Clinical Nutrition 41 (2022) 365–373

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Meta-analyses

Meta-analysis of randomized controlled trials of the effects of probiotics on type 2 diabetes in adults

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ARTICLE INFO

Article history:
Received 13 November 2020
Accepted 26 November 2021

Keywords:
Probiotic
Randomized controlled trials
Type 2 diabetes
Glycemic homeostasis
Meta-analysis

SUMMARY

Background & aims: Despite advancements in preventive medicine and pharmacotherapy, diabetes remains an overwhelming health problem. Evidence from randomized controlled trials (RCTs) suggests that probiotics may offer beneficial effects on glycemic control. Our objective was to perform a systematic review and meta-analysis of RCTs to quantify the effect of probiotic administration on glycemic homeostasis in type 2 diabetes.

Methods: Medline, Web of Science, Google Scholar, and Cochrane Central Register of Controlled Trials were searched for relevant trials published until October 12, 2021. RCTs that lasted ≥3 weeks and assessed the effects of probiotics on the markers of glycemic homeostasis in type 2 diabetes were included. Data were pooled using the generic inverse variance method and expressed as mean differences (MDs) with 95% confidence intervals (CIs). Heterogeneity was assessed using Cochran's Q statistic and quantified using the I^2 statistic. The Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach was used to evaluate the certainty of evidence.

Results: A total of 33 eligible trial comparisons (n = 1927) were included in this meta-analysis. Our results revealed that compared with placebo, a median probiotic dose of ~10^9 cfu/day significantly reduced the glycated hemoglobin (HbA_{1c}) levels (MD: −0.19% [95% CI: −0.32, −0.07]; $P = 0.003$), fasting blood glucose levels (MD: −1.00 mmol/L [95% CI: −1.45, −0.56]; $P < 0.0001$), fasting insulin levels (MD: −5.73 pmol/L [95% CI: −12.17, 0.72]; $P = 0.08$), and HOMA-insulin resistance (IR) (MD: −1.00 [95% CI: −1.32, −0.68]; $P < 0.00001$). The certainty of evidence was graded low for HbA_{1c} and fasting glucose, moderate for fasting insulin, and high for HOMA-IR. Probiotic supplements do not induce clinically significant reductions in HbA_{1c} levels, but lead to marginally clinically significant reductions in fasting glucose and fasting insulin levels in patients with type 2 diabetes. Compared with single-strain and low-dose probiotics, multi-strain and high-dose probiotics have a greater beneficial effect on glycemic homeostasis. In addition, probiotic treatment may be more effective in patients with a high baseline body mass index and age.

1. Introduction

Diabetes is one of the most common chronic diseases worldwide, reported to have affected more than 463 million adults aged 20–79 years worldwide in 2019 (90% of these cases were of type 2 diabetes [T2D]); this number is estimated to increase to 700 million by 2045 [1]. In addition to adversely affecting the quality of life, diabetes places a huge economic burden on patients

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https://doi.org/10.1016/j.clnu.2021.11.037
0261-5614/

seeking treatment. The annual global medical expenditure for diabetes in 2019 was US $760 billion [1]. In recent years, the role of intestinal flora in regulating metabolism has become a hot research topic [2–4]. Probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [5]. Diet and lifestyle are the main pillars for managing T2D [6,7]; however, probiotic interventions for managing T2D are a more attractive option as their acceptability is higher and compliance to them is easier [8]. Several trials have evaluated the effects of probiotic supplementation on ameliorating T2D, and their findings suggest that probiotics regulate glycemic homeostasis by acting on the gut microbiota via the production of metabolites, such as short-chain fatty acids [9], or directly affecting host metabolism by interacting with Toll-like receptors [10].

Some recently published randomized controlled trials (RCTs) on the effects of probiotics against T2D have reported conflicting results [11–14]. Moreover, although some meta-analysis studies have reported favorable effects of probiotics in alleviating T2D, their clinical efficacy is still controversial due to small sample sizes, high risks of bias, and high heterogeneity in the individual trials and limitations of the analysis methods used [15–18].

