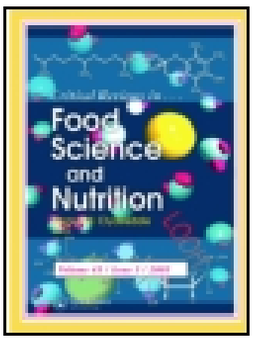

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Efficacy of Synbiotic Supplementation in Patients with Nonalcoholic Fatty Liver Disease: A Systematic Review and Meta-Analysis of Clinical Trials

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Abstract

Objective: We systematically reviewed available randomized clinical trials (RCTs) to elucidate the overall effects of synbiotic supplementation in patients with nonalcoholic fatty liver disease (NAFLD).

Methods: PubMed, Scopus, ISI Web of science and Google Scholar were searched up to December, 2017. All RCTs using synbiotic supplements to treat NAFLD included in this systematic review and meta-analysis. Mean Difference (MD) was pooled using a random-effects model.

Results: Eleven eligible databases from seven RCTs were identified for the present meta-analysis. Our results showed that synbiotic supplementation can decrease body weight, fasting blood sugar, insulin, low density lipoprotein cholesterol, total cholesterol, triglyceride, high-sensitivity C-reactive protein, tumor necrosis factor alpha, alanine transaminase and aspartate transaminase levels among patients with NAFLD. In contrast, synbiotic did not have favorable effects on body mass index (BMI), waist circumference, homeostasis model assessment for insulin resistance (HOMA-IR), and high density lipoprotein cholesterol (HDL) levels compared with the placebo group.

Conclusion: The current study revealed that synbiotic supplementation has favorable effect on inflammatory factors, liver enzymes and some anthropometric indices, lipid profiles and glucose homeostasis parameters in patients with NAFLD.

Keywords: Synbiotic, inflammation, insulin, NAFLD

Introduction

Nonalcoholic fatty liver disease (NAFLD) is a silent and progressive disorder associated with excessive deposition of fatty acids, within hepatocytes in the absence of significant alcohol consumption. NAFLD encompasses a broad spectrum of clinical presentations ranging from benign steatosis to hepatocellular carcinoma (HCC) (Loomba and Sanyal, 2013).

The prevalence of NAFLD is on the rise; recent estimated prevalence in developed and developing countries is approximately 20–30% and 10–20%, respectively (Younossi et al., 2017). On the other hand, given the alarmingly high prevalence of obesity, metabolic syndrome and type 2 diabetes mellitus (T2DM) worldwide, as the main risk factors of NAFLD, it is expected that NAFLD will become the commonest human affliction in the near future (Beltrán-Sánchez et al., 2013; Hales et al., 2017; Unnikrishnan et al., 2017).

Thus, a comprehensive care and treatment plan for controlling the mentioned dysfunction is required. In this regard, lifestyle modification, in particular diet, is considered as the first line and the best treatment strategy in NAFLD (Ghaemi et al., 2013). However, the difficulty in implementing and maintaining these lifestyle interventions in clinical practice in NAFLD patients is well documented (Suzuki et al., 2005). As a result, a convenient and effective adjunctive therapy with minimal side effect is needed.

Given the critical role of gut microbiota in the pathogenesis of various metabolic diseases (Entezari et al., 2017; Miraghajani et al., 2017b; Nasri, 2016), designing the intestinal microbiome modulation strategies to improve NAFLD can be considered as a new therapeutic option in these patients (Leung et al., 2016; Sekirov et al., 2010). In this context, probiotics, prebiotics and synbiotics have attracted a great deal of attention.

According to the Food and Agriculture Organization of the United States (FAO), probiotics defined as a culture of living microorganisms which could have health benefits for the host if

consumed in adequate amounts and duration (Miraghajani et al., 2017b). Prebiotics contain a group of fermentable dietary fibers that confer a health benefit on the host by stimulating the growth and survival of probiotics (Khalesi et al., 2017). Synbiotic refers to nutritional supplements combining probiotic and prebiotic in a form of synergism, hence synbiotic. Previous studies have reported that synbiotics have a potentially stronger effect in modulating the gut microbiota than either probiotics or prebiotics alone (Khalesi et al., 2017; sadat Ebrahimi et al., 2017; Tabrizi et al., 2017). A huge body of evidence suggests that synbiotic supplementation may improve lipid metabolism, insulin resistance, inflammatory mediators and liver enzymes markers by alternating the form and/or function of the gut microbiota (Ferolla et al., 2016; Khalesi et al., 2017; Mofidi et al., 2017; Tabrizi et al., 2017). However, existing evidence diverge on the possible effects of synbiotic supplementation in the clinical management of patients with NAFLD.

Although, several meta-analysis showed that probiotics significantly reduced the development of the NAFLD in adults and children (Gao et al., 2016; Lavekar et al., 2017; Ma et al., 2013), according to our search in databases, no systematic review or meta-analysis has performed regarding the separate role of synbiotic in this context. Therefore, present paper was conducted to collate and evaluate the overall effect of synbiotic supplementation on anthropometric indices, lipid profiles, glucose homeostasis parameters, inflammatory factors and liver enzymes in patients with NAFLD.

Methods

Search strategy

This systematic review and meta-analysis was performed based on the Preferred Reporting Item for Systematic Review and Meta-analysis (PRISMA) guideline. A keyword search was undertaken by one of the researchers (A.H) in four major electronic databases: PubMed

(http://www.ncbi.nlm.nih.gov), Scopus (http://www.scopus.com), ISI Web of science (http://www.web of science.com) and Google Scholar (http://scholar.google.com). All the above databases looked at from their respective inceptions to December 15, 2017. Our search strategy included all possible combinations of following keywords: 'synbiotic', 'symbiotic' and 'nonalcoholic fatty liver disease', 'NAFLD', 'fatty liver', 'nonalcoholic steatohepatitis' or 'NASH'. In order to locate additional studies that could have been missed during the electronic search, we inspected the reference lists of relevant articles by hand-scanning.

Study selection

After excluding the duplicates using EndNote ® X7 software (Thomson Reuters, New York, USA), in the first stage of the screening process, titles and abstracts of the remaining articles were assessed to select appropriate randomized controlled trials (RCTs). When the abstracts were not clear to decision the eligibility of articles, in the second stage, the full text were reviewed. Finally, all human RCTs that examined the effects of synbiotic supplements on NAFLD or NASH were included in this systematic review. We defined the followings as exclusion criteria: *1)* duplicated papers; *2)* studies that target participants were children or adolescents under 18 years; and *3)* synbiotic was used in combination with any other medication. All stages of the selection process were performed by two authors (A.H and H.M), independently. In this process, all differences in opinion were settled by face-to-face discussion.

Data extraction and quality assessment

To reduce human errors, the data extraction for each study was separately done by two investigators (A.H and H.M) using a pre-designed data collection check form. The data included the first author's name, study design, country and year of publications, sample size, participants' gender and mean age, doses of synbiotic administered, follow-up duration, type

and dose of intervention in the comparison group and main results. Trials were abstracted as a whole once if they had more than one publication. Two authors (A.H and H.M) independently and critically evaluated the quality of the selected articles using a validated scale for RCTs (Jadad scale) (Jadad et al., 1996). This 5-point quality scale included method of randomization concealment (0-2 points), blinding (0-2 points), and dropout rate (0-1 point). Clinical trials with score of 3 or more and 2 or less were considered as high and low quality studies, respectively. There was little disagreement between the two researchers over data extraction or quality assessment, which were resolved by discussion and consensus.

Statistical analysis

For each parameter we used mean (SD) at baseline and post-intervention in synbiotic and control groups. To calculate pooled effect size for anthropometric indices [body mass index (BMI), waist circumference (WC) and weight], lipid profile [high density lipoprotein (HDL-C), low density lipoprotein (LDL-C), total cholesterol (TC) and triglyceride (TG)], glycemic indices [fasting blood sugar (FBS) and homeostasis model assessment for insulin resistance (HOMA-IR)], and liver enzymes [alanine transaminase (ALT) and aspartate transaminase (AST)], we used random effects model. Also, Hedges's adjusted *g* was used to calculate pooled effect size for insulin, high-sensitivity C-reactive protein (hs-CRP) and tumor necrosis factor alpha (TNF-α). Between-study heterogeneity was evaluated using I-square (I^2) test. To find the potential sources of between-study heterogeneity, we carried out a pre-planned subgroup analysis based on baseline BMI (BMI ≥ 30 and BMI < 30), study duration (> 8 weeks and < 8 weeks) and lifestyle modification (with and without lifestyle modification). Heterogeneity between subgroups was evaluated using fixed-effect model. Proportion of each study in overall effect was assessed by sensitivity analysis. We used Begg's rank correlation test and Egger's regression asymmetry test to evaluate publication bias. Statistical analysis was performed using STATA 11.2 software (StataCorp, College Station, Texas, USA).

