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RESEARCH ARTICLE

A Meta-Analysis of the Effects of Probiotic Supplementation on Type 2 Diabetes Parameters

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ABSTRACT

We aimed to assess the effect of probiotic supplementation on type 2 diabetes (T2D) related variables. Randomized controlled trials (RCTs) were systematically searched from PubMed, Springer-link and Scopus data bases published between 2016 and 2020. Collected studies were independently assessed for eligibility, data extracted and evaluated for risk of bias. Statistical analysis was performed using RevMan 5.4.1. The effects of probiotics were assessed for: body mass index (BMI), fasting blood glucose (FBG), glycated hemoglobin (HbA1c), homeostasis, model assessment-estimated insulin resistance (HOMA-IR), insulin, triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), systolic blood pressure (SBP), diastolic blood pressure (DBP) and high-sensitivity C-reactive protein (hs-CRP). From 1702 identified studies, eight trials were eligible for systematic review and six were included in the final meta-analysis. Probiotic strain levels increased without any change on microbiota diversity. Abundant phyla were *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, *Verrucomicrobia*, and *Cyanobacteria*. Significant effects were found for FBG (SMD –0.53) and HOMA-IR (SMD –0.61). No significant effect were found for BMI (MD –0.55 kg/m^2), HbA1c (MD –0.13%), insulin (MD –1.77 μU/mL), TG (SMD –0.17), TC (SMD –0.13), LDL-C (SMD –0.09), HDL-C (SMD 0.21), SBP (MD –3.42 mmHg), DBP (MD –2.27 mmHg) and hs-CRP (SMD –0.05). No serious adverse events were reported for eligible trials, no important heterogeneity for FBG and HOMA-IR and no significant publication bias were found. Our findings indicate that the probiotic has a significant effect on FBG and HOMA-IR. Also, they improve BMI, TG, TC, LDL-C, HDL-C, HbA1c, insulin, hs-CRP, SBP and DBP.

Keywords: Meta-analysis, Microbiota diversity, Probiotics, Supplementation, Type 2 diabetes

Introduction

The type 2 diabetes (T2D) non-insulin dependent is a lifestyle disease in genetically defenseless persons defined by high blood glucose, insulin resistance and relative insulin deficiency,[1] with increased TG and decreased levels of HDL-C.[2] T2D contributes to difficult complications such as cardiovascular disease, kidney insufficiency, amputation, and other negative impacts.[3] As recently indicated, it is projected that by the year 2035, approximately 592 million individuals worldwide will be diagnosed with diabetes.[4] In addition, this disease continues to be one of the main causes of death.[4,5] Not long ago, an ever increasing number of studies attributed beneficial effects to probiotic use in glycemic control in human studies and in T2D related variables, such as decrease of FBG, HbA1c, body weight, inflammatory markers, SBP, DBP and HOMA-IR in animal studies.[2,6,7] Probiotics are “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” as defined by the Food and Agriculture

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Organization/World Health Organization (FAO/WHO) and are "a live organism that provides a benefit to the host when provided in adequate quantities" as defined by the International Scientific Association for Probiotics and Prebiotics (ISAPP). *Lactobacillus* and *Bifidobacterium* are the most common genera used as probiotics,[8] followed by *Streptococcus*, *Enterococcus*, *Propionibacterium*, *Bacillus*, *Escherichia coli* and some yeasts such as *Saccharomyces boulardii* and *Saccharomyces cerevisiae*.[9] Beneficial effects of probiotics are due to their ability to regulate the gut microbiota, reinforce gut barrier function and their immunomodulatory actions.[2,10] Probiotic supplementation could be administered as capsules, tablets, powders, and as food ingredient.[9] More recent meta-analyses, including probiotic capsules, revealed useful effects in T2D and obesity.[11] The current meta-analysis aimed to assess the effect of probiotic supplementation on T2D related variables.

Materials and methods

Work protocol

The present meta-analysis was conducted according to the instructions listed in the Cochrane Handbook for Systematic Reviews of Interventions[12] and listed in Preferred Reporting Items for Systematic Reviews and Meta Analyzes (PRISMA).[13] Literature search was carried out in databases: PubMed, Springer-link and Scopus. The purpose behind this research was to identify relevant articles published between 2016 and 2020 using the keywords: "probiotic", "diabetes", "diabetes type 2", "T2D", "hyperglycemia", "glycemia", "obesity", "microbiota", "gut microbiota".

Eligibility selection

The identified articles were first assessed for eligibility to select in English and French language RCTs studying probiotic supplementation, T2D, obesity and gut microbiota after reading tile, abstract, and full lecture. Moreover, full texts were secondly analyzed.[7] Duplicate articles, editorials, literary reviews, non-RCTs and trials on a non-human model, articles presented only as summaries, and articles insufficient for data extraction were excluded.[14]

Data extraction

The elements extracted concerned: authors, year, location, study design, participants (number, age, gender and randomization), interventions and comparators (probiotic, source, dose), main outcomes, serious adverse events, follow-up or duration (week).[4]

Quality assessment

Quality and risk of bias were independently assessed in duplicate based on a Cochrane Handbook for Systematic Reviews of Interventions.[12] Obtained differences were resolved through discussion and a third investigator was involved to resolve the controversy. This assessment was performed using RevMan 5.4.1.[15] Seven items of bias were assessed: random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias) and other bias (control for dietary intake and baseline parameters). These items were classified as low risk of bias (circles colored green and marked with "+"), as high risk of bias (circles colored red and marked with "−"), or as unclear risk of bias (circles colored yellow and designated with "?"). The overall quality of the studies was assessed based on their risk of bias.[12]