The objective of this meta-analysis was to evaluate the effect of probiotic supplements on the glycemic parameters of T2D patients and the association of patient characteristics (i.e., age, sex, baseline body mass index [BMI], baseline fasting glucose, and baseline glycated hemoglobin [HbA_{1c}]) and intervention characteristics (i.e., probiotic dose, number of probiotic strains, and treatment duration) with the effect of probiotics on T2D.

2. Research design and methods

2.1. Protocol

This study was performed according to the Cochrane Handbook for Systematic Reviews of Interventions [19]. The reporting of results followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [20].

The protocol for this meta-analysis was registered in advance with PROSPERO (No. CRD42020184928).

2.2. Data sources and search strategy

We searched Medline, Google Scholar, Web of Science, and the Cochrane Central Register of Controlled Trials for relevant trials published until October 12, 2021, and manually searched their reference lists for relevant reviews. No language restrictions were applied. The search terms we used are listed in Supplementary Table 1. The management of study details was conducted using EndNote X8.

2.3. Study selection and eligibility criteria

RCTs were included if they fulfilled the following criteria: included patients with T2D aged ≥18 years with ≥3 weeks in duration [21] and investigated the effects of probiotic supplementation (*Lactobacillus*, *Bifidobacterium*, or yeast) on at least one of the measures-HbA_{1c}, fasting insulin, fasting glucose, and HOMA-insulin resistance (HOMA-IR)-in comparison with an appropriate control (i.e., probiotic-free administration or placebo). For multi-arm trials, the trials distinguishing the effects of probiotic supplements from those of the control treatments were included. We determined the criteria of glycemic homeostasis outcomes in accordance with the guidelines of the American Diabetes Association [22].

2.4. Data extraction

Two authors (C.C.Z. and Q.X.Z.) independently identified and extracted data from the included studies. The extracted data elements included study characteristics (design, blinding, background diet, sample size, country where the research was conducted, statistical analysis, and funding sources), participants' characteristics (i.e., sex, age, baseline BMI, baseline fasting glucose, and baseline HbA_{1c}), and intervention characteristics (i.e., probiotic dose, number of probiotic species, treatment duration, and dropout rate). Disagreement between reviewers was resolved by discussing with a third researcher. The mean and standard deviation (SD) values were extracted for HbA_{1c}, fasting glucose, fasting insulin, and HOMA-IR as change from baseline for both intervention and control groups. For trials that did not report the SD values, we calculated them from the available data (95% confidence intervals [CIs] or standard error of the mean [SEM]) using the standard formulas [23]. For any other missing outcome data, the authors were contacted directly.

2.5. Data synthesis and analysis

Review Manager 5.3 (Cochrane Collaboration, Oxford, UK) was used for primary data analyses and subgroup analyses of the pooled data. Comprehensive Meta-Analysis 2.0 (Biostat, Englewood, NJ, USA) was used for dose–response, sensitivity, meta-regression, and publication bias analyses. The mean difference (MD) with 95% CI was used to express the outcome, and $P < 0.05$ was considered as significant. When the changes in parameters from baseline were not reported, they were calculated as the difference between baseline values and post-intervention values for HbA_{1c}, fasting insulin, fasting glucose, and HOMA-IR. When HOMA-IR was not reported, it was calculated using the following equation: HOMA-IR = fasting glucose (mmol/L) × fasting insulin (mU/L)/22.5 [23]. Correlation coefficients between baseline and end-of-treatment values within each individual crossover trial were derived from the reported within- and between-treatment SD according to a published formula. These correlation coefficients were transformed into z-scores ± SD, meta-analyzed using inverse-variance weighing and back-transformed to derive the pooled correlation coefficient. For end points when a pooled correlation coefficient for imputing missing SD could not be derived, a value of 0.50 was assumed, as it is a conservative estimate for an expected range of 0–1. To assess the robustness of the effect size, sensitivity analysis was performed by varying the correlation coefficient values (0.25 and 0.75).