Results

Literature Search

The PRISMA flow diagram summarizes the results of study selection process (**Fig.1**). Electronic database searches identified 975 records, of which there were 542 records after removing duplicates. These records were screened by title/abstract, which 15 articles were identified for full-text assessment. Further assessment revealed that 4 records did not meet our inclusion criteria. Finally**,** 11 datasets from 7 trials were eligible for inclusion in this review (Asgharian et al., 2016; Asgharian et al., 2017; Ekhlasi et al., 2016; Ekhlasi et al., 2017; Eslamparast et al., 2013; Eslamparast et al., 2014; Ferolla et al., 2016; Javadi et al., 2017a, b; Malaguarnera et al., 2012; Mofidi et al., 2017). Characteristics of each study are illustrated in **Table 1**.

Study characteristics

These included trials were conducted between 2012 and 2017. In 5 and 2 trials, participants consisted of NAFLD (Asgharian et al., 2017; Ekhlasi et al., 2017; Eslamparast et al., 2014; Javadi et al., 2017a; Mofidi et al., 2017) and NASH (Ferolla et al., 2016; Malaguarnera et al., 2012) respectively at aged 42-57 in both genders. Five trials were conducted in Iran (Asgharian et al., 2017; Ekhlasi et al., 2017; Eslamparast et al., 2014; Javadi et al., 2017a; Mofidi et al., 2017) and the remaining 2 trials consisted of participants from Italy (Malaguarnera et al., 2012) and Brazil (Ferolla et al., 2016). All included studies were used parallel design. Trials used synbiotic supplements that contained 2×10^7 CFU - 5×10^9 bacterial strains and 125 mg- 10g prebiotic. In all trials, adverse effects of synbiotic supplements were not reported. The quality score of each clinical trial was assessed using a validated scale for RCTs (Jadad scale). According to Jadad score, all studies had high quality except, Ferolla's study (Ferolla et al., 2016) which had low quality.

The effects of synbiotic supplementation on anthropometric indices (BMI, WC and weight)

The effect of the synbiotic supplementation on BMI was examined in 6 clinical trials (Asgharian et al., 2017; Ekhlasi et al., 2017; Eslamparast et al., 2013; Ferolla et al., 2016; Javadi et al., 2017b; Malaguarnera et al., 2012). Overall, meta-analysis showed that there was no significant effect of the synbiotic supplementation on BMI compared to the placebo (MD: -0.08 kg/m^2; 95% CI: -0.30, 0.14, p= 0.46) (**Fig.2a**). There was no evidence of heterogeneity between the effect sizes of included studies (I^2=12.0%, p= 0.33). Findings from the sensitivity analysis revealed that the exclusion of Malguarnera's study (Malaguarnera et al., 2012) from the analysis alters the overall effect size (MD: -0.11 kg/m^2; 95% CI: -0.22, -0.003).

Four trials reported the data on WC (Asgharian et al., 2017; Ekhlasi et al., 2017; Eslamparast et al., 2013; Ferolla et al., 2016). Meta-analysis showed no significant effects of synbiotic supplementation on WC (MD: -0.96 cm; 95% CI: -2.63, 0.70, p= 0.25). There was a significant heterogeneity between studies (I^2 = 73.7%, p= 0.010). When the meta-analysis was subgrouped by study duration (**Fig.2b**), heterogeneity was attenuated in studies with > 8 weeks duration (I^2=0.0%, p= 0.33). However, there was not significant between-subgroup heterogeneity (p= 0.32). In addition, sensitivity analysis revealed that exclusion of Ekhlasi's study (Ekhlasi et al., 2017) from the analysis alters the overall effect size (MD: -1.84 cm; 95% CI: -3.12, -0.56).

Five trials assessed the effect of synbiotic supplementation on weight (Asgharian et al., 2017; Ekhlasi et al., 2017; Eslamparast et al., 2013; Ferolla et al., 2016; Javadi et al., 2017b). Pooled effect size indicated a significant reduction in weight after taking synbiotic in patients with NAFLD (MD: -2.98 kg; 95% CI: -3.78, -2.19, p <0.001) (**Fig.2c**). The effect was

homogenous across the included trials (I^2 = 0.0%, p= 0.44). Also, findings from the sensitivity analysis revealed that the exclusion of any single study from the analysis did not alter the overall effect.

No evidence of publication bias was found for weight (p= 0.62, Begg's test and p= 0.10, Egger's test), BMI (p=0.57, Begg's test and p= 0.71, Egger's test), and WC (p= 0.49. Begg's test and p= 0.19, Egger's test). The result of subgroup analysis on anthropometric indices are presented in **Table 2**.

The effects of synbiotic supplementation on glycemic indices (FBS, insulin, HOMA-IR)

The pooled mean difference of 7 datasets (Asgharian et al., 2017; Ekhlasi et al., 2016; Eslamparast et al., 2014; Ferolla et al., 2016; Javadi et al., 2017b; Malaguarnera et al., 2012; Mofidi et al., 2017) for the effects of synbiotic on FBS compared to the placebo group was (MD: -8.23 mg/dl; 95% CI: -14.06, -2.41, p= 0.006) with a maximum heterogeneity (I^2 = 83.7%, p< 0.001) (**Fig.3a**). When the meta-analysis was subgrouped by baseline BMI (**Fig.3a**), heterogeneity was attenuated in studies with baseline BMI ≥ 30 kg/m^2 (I^2=0.0%, P= 0.43). However, there was not significant between-subgroup heterogeneity (p= 0.49). Findings from the sensitivity analysis revealed that the exclusion of any single study from the analysis did not alter the overall effect.

Five trials reported the effect of synbiotic supplementation on insulin (Ekhlasi et al., 2016; Eslamparast et al., 2014; Javadi et al., 2017b; Malaguarnera et al., 2012; Mofidi et al., 2017). Pooled effect size indicated a significant reduction in fasting insulin after taking the synbiotic supplement in patients with NAFLD compared to the placebo (MD:-0.83 Hedges's; 95% CI: -1.54, -0.13, p< 0.02) (**Fig.3b**). The effect was not fairly consistent across the mentioned trials (I^2 = 84.0%, p< 0.001). When the meta-analysis was subgrouped based on studies with/without lifestyle modification, heterogeneity was attenuated in studies without lifestyle modification (I^2=59.5%, p= 0.11). There was a significant between-subgroup heterogeneity

(p< 0.001). To examine the effect of each study on the pooled effect size, sensitivity analysis showed that 3 clinical trials alter the overall effect size (MD: -0.89 Hedges's; 95% CI: -1.88, 0.08) (Malaguarnera et al., 2012), (MD: -0.52 Hedges's; 95% CI: -1.10, 0.05) (Ekhlasi et al., 2016) and (MD: -0.71 Hedges's; 95% CI: -1.53, 0.10) (Javadi et al., 2017b), respectively.

The effect of the synbiotic supplementation on HOMA-IR was examined in 5 clinical trials (Ekhlasi et al., 2016; Eslamparast et al., 2014; Javadi et al., 2017b; Malaguarnera et al., 2012; Mofidi et al., 2017). Pooled effect size revealed that synbiotic supplementation did not have a noticeable effect on the HOMA-IR (MD: -0.02.; 95% CI: -0.51, 0.46, p= 0.92), with high heterogeneity across studies (I^2 = 93.7%, p< 0.001) (**Fig.3c**). When the meta-analysis was subgrouped by baseline BMI (**Fig.3c**), heterogeneity was attenuated in the studies with BMI ≥ 30 kg/m^2 (I^2=0.0%, p= 0.57). There was a significant between-subgroup heterogeneity (p= 0.01). We identified a significant decrease in HOMA-IR in the studies that reported data among subjects with BMI ≥ 30 compared to BMI < 30 (MD: -0.48 vs. 0.22, respectively). Sensitivity analysis showed that the exclusion of Mofidi's study (Mofidi et al., 2017) from the analysis changed the overall effect (MD: -0.32 ; 95% CI: -0.61,-0.03).