Meta-analysis

Statistical analysis was performed using RevMan 5.4.1.[15] For RCTs involving two probiotic treatment groups, outcomes from the two groups were combined to create a single pair wise comparison with the placebo group.[16] For trials that show the standard error data, a standard deviation was obtained from the standard error of a mean by multiplying by the square root of the sample size $SD = SE \times \sqrt{N}$. $p < 0.05$ was regarded as statistically significant. For continuous outcomes, a statistical random effects model was used to estimate the mean difference (MD) when trials used the same scale or the standardized mean difference (SMD) between RCTs with a 95% confidence interval (CI). Heterogeneity was measured using the chi-square test ($\chi 2$ or chi2). Heterogeneity between RCTs was evaluated by I2 statistics and categorized as flow: 0 to 40% (not important), 30 to 60% (moderate heterogeneity), 50 to 90% (substantial heterogeneity) and 75 to 100% (considerable heterogeneity).[12] A funnel plot using fixed-effects models was used for the visual assessment of publication bias.[6]

Results and Discussion

Literature search and screening

A total of 1702 studies were identified: 671 studies from PubMed, 481 studies from Springer-link, 452 studies from Scopus and 98 studies from manual search. 162 duplicate studies were removed, 1173 studies were excluded on the basis of title and abstract

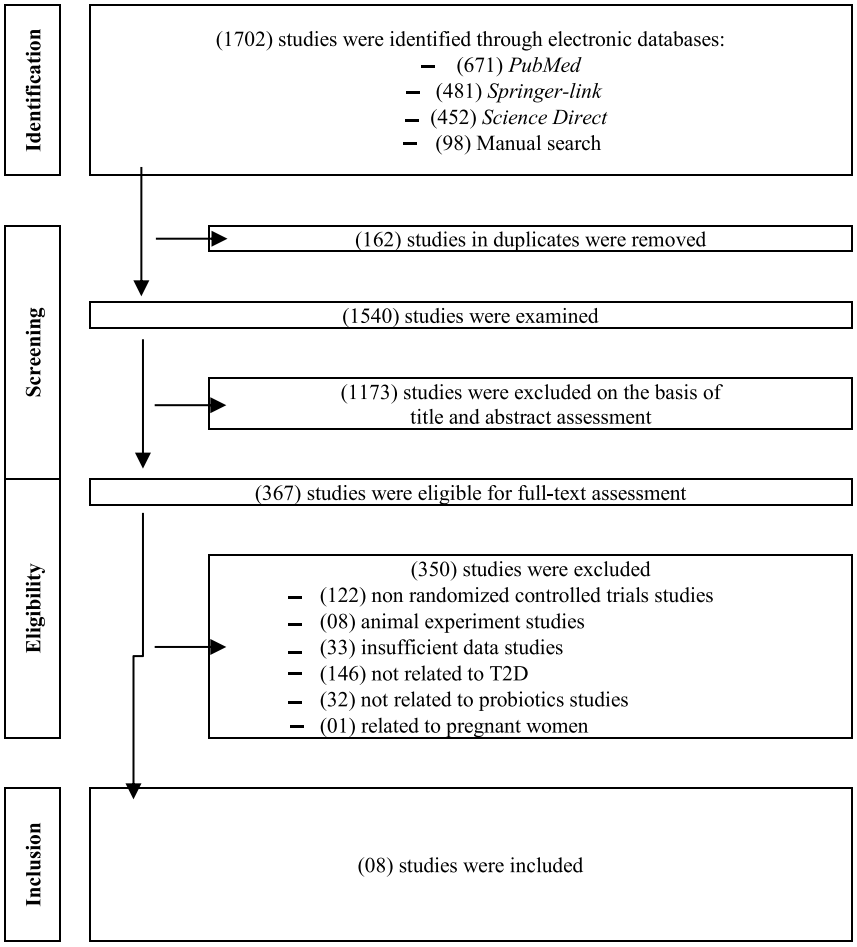

Fig. 1. Diagram of studies search and screening process.

assessment, and the remaining 367 studies were eligible based on the inclusion and exclusion criteria for full-text assessment. From these, 350 studies were excluded for various reasons: 121 studies were non-RCTs, eight animal experiments, 33 studies had insufficient data, 146 studies were not related to T2D, 32 studies were not related to probiotic studies and one study was related to pregnant women. Thereafter, eight studies were maintained Fig. 1.

Data extraction

The eligible eight trials were from Asia and Europe: five from Iran[16–18] one from Malaysia,[19] one from Sweden[1] and one from Spain.[10] They were RCTs: two double-blind placebo controlled trials with three parallel groups, four double-blind placebo controlled trials with two parallel groups, one double-blind crossover placebo-controlled single-center trial and the last was controlled trials with four parallel groups (no placebo) and report the blinding process in the clinical trial protocol consulted from the Iranian Registry of Clinical Trials. Included trials were with (n = 331) patients with T2D with (n = 170) patients in intervention groups and (n = 161) in control groups. Patients were aged 25-75 years. Follow-up duration of intervention ranged from six to 24 weeks, using probiotic genera *Lactobacillus*, *Bifidobacterium* and *Streptococcus* in 10^8 to 10^{10} colony-forming Units/day amounts (CFU/d). Four studies used multispecies probiotics, three studies used single species probiotics, whereas one study used yogurt as a source of probiotics. The trials reported anthropometric, glycemic control, lipid profile and inflammatory marker measurements. Six trials were included in the final meta-analysis and the common reported data were: FBG for six trials (n = 331). BMI, TG, TC, HDL-C and LDL-C for five trials (n = 291). HbA1c for four trials (n = 225), HOMA-IR and insulin for four trials, both with (n = 247) and hs-CRP for four trials (n = 231). SBP and DBP for three trials (n = 185). Moreover, no serious adverse events were registered. The only adverse event was reported in four studies: gastrointestinal complications (flatulence, dysphagia,

and dyspepsia), infection, hypoglycemia, headache and musculoskeletal symptoms, and higher sexual desire Table 1.