The generic inverse variance method with random-effects model was used for pooled estimates. When an outcome was reported in five or fewer of the RCTs, the fixed-effects model was used. The I^2 statistic and Cochran's Q-test were used to assess heterogeneity, where $I^2 > 50\%$ and $P < 0.10$ were considered to indicate substantial heterogeneity and significance, respectively [19]. Sources of heterogeneity in participants' characteristics (i.e., sex, age, and baseline values), intervention characteristics (i.e., probiotic dose, number of probiotic species, and treatment duration), and food matrix (i.e., capsule, tablet, and food) were explored with subgroup analyses or meta-regression when 10 or more trials were included for an outcome. To determine whether a single study had an undue influence on the overall results, we conducted a sensitivity analysis, explored heterogeneity by removing trials one by one, and reassessed the pooled effect estimates. Dose–response analysis was conducted using random-effects meta-regression to obtain dose estimates. First, larger studies have more influence on the relationship than smaller studies, since studies are weighted by the precision of their respective effect estimate. Second, it is wise to allow for the residual heterogeneity among intervention effects not

modelled by the explanatory variables. The regression coefficient obtained from a meta-regression analysis will describe how the outcome variable (the intervention effect) changes with a unit increase in the explanatory variable (the potential effect modifier). The statistical significance of the regression coefficient is a test of whether there is a linear relationship between intervention effect and the explanatory variable. To evaluate publication bias in each included study, visual inspection of funnel plots was performed using Begg's rank correlation test and Egger's regression intercept test. If funnel plot asymmetry was suspected, asymmetry was corrected for and missing study data were imputed using the trim-and-fill method by Duval and Tweedie. P values < 0.05 were considered to indicate significance.

2.6. Quality assessment

The risk of bias of studies was assessed using the Cochrane risk-of-bias tool [19]. Domains of bias assessment included randomized sequence generation, allocation concealment, blinding of participants and outcome assessment, incomplete outcome data, and selective reporting. A trial was considered to have a low risk of bias when proper methods were used to reduce bias, a high risk of bias when improper study methods likely affected the true outcome, and an unclear risk of bias when no sufficient information was provided to judge the bias level.

The overall quality of evidence was evaluated using the GRADE method [19], wherein RCTs are graded to have very low, low, moderate, or high quality of evidence and then downgraded based on their risk of bias (weight of studies assessed by the Cochrane risk-of-bias tool), inconsistency (substantial unexplained inter-study heterogeneity, $I^2 > 50\%$, $P < 0.10$), indirectness (presence of factors that limit the generalizability of the results), imprecision (95% CI for the pooled effect estimates that are wide or overlap the minimum clinically important differences of 0.3% for HbA_{1c} [24], 0.5 mmol/L for fasting glucose, and 5 pmol/L for fasting insulin [25], and publication bias (significant evidence of small-study effects).

3. Results

3.1. Search results

The literature search and study selection process are presented in Fig. 1. Our initial search identified 2966 reports, 2905 of which were excluded based on the review of title/abstract and elimination of duplicates. Whole texts of the remaining 61 studies were reviewed, 29 of which (33 trial comparisons) were included in the meta-analysis ($n = 1927$) [11–14,26–50]. 29 trails were identified, 4 of which included two intervention groups. These groups were treated as separate studies, resulting in the inclusion of 33 trials in the meta-analysis. Of the 33 trial comparisons, 19 reported HbA_{1c} ($n = 1138$) [11,12,27,28,31,32,35,37,38,40,41,43,44,46,48–50] 33 reported fasting glucose ($n = 1927$) [11–14,26–50], 21 reported fasting insulin ($n = 1196$) [11–14,26–33,35,38,41,42,44,46], and 16 reported HOMA-IR directly or provided enough information for calculation ($n = 957$) [11–14,26,28–30,33,38,39,42,43,46].