Begg's and Egger's tests did not confirm the publication bias for FBS (p= 0.19, p= 0.33, respectively), insulin (p= 0.32, and p= 0.19 respectively), and HOMA-IR (p= 0.32, and p= 0.32, respectively). The result of subgroup analysis on glycemic indices are presented in **Table 2**.

The effects of synbiotic supplementation on lipid profile (HDL, LDL, TC, and TG)

Six trials reported the effect of synbiotic on HDL (Asgharian et al., 2017; Ekhlasi et al., 2016; Ferolla et al., 2016; Javadi et al., 2017b; Malaguarnera et al., 2012; Mofidi et al., 2017). Meta-analysis showed that synbiotic supplementation could not result in increasing HDL level (MD: 1.54 mg/dl; 95% CI: -1.29, 4.36; I^2= 57.1%, p= 0.04) (**Fig.4a**). However, in

subgroup analysis, we found a significant increase in HDL in studies with > 8 weeks duration (**Fig.4a**). In addition, sensitivity analysis revealed that Asgharian's study (Asgharian et al., 2017) significantly affected the summary effects (MD: 2.59 mg/dl; 95% CI: 0.48,4.90).

The effect of the synbiotic on LDL was examined in 6 clinical trials (Asgharian et al., 2017; Ekhlasi et al., 2016; Ferolla et al., 2016; Javadi et al., 2017b; Malaguarnera et al., 2012; Mofidi et al., 2017). Meta-analysis revealed a significant effect of the synbiotic supplementation on LDL (MD: -17.01 mg/dl; 95% CI: -20.50, -13.52, p< 0.001) (**Fig.4b**). There was a significant heterogeneity between studies (I^2=75.6%, p= 0.001). When the meta-analysis was subgrouped by baseline BMI (**Fig.4b**), heterogeneity was attenuated in studies with BMI < 30 kg/m^2 (I^2=20.6%, p= 0.28). There was a significant between-subgroup heterogeneity (p= 0.01). Sensitivity analysis showed that no particular study prominently affected the overall effects.

Pooled effect size of 6 datasets (Asgharian et al., 2017; Ekhlasi et al., 2016; Ferolla et al., 2016; Javadi et al., 2017b; Malaguarnera et al., 2012; Mofidi et al., 2017) reported a significant effect of the synbiotic supplementation on TC (MD: -17.81 mg/dl; 95% CI: -25.11, -10.50, p< 0.001) (**Fig.4c**), but the trials were substantially heterogeneous (I^2= 53.1%, p= 0.058). Subgroup analysis based on baseline BMI attenuates the heterogeneity in BMI < 30 kg/m^2 category (I^2=17.3%, p= 0.30). There was a significant between-subgroup heterogeneity (p= 0.04). Based on sensitivity analysis, no one trial modified the pooled effect size.

The pooled mean difference of 6 datasets for the effects of synbiotic on TG was (MD: -22.85 mg/dl; 95% CI: -33.01, -12.70, p< 0.001), with no heterogeneity (I^2= 0.0%, p= 0.941) (**Fig.4d**). Also, findings from the sensitivity analysis revealed that the exclusion of any single study from the analysis did not alter the overall effect.

No evidence of publication bias was found for HDL (p= 0.85, Begg's test and p= 0.79, Egger's test), LDL (p=0.85, Begg's test and p=0.79, Egger's test), TC (p= 0.85, Begg's test and p= 0.97, Egger's test), and TG (p =0.57, Begg's test and p= 0.75, Egger's test). The result of subgroup analysis on lipid profile are presented in **Table 2**.

The effects of synbiotic supplementation on inflammatory factors (hs-CRP and TNF-α)

The quantitative analysis of hs-CRP values (4 trials) (Asgharian et al., 2016; Eslamparast et al., 2014; Malaguarnera et al., 2012; Mofidi et al., 2017) indicated a significant reduction in hs-CRP in the synbiotic group compared to the placebo group (MD: -0.58 Hedges's; 95% CI: -1.01, -0.14, p= 0.01) with high heterogeneity across studies (I^2 = 62.7%, p= 0.045) (**Fig.5a**). We could not perform a subgroup analysis to assess the source of heterogeneity as there were few studies for comparisons. Sensitivity analysis showed inferences might not depend on a particular study.

Furthermore, meta-analysis analysis on 4 datasets (Ekhlasi et al., 2017; Eslamparast et al., 2014; Malaguarnera et al., 2012; Mofidi et al., 2017) illustrated that synbiotic supplementation has a noticeable effect on the reduction of TNF-α level (MD: -1.12 Hedges's; 95% CI: -1.98, -0.27, p= 0.01) with high heterogeneity across studies (I^2 = 86.1%, p< 0.001) (**Fig.5b**). Due to few studies in this field, subgroup analysis was not performed. We performed a sensitivity analysis and found that the exclusion of Malguarnera's (Malaguarnera et al., 2012) (MD: -0.95 ; 95% CI: -2.01,0.09) and Ekhlasi's studies (Ekhlasi et al., 2017) (MD: -0.78 ; 95% CI: -1.61,0.04) from the analysis changed the overall effect.

No evidence of publication bias for hs-CRP (p= 0.58, Begg's test and p= 1.00, Egger's test), and TNF-α (p= 1.00, Begg's test and p= 0.47, Egger's test) was seen. The result of subgroup analysis on inflammatory factors are presented in **Table 2**.

The effects of synbiotic supplementation on liver enzymes (ALT and AST)

Seven datasets evaluated the effect of the synbiotic supplementation on ALT level among NAFLD patients (Asgharian et al., 2016; Ekhlasi et al., 2017; Eslamparast et al., 2014; Ferolla et al., 2016; Javadi et al., 2017a; Malaguarnera et al., 2012; Mofidi et al., 2017). Overall, there was a significant reduction in the mean difference of ALT in the synbiotic group compared to the placebo group (MD: -10.78 IU/L; 95% CI: -15.59, -5.96, p< 0.001) (**Fig.6a**). High heterogeneity across studies was seen (I^2= 88.5%, p< 0.001). Although, the influence of several known subgroups on pooled effect size was assessed, subgrouping based on baseline BMI resulted in reduced heterogeneity in BMI ≥30 kg/m^2 category (I^2= 62.9%, p= 0.06). There was a significant between-subgroup heterogeneity (p<0.001). Sensitivity analysis showed that no particular study prominently affected the overall effects. Begg's (p = 0.88) and Egger's test (p= 0.35) suggested no publication bias.

Consistently, a forest plot of 7 datasets (Asgharian et al., 2016; Ekhlasi et al., 2017; Eslamparast et al., 2014; Ferolla et al., 2016; Javadi et al., 2017a; Malaguarnera et al., 2012; Mofidi et al., 2017) indicated a significant reduction in AST level after taking synbiotic supplement compared to the placebo (MD: -12.20 IU/L; 95% CI: -18.03, -6.37, p<0.001) (**Fig.6b**). This finding was not consistent between the trials (I^2 = 92.6%, p< 0.001). The effects of lifestyle modification on AST were evaluated by subgroup analysis. Although heterogeneity was attenuated in the studies without-lifestyle modification (I^2 = 40.8%, p= 0.185), there was not a significant between-subgroup heterogeneity (p= 0.71). Findings from the sensitivity analysis showed that the exclusion of any single study from the analysis did not change the overall effect. Begg's (p= 0.88) and Egger's test (p= 0.43) did not report the publication bias. The result of subgroup analysis on liver enzymes are presented in **Table 2**.

Discussion

Historical evidence spanning eight decades has suggested that an imbalance between the gut microbiome could be involved in the liver damage from early life into adulthood. Although, the pathogenesis and mechanisms underlying NAFLD development are complex, manipulation of the bacteria in the GI system to ensure a non-dysbiotic state, decreased abundance of beneficial bacteria with increased prevalence of pathogenic bacteria, offers novel therapeutic for patients with NAFLD (Houghton et al., 2016).