Gut microbiota assessment

In only three studies, gut microbiota was characterized using fecal samples before and after intervention. Probiotic strains were measured using 16S rRNA sequencing performed in accordance with the Illumina protocol,[1,10] or plate-counting method combined with polymerase chain reaction (PCR) at baseline and week 12 to demonstrate the successful passage of the supplements from gastrointestinal tract and also microbiota diversity or overall microbiota composition as a consequence of treatment.[19] The last two authors found marginal (placebo group) or significant (probiotic group) increases in probiotic strain levels, but they did not observe any change in diversity or overall microbiota composition because of probiotic use. Whereas, *Firmicutes* and *Bacteroidetes* were the most abundant phyla, followed by *Proteobacteria*, *Actinobacteria*, *Verrucomicrobia*, and *Cyanobacteria*[10] Table 2.

Quality assessment

All studies exhibited low risk regarding the possible bias from sequence generation using either computer generated random sequence or block randomization and they mentioned allocation concealment, also blinding of participants, personnel and outcome assessment using a masking double for the participant, investigator or quadruple for participant, care provider, investigator, and outcomes assessor. Regarding incomplete outcome data, one study has a high risk because of the loss of participants to follow-up the study.[17] As regards to selective reporting bias, two studies have a high risk because the pre-specified outcomes were not reported[10,17] and one study have an unclear risk because they did not register their clinical trial protocol.[5] Concerning other bias, two studies obtained a high risk due to non-reporting of control dietary intake data.[10,17] All studies have mentioned the baseline parameter measurements. Based on overall quality, two studies were judged high risk of bias and excluded,[10,17] while five trials were judged low risk of bias[1,2,16] and one some concerns; the trial is assessed to raise some concerns in at least one domain regarding this result, but it is not considered to have a high risk of bias in any domain, as outlined in the Cochrane Handbook for Systematic Reviews of Interventions, section 8.2.4, which addresses reaching an overall risk-of-bias judgment for a result.[5] Finally, six trials were included in the meta-analysis[1,2,5] Table 3 and Fig. 2.

Meta-analysis

The effect estimate of probiotic intervention on the anthropometric parameter BMI in T2D patients measured on six trials, probiotic group (n = 150) and control group (n = 141), was not significantly reduced by MD –0.55 kg/m (95% CI: –1.83 to 0.73; $p = 0.40$) with moderate heterogeneity (I2 = 26%, $p = 0.12$). Differences between probiotic intervention and control groups were significant in two of the six trials.

The effects estimate of the glycemic control before and after supplementation of probiotics yielded a significant reduction in T2D patients: FBG by SMD –0.53 (95% CI: –0.76 to –0.30; $p = 0.00001$), a non-significant statistical heterogeneity (I2 = 4%, $p = 0.39$) between groups probiotic (n = 170) and control (n = 161) on six trials; HOMA-IR by SMD –0.61 (95% CI: –0.87 to –0.36; $p = 0.00001$) with a non-significant inter trials heterogeneity (I2 = 0%, $p = 0.47$) detected between probiotic (n = 121) and control groups (n = 126) on four trials Figs. 3 and 4.

Whereas, no significant reduction was observed for the MD values of HbA1c across all groups of probiotic (n = 117) and control group (n = 108) of four trials by MD –0.13% (95% CI: –0.61 to 0.34; $p = 0.58$) and substantial heterogeneity (I2 = 57%, $p = 0.07$). The same observation for insulin levels across the groups of probiotic (n = 121) and control (n = 126) of four trials by MD –1.77 μU/mL (95% CI: –4.24 to 0.70; $p = 0.16$) and moderate heterogeneity (I = 86%, $p = 0.0001$). Differences between probiotic and control groups were significant in two to three of the four trials.

On four trials including probiotic (n = 150) and control groups (n = 141), the effect estimate in T2D patients showed non-significant reduction with a non-significant heterogeneity: of TG by SMD –0.17 (95% CI: –0.46 to 0.12; $p = 0.25$) (I2 = 54%, $p = 0.07$); of TC by SMD –0.13 (95% CI: –0.36 to 0.11; $p = 0.28$) (I2 = 0%, $p = 0.67$); of LDL-C by SMD –0.09 (95% CI: –0.32 to 0.15; $p = 0.46$) (I2 = 0%, $p = 0.55$), except HDL-C which increased by SMD 0.21 (95% CI: –0.02 to 0.45; $p = 0.07$) (I2 = 0%, $p = 0.59$). On three trials including probiotic (n = 97) and control groups (n = 88), SBP and DBP were not significantly reduced with substantial heterogeneity by MD –3.42 mmHg (95% CI: –11.76 to 4.92; $p = 0.42$) (I = 67%, $p = 0.05$) and by MD –2.27 mmHg (95% CI: –6.36 to 1.82; $p = 0.28$) (I2 = 53%, $p = 0.12$). Differences between probiotic

Table 1. Data extraction of eligible studies.