3.2. Trial characteristics

The characteristics of the included studies are provided in Table 1. Most of the trials were conducted in clinics: 3 (10.3%) in Europe, 7 (24.1%) in Asia, 1 (3.4%) in South America, and 18 (62.1%) in the Middle East. The median duration of the trials was 8 weeks (range 4–16). The median dose of probiotic administration was 10^{10} cfu/day (range 10^7–10^{12}). Most participants were middle aged (median age: 53.0 [range 25–75] years). The median baseline BMI and HbA_{1c} levels of the participants across the trials were 29.8 (range 24.4–31.3) kg/m^2 and 7.4% (range 6.9%–9.1%), respectively. The Cochrane risk-of-bias tool (Supplementary Fig. 1) showed that 23 trials (69.7%) had unclear risk of bias and 10 trials (30.3%) had low risk of bias for random sequence generation. Further, 23 trials (69.7%) had unclear risk of bias for allocation concealment; 21 (63.6%) had unclear risk, 10 trials (30.3%) low risk of bias, and 2 trials (6.1%) high risk of bias for blinding of participants and personnel; and 17 trials (61%) had low risk of bias, 6 (18.2%) had unclear risk of bias, and 5 (15.2%) had high risk of bias for incomplete outcome data. 20 trials (60.6%) had low risk of bias and 3 (9.1%) had high risk of bias for selective outcome reporting. Funding sources included agency for 14 trials (42.4%), industry for 5 (15.2%), agency–industry for 5 (15.2%), and were not reported for 4 (12.1%).

3.3. Effect on HbA_{1c}

Nineteen studies (19 comparisons) with a total of 1138 patients assessed the effects of probiotics on the HbA_{1c} levels of T2D patients. Compared with placebo, a median probiotic dose of 3×10^9 cfu/day for a median duration of 8 weeks was found to marginally reduce the HbA_{1c} levels (MD: −0.19% [95% CI: −0.32, −0.07]; $P = 0.003$), with evidence of substantial inter-study heterogeneity ($I^2 = 84\%$, $P < 0.00001$) (Fig. 2A). Systematic removal of individual studies one by one did not change the results or explain the heterogeneity. Subgroup analysis showed that probiotic administration in capsule/tablet matrix (MD: −0.55% [95% CI: −0.88, −0.22]; $P = 0.001$) and higher baseline BMI (MD: −0.29% [95% CI: −0.60, 0.02]; $P = 0.0009$) were significantly associated with reduced HbA_{1c} levels (Supplementary Table S2). However, multivariable meta-regression did not reveal any effect of participants' age, baseline HbA_{1c}, baseline BMI, treatment duration, probiotic dose, or number of probiotic strains on the amount of change in HbA_{1c} levels ($P > 0.05$ for all) (Supplementary Table S6).

3.4. Effect on fasting glucose

Thirty-three studies (33 comparisons) with a total of 1927 patients assessed the effects of probiotic administration on the fasting glucose levels of T2D patients. Compared with placebo, a median probiotic dose of 10^9 cfu/day for a median duration of 8 weeks was found to significantly reduce the fasting glucose levels (MD: −1.00 mmol/L [95% CI: −1.45, −0.56]; $P < 0.0001$), with evidence of substantial heterogeneity ($I^2 = 94\%$, $P < 0.00001$) (Fig. 2B). Systematic removal of individual trials one by one did not change the results or explain the heterogeneity. Subgroup analysis showed that multi-species probiotics significantly decreased the fasting glucose levels (MD: −0.92 mmol/L [95% CI: −1.31, −0.53]; $P < 0.00001$), but not single-species probiotics (MD: −0.48 mmol/L [95% CI: −1.09, 0.13]; $P = 0.12$) except brewer's yeast (MD: −1.48 mmol/L [95% CI: −2.69, −0.27]; $P = 0.02$) (Supplemental Fig. S2). However, the number of included studies is small for most subgroups. For half of the species identified, the conclusion is drawn from an estimate of a single study, including for *Brewer's* yeast. In addition, a higher probiotic dose ($>1 \times 10^9$ cfu/day), greater treatment duration (>8 weeks), a higher baseline BMI (>29.75 kg/m^2), a higher baseline fasting glucose levels (>8.56 mmol/L), and probiotic administration in capsule/tablet matrix were significantly associated with reduced fasting glucose levels, with significant heterogeneity still present across studies (Supplementary Table S3). Multivariable meta-regression analysis revealed a greater reduction of fasting glucose levels in trials that administered higher probiotic doses ($P = 0.021$) (Supplementary Table S6).