Current evidence suggests that combinations of pre and probiotic (synbiotic), provide synergistic and effective therapies for NAFLD. Prebiotics can enhance the proliferation of probiotics, to maximize sustainable changes in the human microbiome. Therefore, synbiotic supplementation might improve some indices related to NAFLD by alternating the form and/or function of the gut microbiota.

To the best of our knowledge, no meta-analysis study evaluates effects of synbiotic supplementation on anthropometric indices, lipid profiles, glucose homeostasis parameters, inflammatory factors and liver enzymes among patients with NAFLD. As, existing evidence diverge in this field, the ultimate goal of this review was to determine whether synbiotic supplementation could be recommended as a public health policy to improve NAFLD.

The present meta-analysis of 11 databases from 7 randomized trials indicated that supplementation with synbiotic can decrease body weight, FBS, insulin, LDL, TC, TG, hs-CRP, TNF-α, ALT, and AST levels among patients with NAFLD. However, synbiotic did not have favorite effects on BMI, WC, HOMA, and HDL levels compared with the placebo group. The results of this meta-analysis were in line with the previous review that assessed the inulin-type fructans, galacto-oligosaccharides and related synbiotics on inflammatory markers. This finding revealed that mentioned supplementation was effective on some

inflammatory markers including hs-CRP in adult with overweight or obesity (Fernandes et al., 2017).

Also, in a previous meta-analysis, data from randomized trials was pooled and found synbiotic consumption significantly improves FBS, insulin, HOMA-IR, homeostatic model assessment-B cell function (HOMA-B), quantitative insulin sensitivity check index (QUICKI), TG, and TC in patients with diabetes but have no effect on LDL and HDL concentrations (Tabrizi et al., 2017). This finding is consistent with the findings of previous meta-analysis that showed probiotic consumption, significantly reduced FBS, fasting plasma insulin and HOMA-IR (Ruan et al., 2015). Another synthesis of the results of the trials shows that probiotic consumption significantly reduced body weight and BMI (Zhang et al., 2016). Evaluation of another relevant literature supported the favorite effects of probiotic combined with Metformin on ALT compare to metformin alone in patients with NASH (Shavakhi et al., 2013).

It should be mentioned that the greater effects following supplementation with synbiotic was found after the subgroup analysis of trials based on the baseline BMI, intervention duration, and lifestyle modification on NAFLD related factors. The effectiveness of lifestyle changes is unprecedented with improvements in metabolic control among patients with NAFLD/ NASH. Other review studies noted that pre/probiotics intake accompanied by diet modifications and exercise play a significant role in the function and diversity of the gut microflora leading to the positive effects on NAFLD (Ghaemi et al., 2013; Houghton et al., 2016; Mokhtari et al., 2017, Shavakhi et al., 2013).

The mechanisms explored by which pre/pro/synbiotics may have a favorable effect on NAFLD are diverse. Development of gut dysbiosis which are seen in NAFLD lead to 1) inhibition of fasting-induced adipocyte factor (FIAF), which increases the activity of

lipoprotein lipase (LPL) and lipogenesis; 2) increasing LPS resulting in the induced inflammatory factors and hepatic injury following the activation of NF-kB pathway; 3) enhancement in polysaccharide absorption leading to the production of SCFAs and hepatic lipogenesis; and 4) conversion of choline into methylamines, which reduces choline availability and induces fat accumulation and reactive oxygen species production in the liver (Mokhtari et al., 2017). Also, dysbiosis could increase gut wall permeability, allowing bacterial translocation and uptake of endotoxin inducing metabolic dysfunctions in NAFLD (Ardalan and Nasri, 2014; Woodhouse et al., 2017). It is important to note that improvement in the inflammation, oxidative stress, insulin resistance, glucose intolerance, serum lipids, hepatic enzymes level, and hepatic lipid deposition through modulating gut microbiota and up-regulated genes related to fatty acid oxidation in both the liver and adipose tissue, delaying the progression of NAFLD via LPS/TLR4 signaling pathway and influencing protein expression and decreasing steatohepatitis are caused by probiotic intake. Similarly, prebiotic intake has effects on lowering LPS and cytokine levels, decreasing the hepatic expression of inflammatory and oxidative stress markers, serum ALT, AST, weight gain and insulin level through restoring normal gastrointestinal microflora and intestinal epithelial barrier function (Ma et al., 2017; Rafieian-Kopaei and Baradaran, 2012).

The small divergence in the results described here is potentially due to differences in the probiotic strain, dosages, study duration, ethnic origin, and dietary context which may affect the proliferation and survival of probiotics, prior pre/pro/ synbiotic intake, and the timing of exposure (Miraghajani et al., 2017a; Miraghajani et al., 2017b). Also, the diversified dysbiosis among NAFLD patients may contribute to the variation of therapeutic outcomes (Houghton et al., 2016).

The present meta-analysis had several limitations. The effects of confounding variables including, genetic background and duration of NAFLD on the efficacy of synbiotic

supplements remained unclear. Also, the number and type of probiotic microorganisms and multiple used methods and Eliza kits for measuring variables affected the clinical response.

The strength of the current study was the subgroup analysis and assessment of the baseline BMI, intervention duration, and lifestyle modification on the overall effect sizes. Moreover, existence of homogeneity for body weight, BMI and TG values were reported. In addition, we tried to minimize any biases in the review process by performing a comprehensive search of the literature and also by conducting and reporting the review by adhering to the PRISMA guidelines.

Implications for practice:

The evidence from this meta-analysis suggests that giving synbiotic supplements to NAFLD patients may have beneficial effects on some metabolic and anthropometric indices. However, a particular concern about using the synbiotic/probiotic products in patients with serious medical conditions, including human immunodeficiency virus (HIV) infection should be considered (Guinane, 2013; Haghighat and Crum-Cianflone, 2016; Trinchieri et al., 2017). So, synbiotics supplementation in NAFLD/NASH patients with HIV infection leading to infections might not be a reasonable option.

Implications for research:

Given that the currently available randomized trial data are heterogeneous, there may be needed the appropriately powered randomized trials with sufficient follow-up periods to prove the long-term clinical benefit of synbiotic supplementation in NAFLD patients. Furthermore, for clinical practice; it seems that cost-effective of synbiotic supplementation should be assessed.

Conclusion

Based on our findings, synbiotic supplements that contained 2×10^7 CFU - 5×10^9 bacterial strains and 10 g- 125 mg prebiotic for 8-28 weeks was suggested for NAFLD patients. Although, supplementation can decrease body weight, FBS, insulin, LDL, TC, TG, hs-CRP, TNF-α, ALT, and AST levels among mentioned patients, our analysis of the evidence agrees that there is currently insufficient evidence to make firm conclusions about the effects of supplementation.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

None.

Author Contribution

H.M and A.H contributed to the conception of research. H.M and A.H searched databases, screened articles and extracted data. H.M and M.M performed statistical analysis; EG contributed to revision of manuscript, and all authors contributed to the writing and revision of the manuscript.

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Legends of figures:

Figure 1. PRISMA flow diagram of study selection process

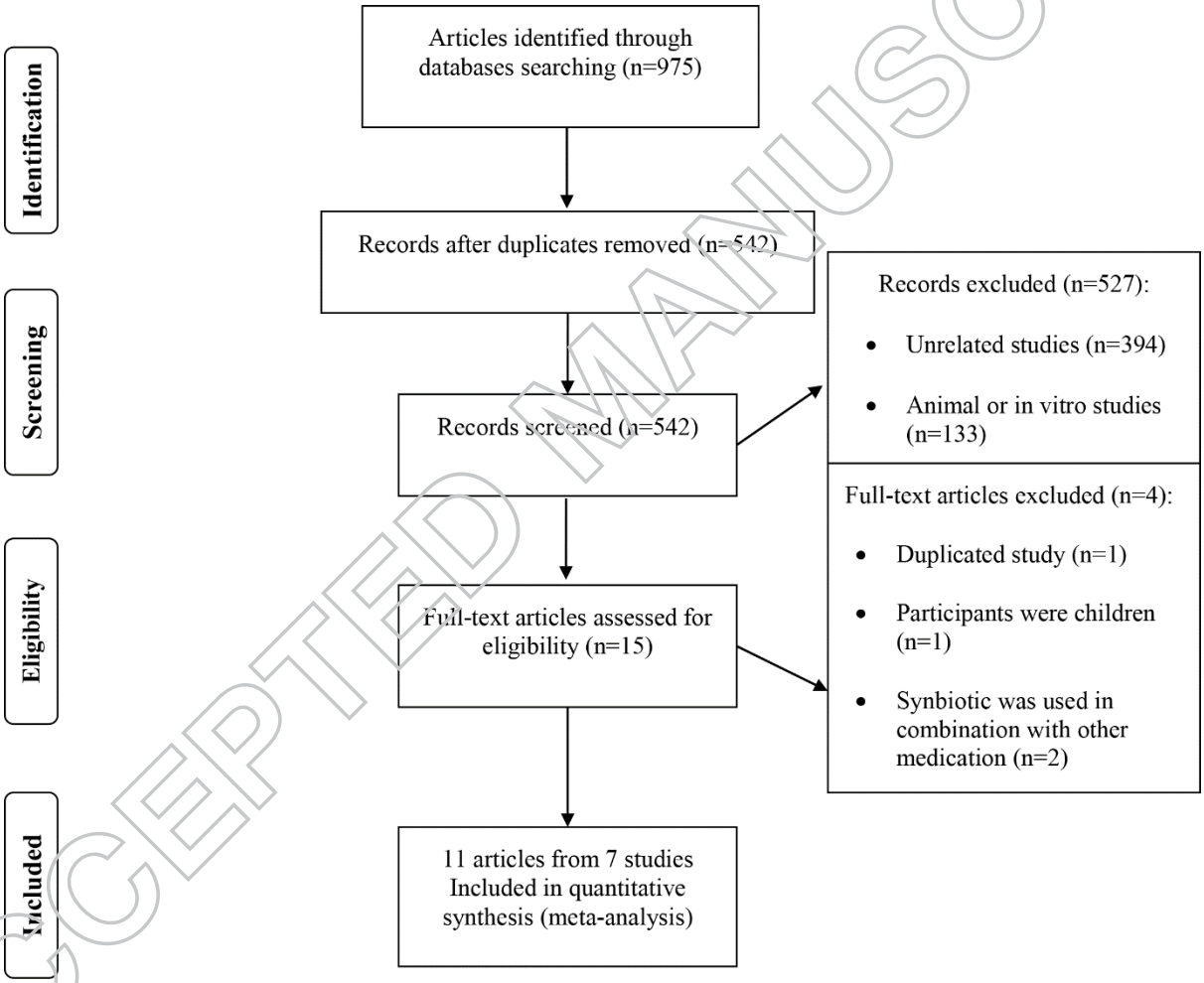
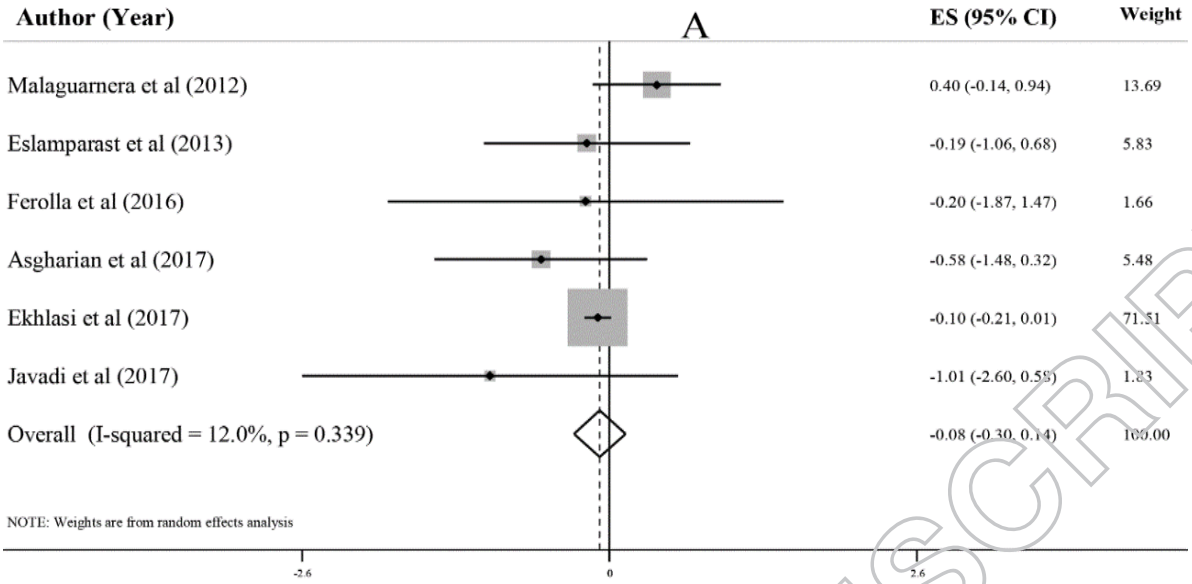
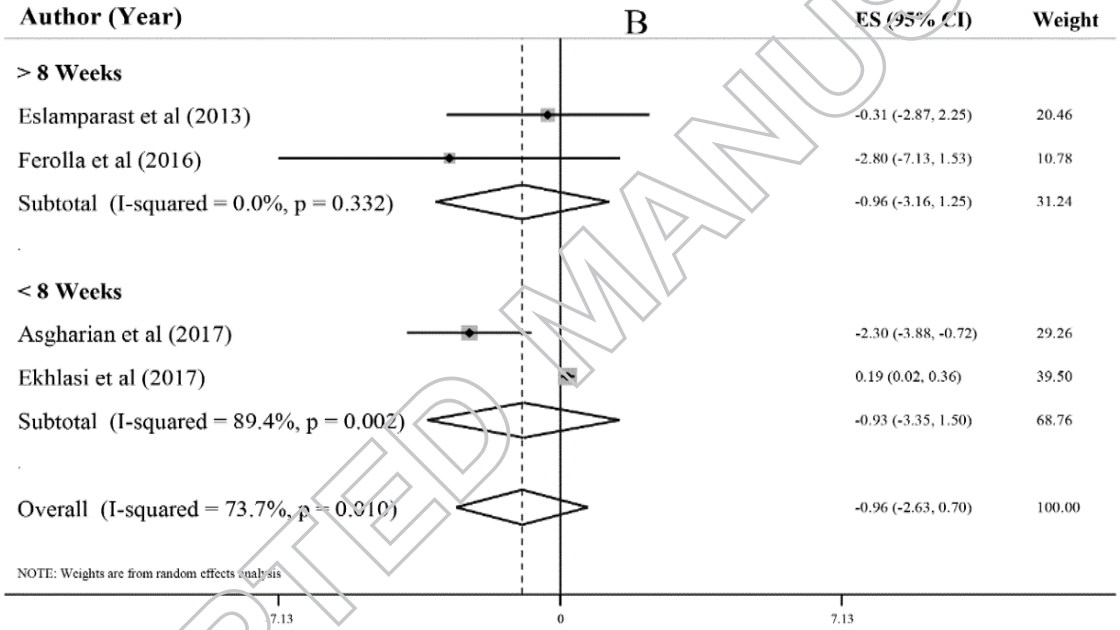


Figure 2. Forest plot of the effect of synbiotic supplementation on anthropometric indices (A: BMI; B: waist circumference; C: weight)





