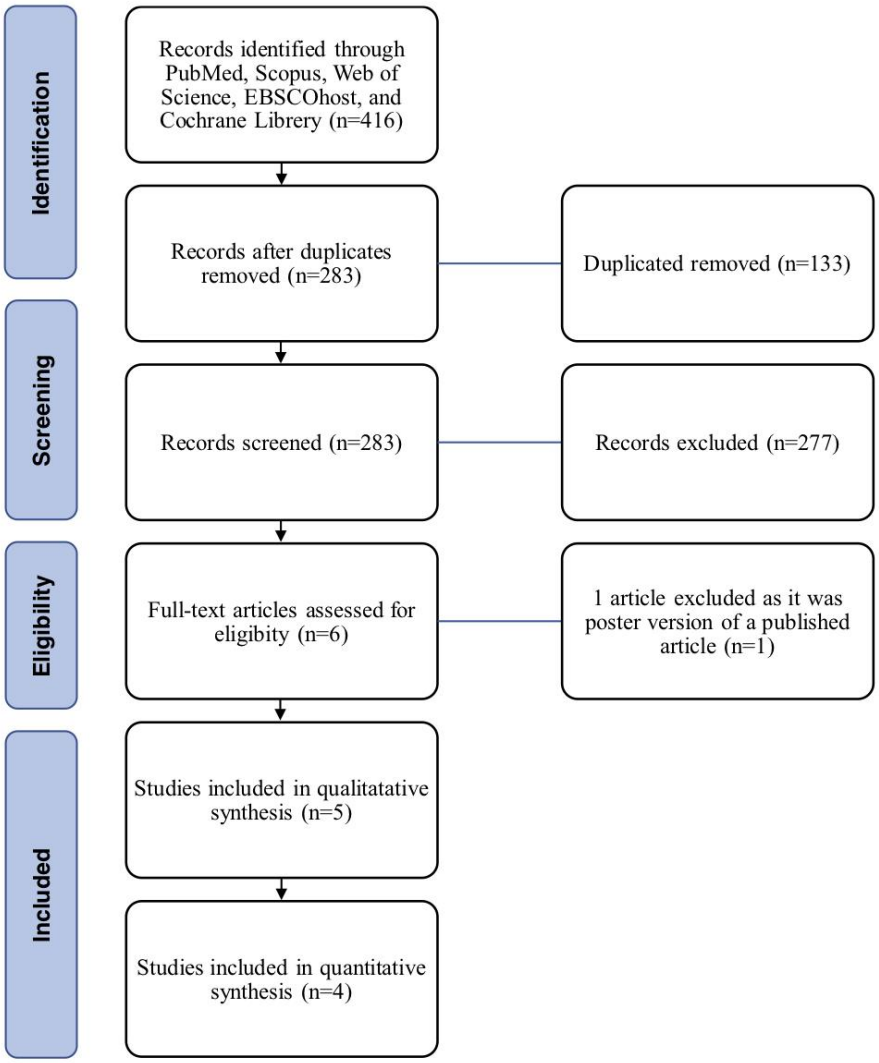
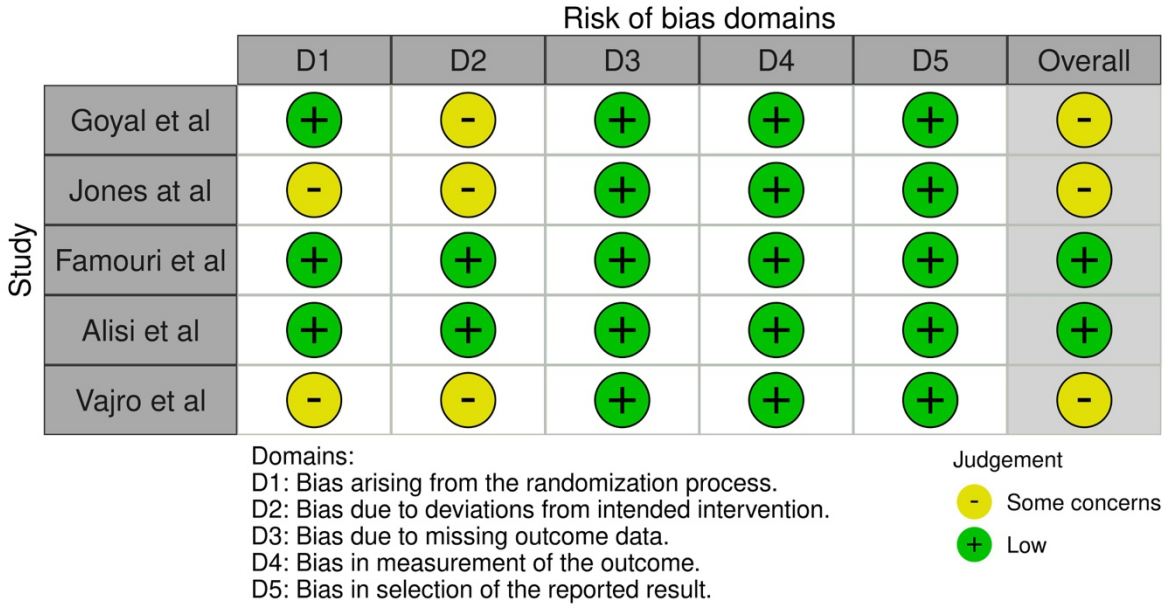
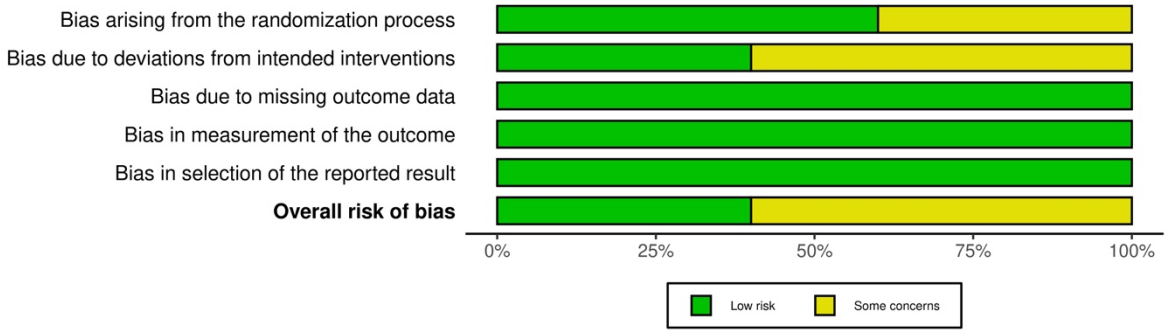