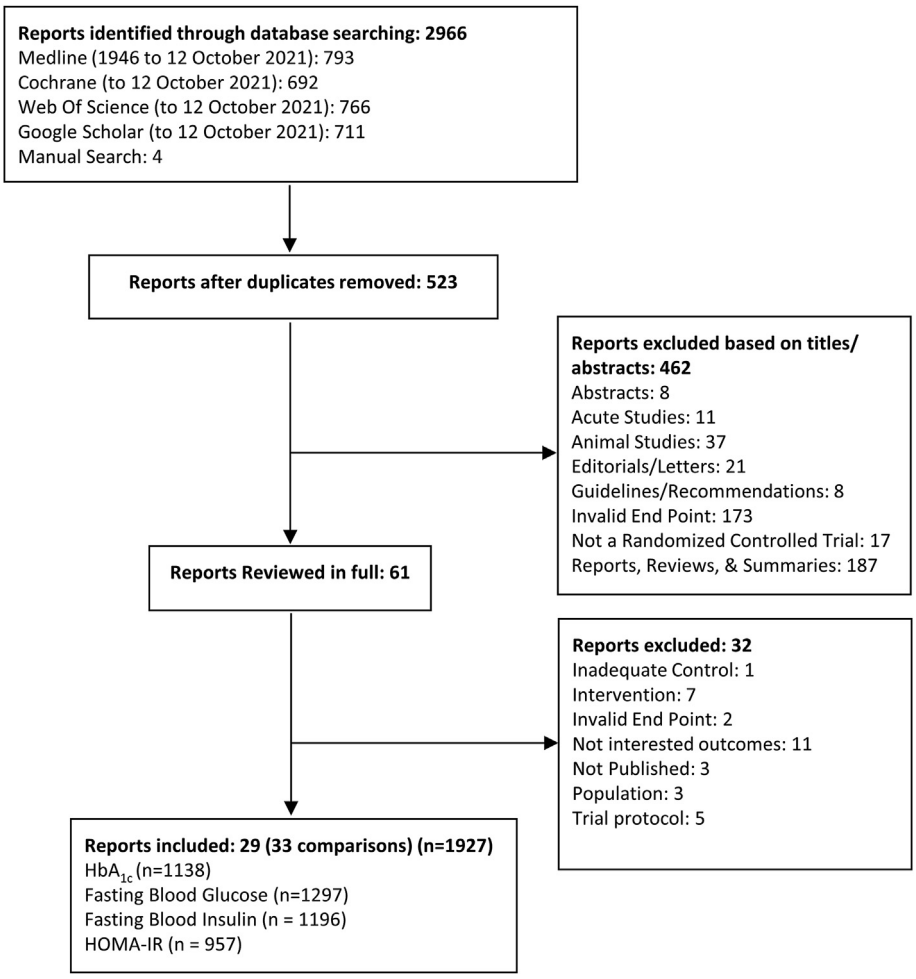

Fig. 1. Flow diagram of identified citations and included studies.

3.5. Effect on fasting insulin

Twenty-one studies (21 comparisons) with a total of 1196 patients assessed the effects of probiotic administration on the fasting insulin levels of T2D patients. Compared with placebo, a median probiotic dose of 8×10^9 cfu/day for a median duration of 8 weeks was found to reduce the fasting insulin levels (MD: −5.73 pmol/L [95% CI: −12.17, 0.72]; $P = 0.08$), although in a non-significant manner, with evidence of substantial interstudy heterogeneity ($I^2 = 91\%$, $P < 0.00001$) (Fig. 2C). A sensitivity analysis indicated that the study of Kobyliak et al. [41] had contributed to the heterogeneity. Elimination of that study reduced the heterogeneity ($I^2 = 86\%$, $P = 0.004$) and modified the effect size (MD: −8.55 pmol/L [95% CI: −14.33, −2.76], $P < 0.0001$) (Supplementary Table S4), but the heterogeneity still remained significant. Subgroup analysis indicated that probiotic administration in capsule/tablet matrix (MD: −10.27 pmol/L [95% CI: −19.88, −0.66], $P = 0.04$), higher mean age (MD: −10.53 pmol/L [95% CI: −18.73, −2.33], $P = 0.01$) and higher mean BMI (MD: −15.24 pmol/L [95% CI: −20.06, −10.42], $P = 0.0007$) were associated with reduced fasting insulin levels (Supplementary Table S4). In particular, the reduction in fasting insulin levels became more significant with the increase in the participants' age and baseline BMI ($P = 0.00$ for age, $P = 0.030$ for baseline BMI) (Supplementary Table S6).