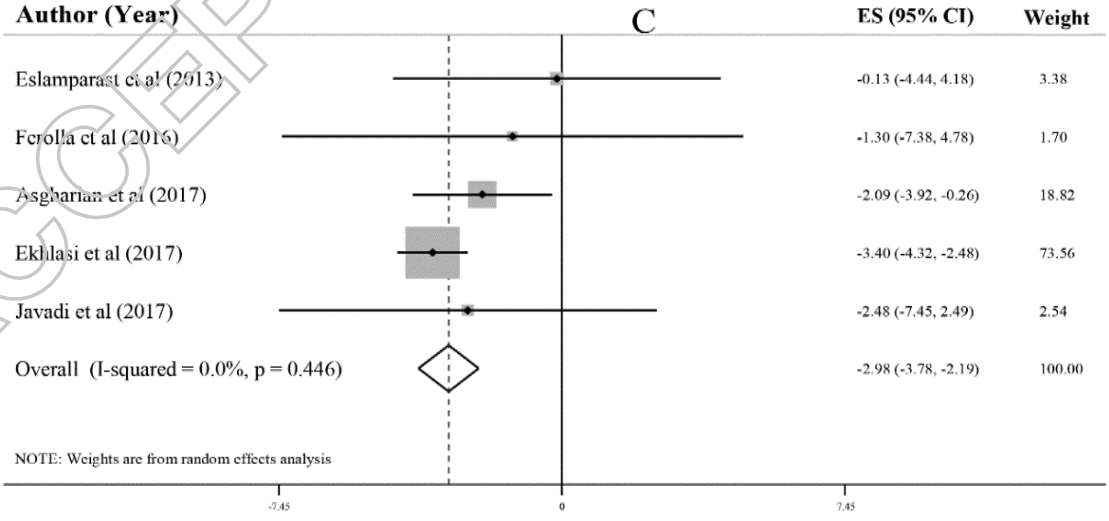
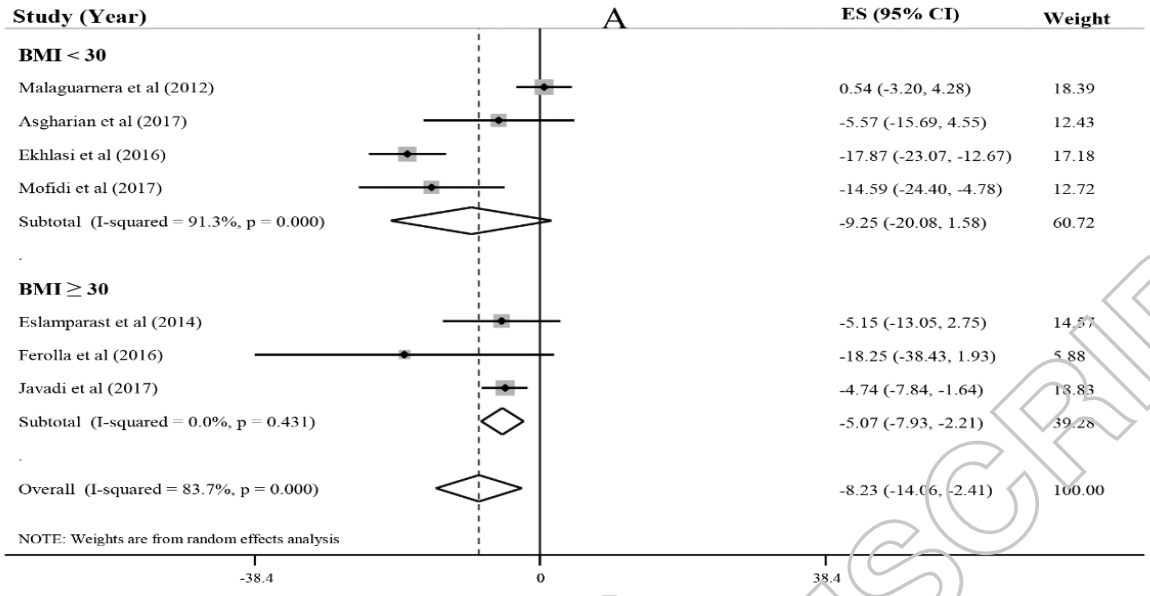
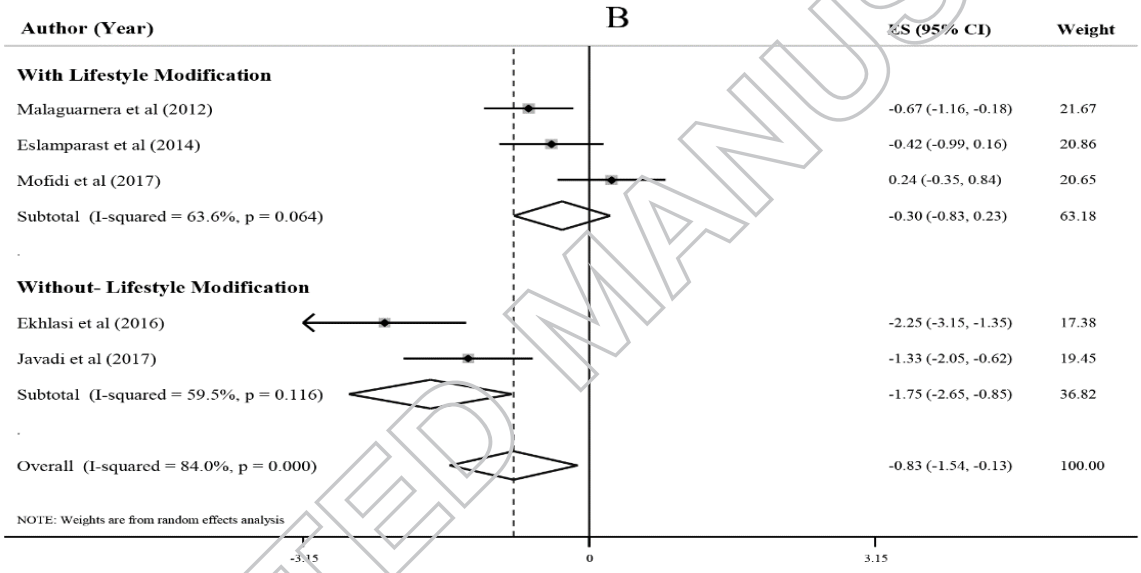


Figure 3. Forest plot of the effect of synbiotic supplementation on glycemic indices (A: FBS; B: insulin; C: HOMA-IR)





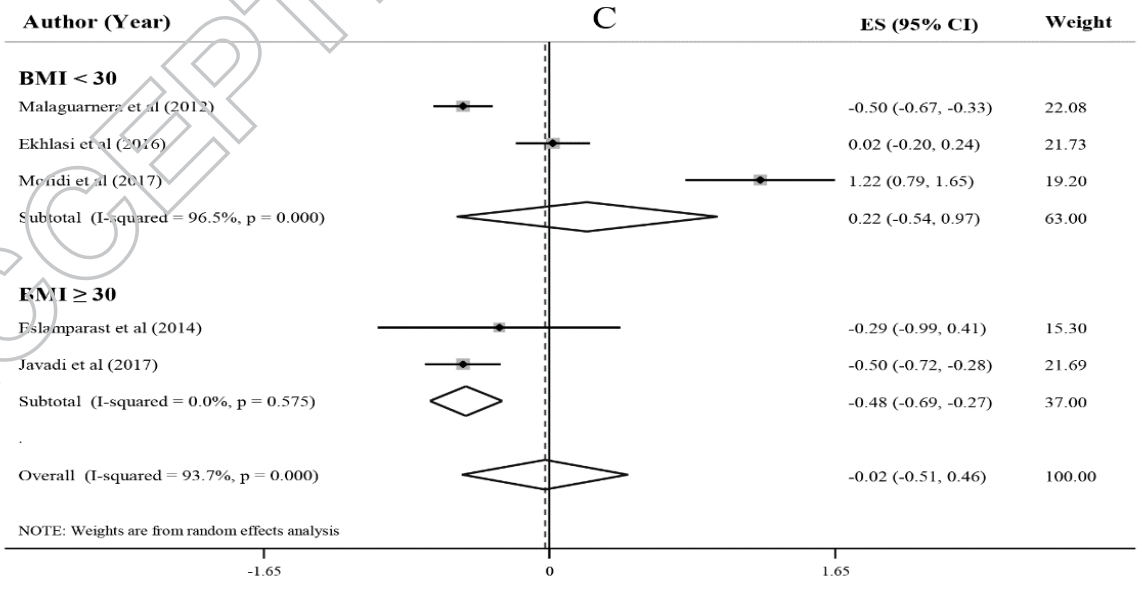


Figure 4. Forest plot of the effect of synbiotic supplementation on lipid profile (A: HDL-C; B: LDL-C; C: total cholesterol; D: triglyceride)