Authors Year	Location	Study design	Participants (number, age, gender and randomization)	Interventions and comparators (probiotic, source, dose)	Main outcomes	Serious adverse events	Follow-up duration (week)
Mobini *et al.* [1]	Sweden	Double-blind, randomized, placebo-controlled trial with three parallel groups	44 patients (34 male and 10 female) with type 2 diabetes Probiotic low dose (n = 15) Probiotic high dose (n = 14) Placebo (n = 15)	– Probiotic low dose (10^8 CFU/d) of powder of *Lactobacillus reuteri* DSM 17938 – Probiotic high dose (10^{10} CFU/d) of powder of *Lactobacillus reuteri* DSM 17938 – Placebo powder with a mild sweet taste Packaged in identical stick pack	Weight, height, waist, body fat and BMI SBP and DBP FBG, TC, HDL-C, LDL-C, TG, HbA1c, ASAT, ALAT and hs-CRP Liver fat content, visceral, subcutaneous, adipose tissue area, adiponectin and leptin Serum bile acids and urine analysis	Nr	12
Razmpoosh *et al.* [2]	Iran	Randomized double blind clinical trial	60 patients (33 male and 27 female) aged 30 to 75 years with type 2 diabetes Probiotic (n = 30) Placebo (n = 30)	– Probiotic as freeze-dried strains: Lactobacillus acidophilus (2×10^9 CFU), Lactobacillus casei (7×10^9 CFU), Lactobacillus rhamnosus (1.5×10^9 CFU), Lactobacillus bulgaricus (2×10^8 CFU), Bifidobacterium breve (3×10^{10} CFU), Bifidobacterium longum (7×10^9 CFU), Streptococcus thermophilus (1.5×10^9 CFU), and 100 mg fructooligosaccharide with lactose as carrier substances. – Placebo as fructo-oligosaccharide and magnesium stearate – Packaged in identical capsules	Weight, chest, waist, hip and BMI FBG, insulin, TC, TG, LDL-C, HDL-C and HOMA-IR	Nr	6
Khalili *et al.* [5]	Iran	Parallel-group, randomized, double-blinded, controlled trial	40 patients (14 male and 26 female) with type 2 diabetes aged 30 to 50 years Probiotic (n = 20) Placebo (n = 20)	– Probiotic as 10^8 CFU/d *Lactobacillus. casei* and maltodextrin as excipient – Placebo as maltodex-trin – Packaged in identical capsules	Weight, waist, WHR and BMI FBG, HbA1c, insulin, HOMA-IR, fetuin-A and SIRT1	Nr	8
Tenorio-Jiménez *et al.* [10]	Spain	Randomized, double-blind, crossover, placebo-controlled, single-center trial	53 patients with metabolic Syndrome (28 male and 25 female) (type 2 diabetes) Probiotic (n = 28) Placebo (n = 25)	– Probiotic as Lacto-bacillus reuteri V3401 (5×109 CFU) – Placebo as (maltodex-trin) – Packaged in identical capsules	Weight, height, waist and BMI SBP and DBP FBG, insulin, TC, TG, LDL-C, HDL-C, HbA1c, *hs*-CRP and HOMA-IR GOT, GPT, γGT, I-6, I-8, TNFα, tPAI1, adiponectin, leptin, resistin, P-selectin, HGF, MCP-1, MPO, sICAM, sVCAM, LPS and LBP		12

(Continued)

Table 1. Continued.

Authors Year	Location	Study design	Participants (number, age, gender and randomization)	Interventions and comparators (probiotic, source, dose)	Main outcomes	Serious adverse events	Follow-up duration (week)
Bayat *et al.* [16]	Iran	Parallel-group randomized controlled clinical trial	80 patients (28 male and 52 female) with type 2 diabetes aged 25 to 75 years Probiotic yogurt (n = 20) Control (n = 20) C. *ficifolia* (n =20) C. *ficifolia* + probiotic yogurt (n = 20)	– Probiotic yogurt (150 g) – Control (dietary advice) – *Cucurbita ficifolia* (100 g) – *Cucurbita ficifolia* (100 g) + probiotic yogurt (150 g)	SBP and DBP FBG, hsCRP. TC, HDL-C, LDL-C, TG, HbA1c	Nr	8
Kassaian *et al.* [17]	Iran	Double-blind, placebo-controlled randomized, three-arm, parallel-group clinical trial	120 patients (50 male and 70 female) prediabetic aged 35 to 70 years (type 2 diabetes) Probiotic (n = 40) Symbiotic (n = 40) Placebo (n = 40)	– Probiotic 6 g/d of freeze-dried Lactobacillus acidophilus, Bifidobacter bifidum, Bifidobacter lactis, and Bifidobacter longum (1.5×10^9 for each) with maltodextrin as filler – Symbiotic (6 g/d inulin as prebiotic + mentioned-above probiotic) – Placebo (maltodextrine 6 g/d) Packaged in identical sachet	Frequency percentage of metabolic syndrome and its components (obesity, hyperglycemia, hypertension, hypertriglyceridemia, Low HDL-C	Nr	24
Rabiei *et al.* [18]	Iran	Parallel triple-blind randomized clinical trial.	46 patients (13 male and 33 female) aged 25 to 70 years (type 2 diabetes) Symbiotic (n = 23) Placebo (n = 23)	– Symbiotic 250 mg of Lactobacillus casei, Lactobacillus rhamnosus, Streptococcus thermophilus, Bifidobacterium breve, Lactobacillus acidophilus, Bifidobacterium longum, Lactobacillus bulgaricus, fructooligosaccharide as prebiotic, 125 mg, magnesium stearate (source: mineral and vegetable), vegetable capsule (hydroxypropyl methylcellulose) total viable counts (2×10^8 CFU), for all of the bacteria – Placebo 250 mg of maltodextrine Packed in identical capsules	Weight, height and BMI FBG, TG, TC, HDL-C, LDL-C, insulin, peptide-YY, glucagon like peptide-1, hs-CRP, I-6 and HOMA-IR	Nr	12

(Continued)

Table 1. Continued.