Figure 2. A. B. Risk of bias assessment.

A



B



REVISTA ESPAÑOLA DE
ENFERMEDADES DIGESTIVAS
The Spanish Journal of Gastroenterology

Figure 3. Funnel plot

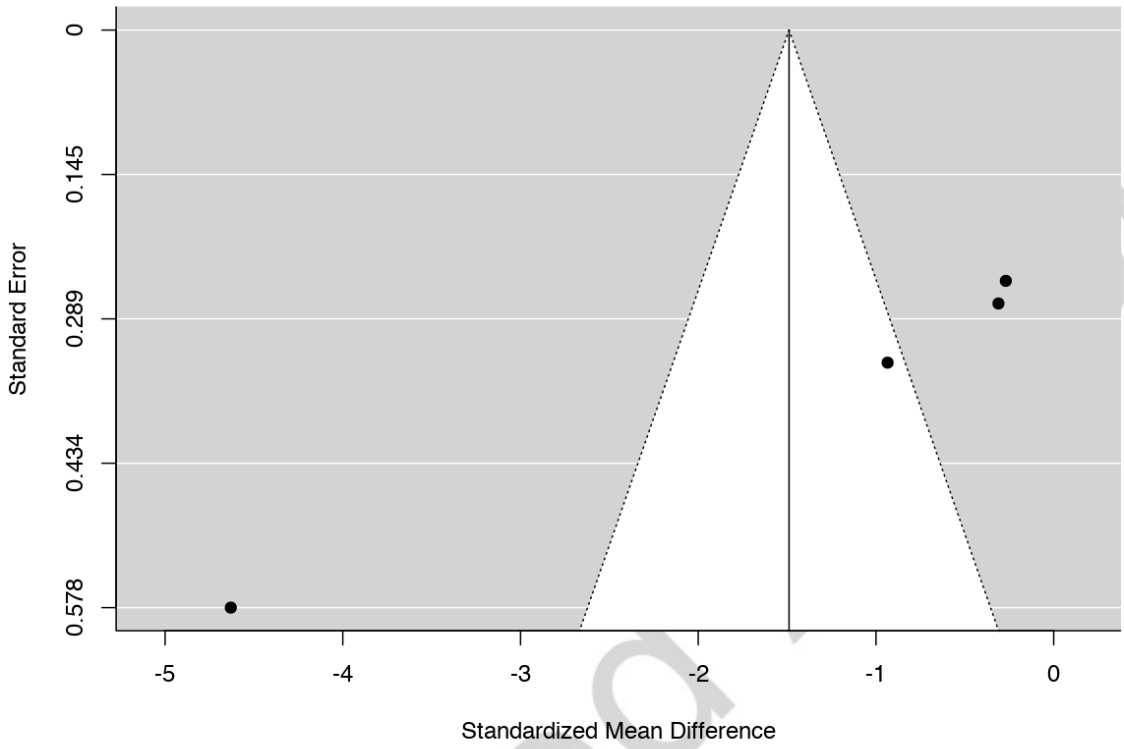


Figure 4. Forrest plot displaying the effect of probiotic therapy on ALT compared to placebo.