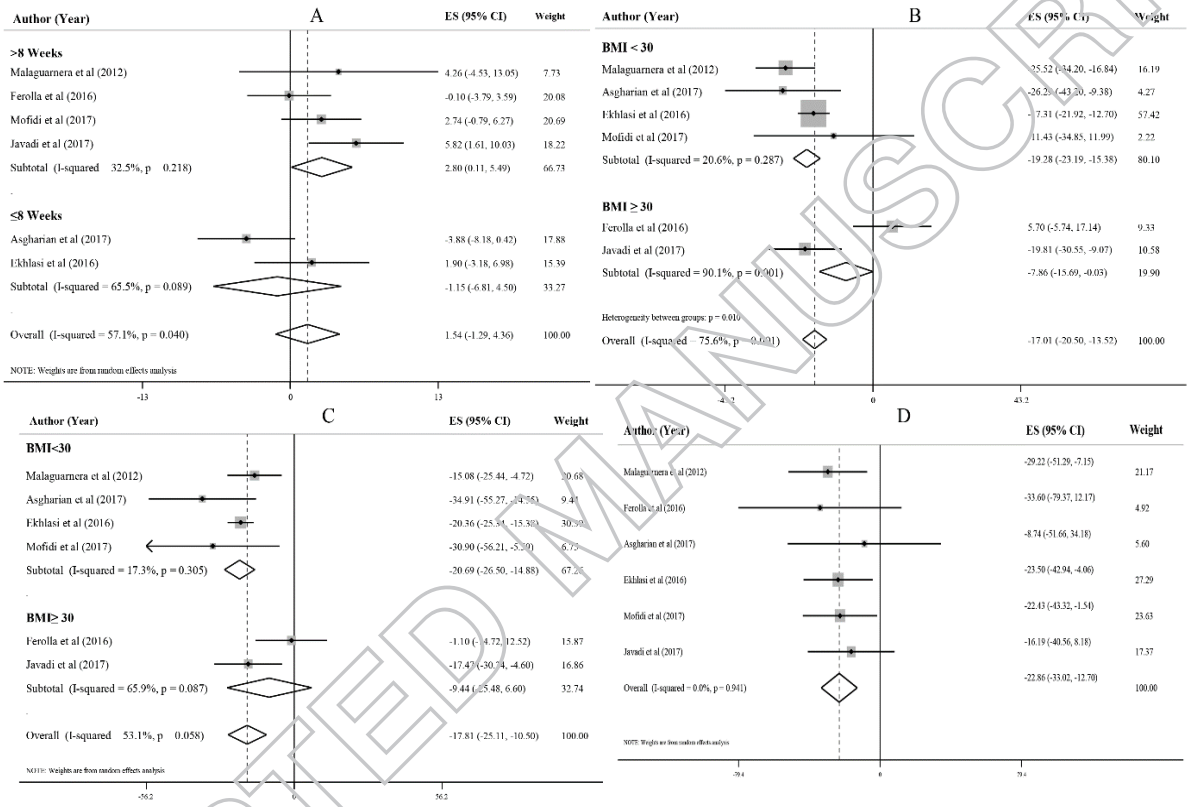


Figure 5. Forest plot of the effect of synbiotic supplementation on inflammatory indices (A: hs-CRP; B: TNF-α)

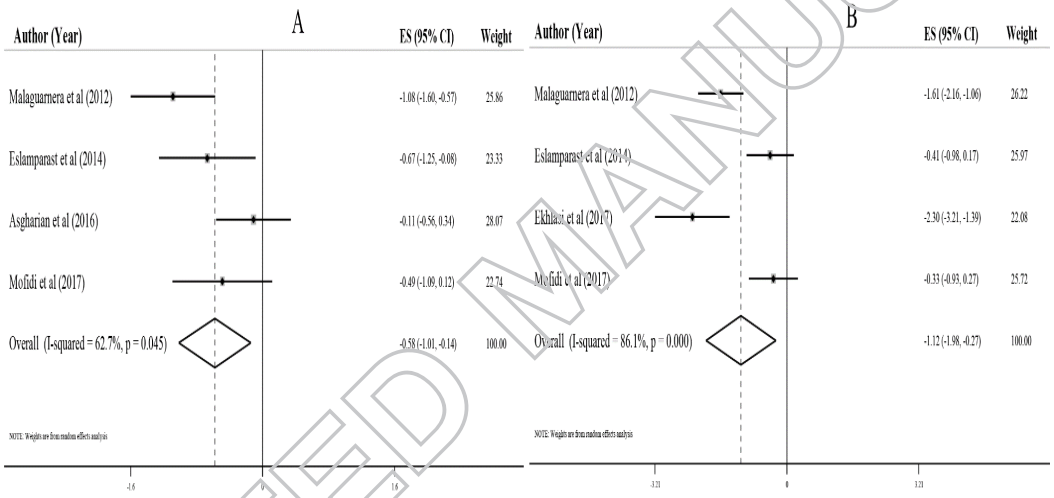
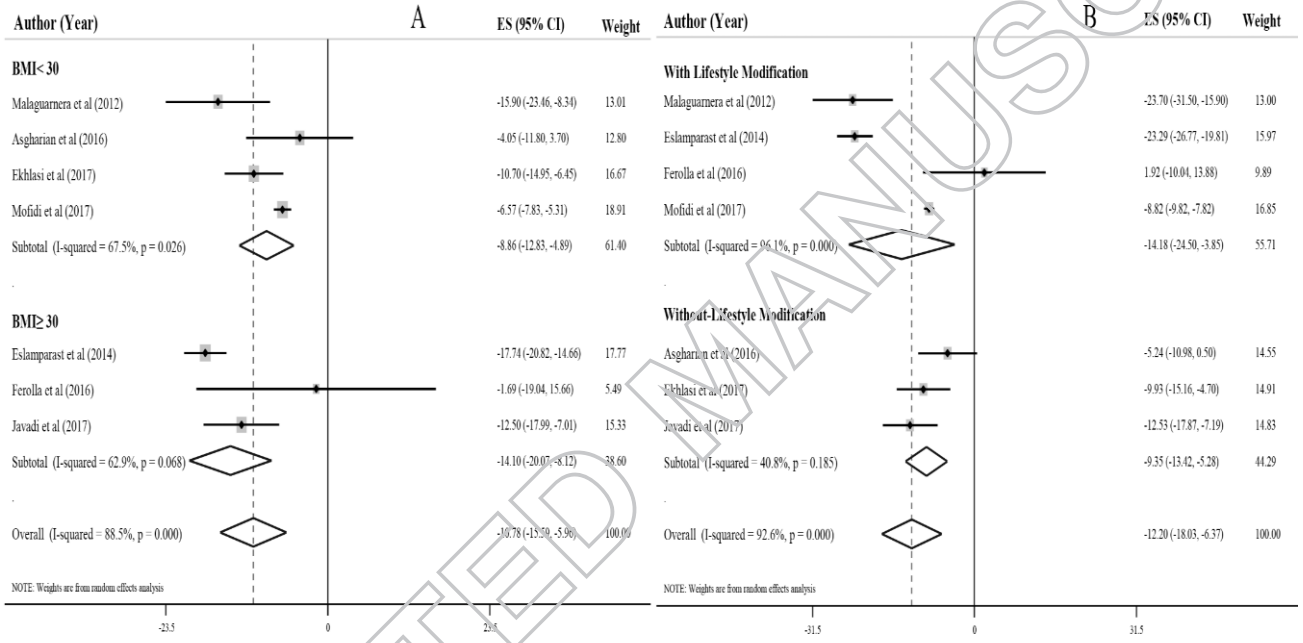


Figure 6. Forest plot of the effect of synbiotic supplementation on liver enzymes (A: ALT; B: AST)



First author (publication year)	Country	Total Sample size (M/F)	Target Population	Age (mean)	RCT design (Blinding)	Duration (wks)	Intervention of experimental group	Intervention of control group	Investigated outcomes	Jaded score
Malaguarnera et al. (2012)	Italy	33M/33F	NASH	46	Parallel (Yes)	24	5×10^9 CFU B. longum + 2.5 g FOS	Placebo (starch)	BMI, FBS, Insulin, HOMA-IR, TAG, TC, LDL-C, HDL-C, ALT, AST, CRP, TNF α	4
Eslamparast et al (2014)	Iran	25M/27F	NAFLD	46	Parallel (Yes)	28	4×10^8 CFU of 7 bacterial strains (L. casei, L. rhamnosus, S. thermophilus, B. breve, L. acidophilus, B. longum, L. bulgaricus) + 197 mg FOS	Placebo (MDX)	Weight, BMI, WC, FBS, insulin, HOMA-IR, ALT, AST, CRP, TNF α	5
Ferolla et al (2016)	Brazil	50.NR	NASH	57	Parallel (NO)	13	1×10^8 CFU L. reuteri + 4 g inulin and guar gum	ND	Weight, BMI, WC, FBS, TAG, TC, LDL-C, HDL-C, ALT, AST	2
Asgharian et al	Iran	19M/55F	NAFLD	46	Parallel	8	1×10^9 CFU of 7 bacterial	Placebo (starch)	Weight, BMI, WC,	5