Authors Year	Location	Study design	Participants (number, age, gender and randomization)	Interventions and comparators (probiotic, source, dose)	Main outcomes	Serious adverse events	Follow-up duration (week)
Firouzi *et al.* [19]	Malaysia	Randomized, double-blind, parallel-group, placebo-controlled trial	101 patients with type 2 diabetes aged 30 to 70 years Probiotic (n = 48) Placebo (n = 53)	– Probiotic as dried 3 10^{10}/d of Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus lactis, Bifidobacterium bifidum, Bifidobacterium longum and Bifidobacterium infantis – Placebo Packaged in identical sachets	Weight, height, waist and BMI SBP and DBP FBG, hs-CRP, TG, TC, HDL-C, LDL-C, HbA1c, insulin, QUICKI, HOMA-IR	Nr	12

BMI: body mass index. FBG: fasting blood glucose. HbA1c: glycated hemoglobin. HOMA-IR: homeostasis, model assessment-estimated insulin resistance. TG: triglycerides. TC: total cholesterol. HDL-C: high-density lipoprotein cholesterol. LDL-C: low-density lipoprotein cholesterol. SBP: systolic blood pressure. DBP: diastolic blood pressure. hs-CRP: high-sensitivity C-reactive protein.QUICKI: quantitative insulin sensitivity check index. WHR: waist to heap ratio. VAT: visceral adipose tissue. SAT: subcutaneous adipose tissue. ISI: insulin sensitivity index. ASAT: aspartate aminotransferase. ALAT: alanine aminotransferase. U-albumin: urine albumin. PYY: peptide YY. GLP-1: glucagon-like peptide–1. GOT: aspartate aminotransferase. GPT: alanine aminotransferase. GT: gamma glutamyltransferase. IL: interleukin. TNF: tumor necrosis factor alpha. tPAI1: plasminogen activator inhibitor–1. HGF: hepatocyte growth factor. MCP-1: monocyte chemoattractant protein 1. sICAM: soluble intracellular adhesion molecules. sVCAM: soluble vascular cell adhesion molecule. MPO: myeloperoxidase. LPS: lipopolysaccharide. LBP: lipopolysaccharide-binding protein. SIRT1: Sirtuin1. NR: no report.

Table 2. Gut microbiota assessment.

Authors/year	Assessment methodology	Samples	Probiotic strain	Levels	
				Baseline	Week 12
Mobini *et al.* [1]	16S rRNA-based Illumina MiSeq sequencing	Fecal	*L. reuteri* DSM 17938 (copies/g)	Placebo 10^3–10^4 Probiotic low dose 10^3–10^4 Probiotic high dose 10^5	Placebo 10^4 Probiotic low dose 10^5–10^6 Probiotic high dose 10^8–10^9
Tenorio-Jiménez *et al.* [10]	16S metagenomics sequencing performed following the Illumina protocol	Fecal	*L. reuteri* V3401 (copies/g)	Ud	Ud
Firouzi *et al.* [19]	Plate-counting method combined to polymerase chain reaction (PCR)	Fecal	*Lactobacillus spp.* (CFU/g) *Bifidobacterium spp.* (CFU/g)	Placebo $1.8 \times 10^7 \pm 0.7 \times 10^7$ Probiotic $6.4 \times 10^6 \pm 1.1 \times 10^6$ Placebo $2.7 \times 10^6 \pm 0.8 \times 10^6$ Probiotic $3.4 \times 10^6 \pm 0.2 \times 10^6$	Placebo $2.1 \times 10^7 \pm 9.2 \times 10^6$ Probiotic $1.2 \times 10^7 \pm 6.4 \times 10^6$ Placebo $5.3 \times 10^6 \pm 1.9 \times 10^6$ Probiotic $1.4 \times 10^7 \pm 1.5 \times 10^7$

Ud: unable to determine the specific presence.

and control groups on lipid profile were significant in no less than two included trials.

No significant reduction with a non-significant heterogeneity was observed for hs-CRP between the probiotic (n = 120) and control group (n = 111) of four trials by SMD –0.05 (95% CI: –0.34 to 0.23; $p = 0.71$) (I2 = 13%, $p = 0.33$). Differences between probiotic and control groups were significant in two trials.

No significant publication bias was shown for FBG and HOMA-IR levels. Funnel plots show the dispersion of studies Figs. 5 and 6.

Discussion

Recently, meta-analyses have examined the effects of probiotics on T2D related variables. Some of them

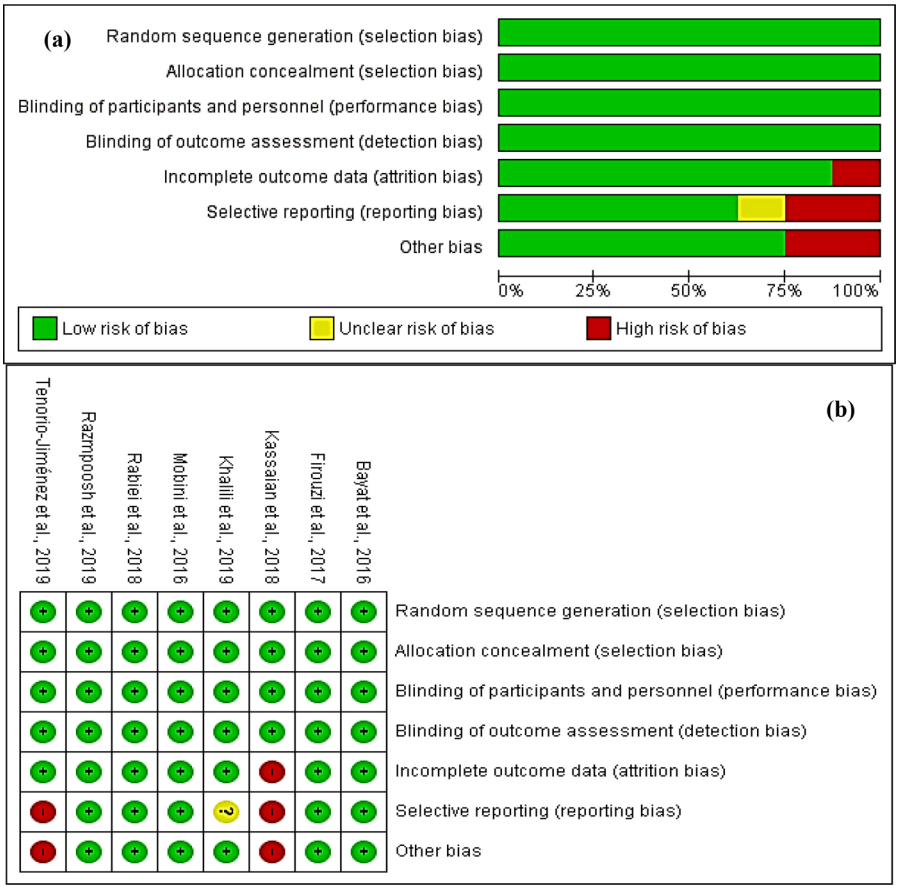

Fig. 2. Risk of bias (A) graph; (B) summary.

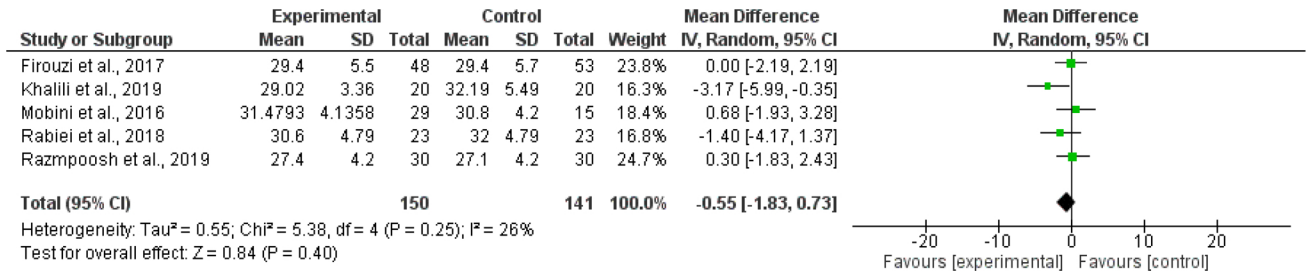

Fig. 3. Forest plot of RCTs comparing the effect estimate of probiotics on FBG with placebo in TD2 patients; mean difference or the standardized mean difference and weight percentage are shown with 95% confidence interval; the random-effect model was applied.

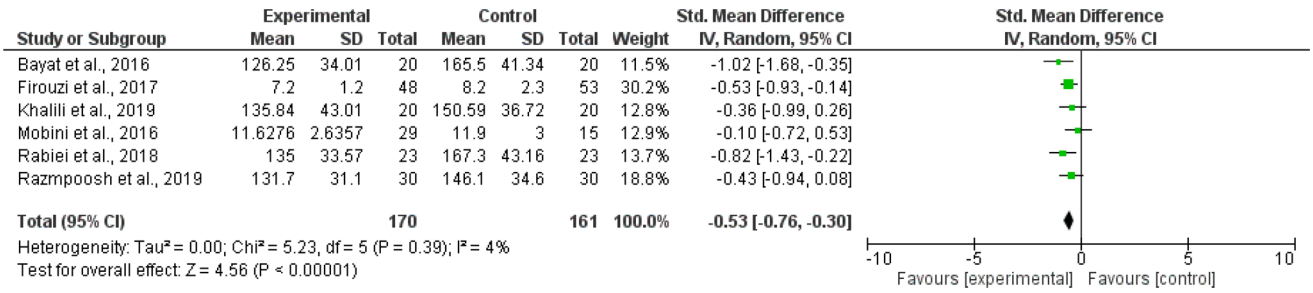

Fig. 4. Forest plot of RCTs comparing the effect estimate of probiotics on HOMA-IR with placebo in TD2 patients; mean difference or the standardized mean difference and weight percentage are shown with 95% confidence interval; the random-effect model was applied.

Table 3. Risk of bias.

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias*	Overall quality
Mobini *et al.*[1]	L	L	L	L	L	L	L	L
Razmpoosh *et al.*[2]	L	L	L	L	L	L	L	L
Khalili *et al.*[5]	L	L	L	L	L	U	L	SC
Tenorio-Jiménez *et al.*[10]	L	L	L	L	L	H	H	H
Bayat *et al.*[16]	L	L	L	L	L	L	L	L
Kassaian *et al.*[17]	L	L	L	L	H	H	H	H
Rabiei *et al.*[18]	L	L	L	L	L	L	L	L
Firouzi *et al.*[19]	L	L	L	L	L	L	L	L

L: low risk. U: unclear risk. H: high risk. SC: some concerns.
*Control for dietary intake and baseline parameters.

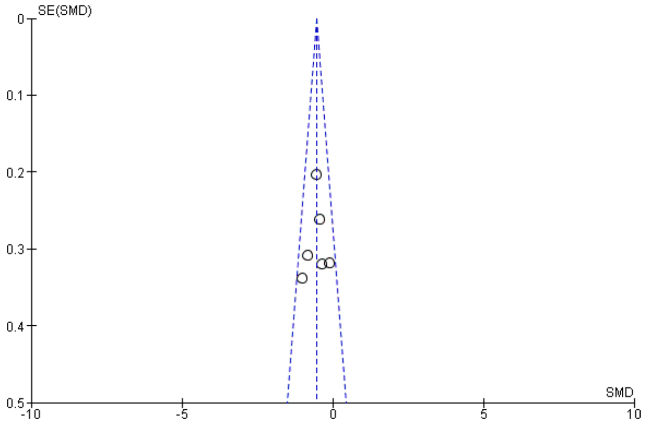

Fig. 5. Funnel plot of assessment of FBG publication bias; standardized mean difference is shown with 95% confidence interval; the fixed effect-model was applied.