(2017)					(Yes)		strains (L casei, L. acidophilus, L. rhamnosus, L. bulgaricus, B. breve, B. longum, S. thermophiles) + FOS (dose NS)]		FBS, TAG, TC, LDL-C, HDL-C, ALT, AST, CRP	
Ekhlasi et al (2017)	Iran	48M/12F	NAFLD	57	Parallel (Yes)	8	2×10^8 CFU of 7 bacterial strains (L. casei, L. rhamnosus, S. thermophilus, B. breve, L. acidophilus, B. longum, L. bulgaricus) + FOS (dose NS)	Placebo (corn starch)	Weight, BMI, WC, FBS, Insulin, HOMA-IR, TAG, TC, LDL-C, HDL-C, ALT, AST, TNF α	5
Mofidi et al (2017)	Iran	23M/19F	NAFLD	54	Parallel (Yes)	28	4×10^8 CFU of 7 bacterial strains (L. casei, L. rhamnosus, S. thermophilus, B. breve, L. acidophilus, B. longum, L. bulgaricus) + 125 mg	Placebo (MDX)	FBS, Insulin, HOMA-IR, TAG, TC, LDL-C, HDL-C, ALT, AST, CRP, TNF α	4

							FOS			
Javadi et al (2017)	Iran	60M/15F	NAFLD	42	Parallel (Yes)	13	2×10^7 CFU of 2 bacterial strains (Bifidobacterium longum and Lactobacillus acidophilus) + 10 g inulin	Placebo (MDX)	Weight, BMI, FBS, Insulin, HOMA-IR, TAG, TC, LDL-C, HDL-C, ALT, AST	5

Table 1. Characteristics of included trials

Abbreviations: RCT, randomized controlled trial; NAFLD, Non-alcoholic Fatty Liver Disease; NASH, Nonalcoholic steatohepatitis; CFU, colony-forming unit; FOS, fructo-oligosaccharides; ND: not determined; MDX, maltodextrin; BMI, body mass index; WC, weight circumference; FBS, fasting blood sugar; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC; total cholesterol; TAG, triacylglyceride; HOMA-IR, homeostasis model assessment for insulin resistance; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; TNF α; tumor necrosis factor α.

Table 2. Subgroup analysis to assess the effect of synbiotic supplementation on anthropometric indices and metabolic profiles of subjects with NAFLD

Subgrouped by	**No. of trials**	**Effect size[1]**	**95% CI**	**I^2 (%)**	**P for heterogeneity**	**P for between subgroup heterogeneity**
BMI						
Baseline BMI						0.46
≥ 30	3	-0.029	-0.42, 0.36	53.7	0.11	
< 30	3	-0.348	-1.04, 0.34	0.0	0.66	
Lifestyle Modification						0.17
With-lifestyle Modification	3	0.205	-0.23, 0.64	0.0	0.46	
Without-lifestyle Modification	3	-0.183	-0.48, 0.12	13.4	0.31	
Duration						0.31
>8 Weeks	4	0.047	-0.46, 0.56	16.7	0.30	
≤8 Weeks	2	-0.124	-0.32, 0.08	7.1	0.29	
WC						
Baseline BMI						0.321
≥ 30	2	-0.956	-3.16, 1.24	0.0	0.33	
< 30	2	-0.926	-3.35,	89.4	0.002	

			1.50			
Lifestyle Modification						0.321
With-lifestyle Modification	2	-0.956	-3.16, 1.24	0.0	0.33	
Without-lifestyle Modification	2	-0.926	-3.35, 1.50	89.4	0.002	
Weight						
Baseline BMI						0.19
≥ 30	3	-1.175	-4.04, 1.69	0.0	0.78	
< 30	2	-2.992	-4.18, -1.80	36.4	0.21	
Lifestyle Modification						0.15
With-lifestyle Modification	2	-0.522	-4.03, 2.99	0.0	0.75	
Without-lifestyle Modification	3	-3.116	-3.92, -2.30	0.0	0.44	
Duration						0.19
>8 Weeks	3	-1.175	-4.04, 1.69	0.0	0.78	
≤8 Weeks	2	-2.992	-4.18, -1.80	36.4	0.21	
FBS						
Lifestyle Modification						0.007
With-lifestyle Modification	3	-6.994	-15.23, 1.24	73.1	0.01	
Without-lifestyle Modification	4	-9.577	-19.17, 0.02	89.1	<0.0001	
Duration						<0.0001
>8 Weeks	5	-5.372	-10.26, -0.48	67.2	0.01	
≤8 Weeks	2	-12.519	-24.47, -0.56	77.7	0.03	
Insulin						
Baseline BMI						0.51
≥ 30	2	-0.852	-1.74, 0.04	74.2	0.04	
< 30	3	-0.844	-2.03, 0.34	90.4	<0.001	
HOMA-IR						
Lifestyle Modification						0.78
With-lifestyle Modification	3	0.145	-1.04, 1.33	96.2	<0.001	
Without-lifestyle Modification	2	-0.240	-0.74, 0.27	90.9	0.001	
HDL-C						
Baseline BMI						0.34
≥ 30	2	2.771	-3.02, 8.57	53.7	0.09	
< 30	4	0.807	-2.84, 4.45	76.7	0.03	
Lifestyle Modification						0.86

With-lifestyle Modification	3	1.607	-0.84, 4.05	0.0	0.45		
Without-lifestyle Modification	3	1.279	-4.56, 7.12	80.1	0.007		
LDL-C							
Duration							0.51
>8 Weeks	4	-13.141	-28.42, 2.14	84.2	<0.001		
≤8 Weeks	2	-17.963	-22.53, -13.39	0.8	0.31		
Lifestyle Modification							0.27
With-lifestyle Modification	3	-10.549	-33.24, 12.14	89.0	<0.001		
Without-lifestyle Modification	3	-18.206	-22.31, -14.09	0.0	0.57		
TC							
Duration							0.06
>8 Weeks	4	-13.859	-23.40, -4.30	45.0	0.14		
≤8 Weeks	2	-24.150	-36.66, -11.63	46.0	0.17		
Lifestyle Modification							0.05
With-lifestyle Modification	3	-13.013	-26.76, 0.73	59.5	0.08		
Without-lifestyle Modification	3	-20.834	-25.98, -15.67	6.2	0.34		
TG							
Baseline BMI						0.77	
≥ 30	2	-20.035	-41.54, 1.47	0.0	0.86		
< 30	4	-23.661	-35.19, -12.14	0.0	0.51		
Lifestyle Modification						0.49	
With-lifestyle Modification	3	-26.427	-40.83,-12.02	0.0	0.86		
Without-lifestyle Modification	3	-19.329	-33.65, -5.01	0.0	0.78		
Duration						0.97	
>8 Weeks	4	-22.709	-37.20, -8.21	0.0	0.74		
≤8 Weeks	2	-20.987	-38.69, -3.27	0.0	0.94		
ALT							
Lifestyle Modification						0.21	
With-lifestyle Modification	4	-11.667	-19.78, -3.54	93.7	<0.001		
Without-lifestyle Modification	3	-9.917	-13.96, -5.86	36.4	0.20		
Duration						0.73	
>8 Weeks	5	-11.919	-18.51, -5.31	92.4	<0.001		
≤8 Weeks	2	-8.197	-14.51, -	54.0	0.14		

			1.88			
AST						
Baseline BMI						<0.001
≥ 30	3	-12.568	-24.25, -0.88	91.4	<0.001	
< 30	4	-11.057	-16.19, -5.91	80.7	0.001	
Duration						0.25
>8 Weeks	5	-13.953	-22.06, -5.83	94.9	<0.001	
≤8 Weeks	2	-7.740	-12.32, -3.15	28.7	0.23	

[1]Calculated by Random-effects model

BMI: body mass index, WC: waist circumference, FBS: Fasting blood sugar, HOMA-IR: Homeostasis model assessment insulin resistance index, HDL-C: high density lipoprotein, LDL-C: low density lipoprotein, TC: total cholesterol, TG: triglyceride, ALT: alanine transaminase, AST: aspartate transaminase