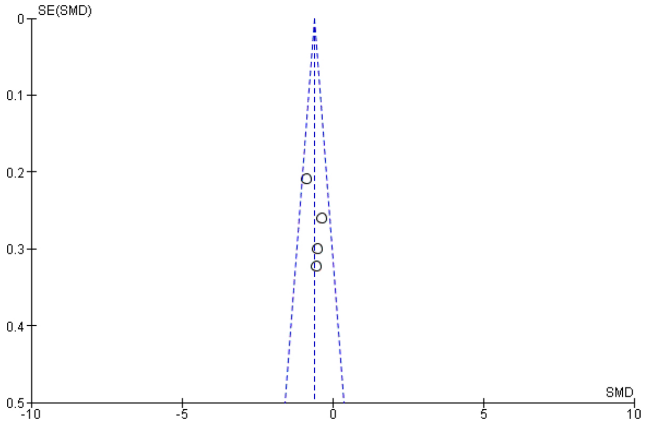

Fig. 6. Funnel plot of assessment of HOMA-IR publication bias; standardized mean difference is shown with 95% confidence interval; the fixed effect-model was applied.

reported that the consumption of probiotics has positive effects on FBG and HOMA-IR. In the current meta-analysis based on six included trials, the effect estimate of probiotic supplementation implies that an experimental intervention has a significant effect on FBG and HOMA-IR levels. The present study has also revealed an improvement in the overall effect favoring the probiotics group, but with no significant effect on BMI, TG, TC, LDL-C, HDL-C, HbA1c, insulin, hs-CRP, SBP and DBP. Therefore, probiotic used strains such as *Lactobacillus*, *Bifidobacterium* and *Streptococcus* become a treatment approach in T2D. In addition, no changes in gut microbiota were observed after treatment with these supplements. These meta-analysis findings may provide important information and improve scientific data by adding the results of six combined data trials. However, further research is needed to confirm the effect of probiotic treatment on T2D patients.

The present meta-analysis combined the results of independent trials regarding the effect of probiotic supplementation on T2D variables. From a total of 1702 from PubMed, Springer-link, Scopus and manual search, eight studies were eligible and only six were included in the final meta-analysis, involving (n = 331) patients allocated randomly between the intervention group (n = 170) and placebo and/or control group (n = 161). Recently, meta-analyses have used electronic databases for research, such as PubMed, Embase, Scopus, Cochrane library, ISI web of knowledge, Medline and others, as used in the present meta-analysis. These meta-analyses aimed to have a considerable scientific paper number and subsequently a maximum of eligible and included trials.[20–22] Therefore, the total scientific papers as well as the number of eligible trials differed among meta-analyses. For example, 17 trials were selected from 1561,[22] eight trials from 571,[21]

11 trials from 442[20] and nine trials were recovered from 551.[11]

In the present meta-analysis, T2D was treated with probiotic genera *Lactobacillus*, *Bifidobacterium* and *Streptococcus* as capsules or sachet supplements and as probiotic yogurt at a dose of 10^8–10^{10} CFU/d. According to FAO/WHO, the optimal use of probiotics can bring health benefits to the host. In general, 10^6–10^8 CFU/d, or 10^8–10^{10} CFU/d of viable cells are considered sufficient and effective.[13]

The flow-up duration of probiotic intervention was six to 24 weeks and 12 different parameters- anthropometric were measured- BMI, blood variables (FBG, HOMA-IR, HbA1c, insulin, TG, TC, HDL-C, LDL-C, SBP and DBP) and inflammatory biomarkers (hs-CRP). Trials aimed to assess the effect of *Lactobacillus*, *Bifidobacterium* and *Streptoccocus* probiotic genera at 10^6 to 10^{12} CFU/d on BMI, FBG, HOMA-IR, HbA1c, insulin, TG, TC, HDL-C, and LDL-C levels.[11,20–22] The most trials used were Iranian cases. About 7.7% of Iranian adults suffer from diabetes.[16]

Trials have detected reductions in FBG and HOMA-IR levels,[20,22] and those using multiple species probiotics indicate a more pronounced reduction. The reduction in FBG would be better at a daily dose $>10^{11}$ CFU and a duration >8 weeks. The present meta-analysis indicated that the probiotic treatment had a beneficial effect on decreasing significantly FBG by SMD –0.53 and HOMA-IR by SMD –0.61.These findings go in line with previous meta-analyses focused on effect estimates of probiotics in the treatment of T2D, that found a significant reduced effect of FBG in T2D patients in comparison with placebo: on eight trials by SMD –1.583,[6] on nine trials by SMD –0.61,[4] on 17 trials by MD –0.31,[22] on 10 trials by SMD –11.27.[20] A few reductions in FBG could have health benefits, but these benefits are unclear; they can be explained by decreased oxidative stress, or the increased of plasma lipopolysaccharides that lead to apoptosis of pancreatic ß-cells and insulin resistance.[4,20,22]

Meta-analyses in such a field of interest revealed that probiotic supplementation reduced significantly HOMA-IR in T2D patients.[6,20–22] They reported identical values to the present study and recorded respectively a significant reduction by SMD –1.05 on six trials, by SMD –1.267 on four trials, by SMD –2.10 on three trials and by MD –0.48 on 17 trials. The beneficial effects of probiotics on glycemic control are also unclear. Such effects can be attributed to their immunoregulatory effects,[6] or to improvement in insulin-resistance and glucose tolerance due to their protective effect on pancreatic ß-cell damage and maintaining the insulin levels.[20] In addition, the beneficial effects of probiotics on glycemic control can be explained by increasing secretion from enteroendocrine L-cells to improve carbohydrate metabolism, increase insulin sensitivity of target cells and decrease glucotoxicity. It can also be deduced that the reported positive effects can normalize the gut microbiota and improve the intestinal epithelium's integrity and reduce the Toll-like receptor 4 pathway and proinflammatory signaling, which enhances sensitivity for insulin.[21]

However, the probiotic treatment had no significant effects on values of: BMI by MD –0.55 kg/m2, HbA1c by MD –0.13%, insulin by MD –1.77 μU/mL, TG by SMD –0.17, TC by SMD –0.13, LDL-C by SMD –0.09, HDL-C by SMD 0.21, SBP by MD –3.42 mmHg, DBP by MD –2.27 mmHg and hs-CRP by SMD –0.05. These results are in accordance with recent meta-analyses reported no significant effect on LDL-C by SMD –0.13, TC by SMD –0.09, TG by SMD –0.22 and HbA1c by SMD 0.06 on nine trials,[4] or suggested that probiotics may not lead to a significant effect on BMI by MD 0.77 on four trials,[7] and on HbA1c by MD –0.366 on six trials by probiotic yogurt.[11] In addition, those revealed no significant reduction on HbA1c by SMD –0.19 and on LDL-C by SMD –0.84 up against TC and TG levels by SMD –8.49 and SMD –23.66 presented a significant reduction as well as an increase of HDL-C by SMD 3.92 on nine trails.[20] Others revealed no significant reduction on TC by SMD 0.12, TG by SMD –0.27 on five trials, LDL-C by SMD 0.37 on four trials, HDL-C by SMD 0.73 and hs-CRP by SMD –1.73 on five trials.[21]

Regarding insulin levels, authors have observed an insignificant reduction, respectively, by MD 0.37 on five trails and by SMD –1.27 on six trials,[11,21] as distinct from those who have reported a significant reduction of insulin after probiotic use, by MD –2.36 μU/mL on two trials, by SMD –1.267 on four trials and by MD –1.29 μU/mL on three trials.[6,13,22] It has also been indicated that probiotics have a significant effect on HDL-C, showing a significant increase by SMD 0.42,[4] by SMD 3.92.[20] In this regard, the beneficial effect of probiotics on lipid profiles may be due to inhibition of absorption of dietary cholesterol and suppression of reabsorption of bile acids in the small intestine.[8]

Meta-analyses examined probiotic health advantages to enhance immune function, blood pressure and lipids.[22] Several animal experiments reported that probiotics reduce TC, TG and LDL-C, but increase HDL-C.[20] On the other hand, numerous RCTs on this topic continue to produce contradictory results.[11] The controversial results may be due to different dosages and strains of probiotics used for the

T2D patients and to the differences between patients' population in their lifestyles (physical activity, diet, sleep, smoking, etc.). Such contradictions complicate conclusions about probiotic use based on these parameters.

Conclusion

The present meta-analysis assessed publication bias only for FBG and HOMA-IR and has shown no significant bias and corresponding funnel plots used as means of detecting bias resemble a symmetrical inverted funnel and show the dispersion of the trials. No change in microbiota diversity or overall microbiota composition was observed and *Firmicutes*, *Bacteroidetes*, *Proteobacteria* and *Actinobacteria* were the most present. It has been demonstrated that most human gut microbiota belong to four major phyla: *Firmicutes*, *Actinobacteria*, *Proteobacteria*, and *Bacteroidetes*.

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Authors' declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for republication, which is attached to the manuscript.
- No animal studies are present in the manuscript.
- Authors sign on ethical consideration's approval.
- Ethical Clearance: The project was approved by the local ethical committee in University of Djillali LIABES.

Authors' contribution statement

M.N., Z.B. and M.B.K. contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

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تحليل بعدي لتأثيرات مكمل البروبيوتيك على معايير مرض السكري نوع 2

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الخلاصة

هدفت الدراسة إلى تقييم تأثير البروبيوتيك على المتغيرات ذات الصلة بمرض السكري نوع 2، حيث جرى البحث بشكل منهجي عن التجارب السريرية العشوائية (RCTs) في قواعد البيانات PubMed و Springer-link و Scopus التي نُشرت بين عامي 2016 و 2020. بعد ذلك، تم تقييم الدراسات المُجمّعة بشكل مستقل لتحديد الاهلية، واستخراج البيانات وتقييم الارتياب. تم إجراء تحليل احصائي باستخدام برنامج RevMan 5.4.1.، من أجل تقييم تأثيرات البروبيوتيك على: مؤشر كتلة الجسم (BMI)، ومستوى غلوكوز الدم الصائم(FBG) ، السكر التراكمي(HbA1c) ، مقاومة الأنسولين(HOMA-IR) ، والإنسولين، الدهون الثلاثية(TG) ، كوليستيرول الدم الكلي(TC) ، البروتين الدهني منخفض الكثافة(LDL-C) ، البروتين الدهني مرتفع الكثافة (HDL-C)، وضغط الدم الانقباضي (SBP)، وضغط الدم الانبساطي (DBP) البروتين المتفاعل عالي الحساسية (hs-CRP) . تجدر الإشارة إلى أنه من بين 1702 دراسة خلصنا إلى أن ثماني تجارب سريرية مؤهلة للمراجعات المنهجية، وأن ستاً منها مؤهلة للتحليل الإحصائي البعدي. وبعد الدراسة تبيّن زيادة مستويات سلالات البروبيوتيك دون تغيير في التنوع ووجود فرق في تراجع مستوى FBG (SMD -0.53) و HOMA-IR (SMD -0.61)، وكذا تراجع في: BMI (MD -0.55 kg/m2)،HbA1c (MD -0.13%)، الإنسولين (MD -1.77 µU/mL) ، TG (SMD -0.17)، TC (SMD -0.13)، LDL-C (SMD -0.09)، HDL-C (SMD 0.21)، SBP (MD -3.42 mmHg)، DBP (MD -2.27 mmHg)و hs-CRP (SMD -0.05). ختاما، تشير نتائج الدراسة إلى أن البروبيوتيك له تأثير ملحوظ على نسب FBG و HOMA-IR. ، وله دور في تحسن BMI و TG، TC، LDL-C، HDL-C، HbA1c ، الإنسولين ، hs-CRP، SBP و.DBP.

الكلمات المفتاحية: تحليل بعدي، تنوع ميكروبيوتا، بروبيوتيك، مكمل، مرض السكري نوع 2.