3.6. Effect on HOMA-IR

Sixteen studies with a total of 957 patients assessed the effects of probiotic supplementation on the HOMA-IR of T2D patients. Compared with placebo, a median probiotic dose of 1.1×10^9 cfu/day for a median duration of 8 weeks was found to significantly reduce HOMA-IR (MD: −1.00 [95% CI: −1.32, −0.68]; $P < 0.00001$), with evidence of substantial interstudy heterogeneity ($I^2 = 62\%$, $P = 0.0006$) (Fig. 2D). Systematic removal of individual trials did not change the results or explain the heterogeneity. Subgroup analysis showed that higher baseline BMI was significantly associated with greater reduction in HOMA-IR (MD: −1.56 [95% CI: −2.16, −0.95], between-subgroup difference, $P = 0.02$) (Supplementary Table S5). However, multivariate meta-regression indicated no significant effects of participants' mean age, baseline HOMA-IR, baseline BMI, probiotic dose, treatment duration, or number of probiotic strains on HOMA-IR ($P > 0.05$ for all) (Supplementary Table S6).

3.7. Dose–response analyses

Our results showed significant dose–response effects on fasting glucose levels ($P = 0.00$) and HOMA-IR ($P = 0.00$), but not HbA_{1c} ($P = 0.40$) and fasting insulin ($P = 0.07$) levels (Supplemental Fig. S4). In particular, an increase in the probiotic dose was found to substantially reduce the fasting glucose levels and HOMA-IR.

Table 1
Characteristics of included trail comparisons.

Trail	Age, years	Participants	Dose (CFU/day)	Duration, weeks	Form	Design, blinding	Control	BMI, kg/m^2	Diet	Founding	Country	Body weight, kg
Khalili et al., 2019	C: 45.0 T: 44.0	40 (13M:26F)	10^8	8	Capsule	P, DB	Maltodextrin	C: 31.94 T:29.50	Usual	NR	Iran	C: 83.45 T: 77.15
Mazloom et al., 2013	C: 51.8 T: 55.4	34	NR	6	Capsules	P, SB	Magnesium stearate	C: 27.24 T: 27.97	NR	NR	Iran	C: 68.55 T: 74.56
Firouzi et al., 2016	C: 54.2 T: 52.9	136 (65M:71F)	6×10^{10}	12	Powder	P, DB	Placebo	C: 28.4 T: 29.5	NCEP	A-I	Malaysia	C: 74.2 T: 75.3
Ebrahimi et al., 2017	C: 58.6 T: 58.7	70 (32M:28F)	NR	9	Capsules	SC, DB	Row starch	C: 27.3 T: 28.13	Usual	A	Iran	C: 74.61 T: 77.59
Asemi et al., 2013	C: 58.6 T: 58.7	54	3.92×10^{10}	8	Capsules	P, DB	Placebo	C: 30.17 T: 29.91	Usual	A	Iran	C: 73.03 T: 72.42
Asemi et al., 2015	52.9 ± 8.1	102	2.7×10^8	6	Package	Cr, DB	Isomalt, sorbitol and stevia	C: 30.15 T: 29.88	Usual	A	Iran	C: 78.28 T: 77.59
Asemi et al., 2014	53.1 ± 8.7	62	2.7×10^8	6	Synbiotic food	Cr, DB	Control food	C: 29.9 T: 29.6	Usual	A	Iran	C: 75.42 T: 74.88
Tonucci et al., 2015	C: 51.0 T: 51.8	45 (26M:19F)	10^9	6	Fermented goat milk	P, DB	Conventional fermented goat milk	C: 27.94 T: 27.49	Usual	A	Brazil	C: 77.15 T: 71.7
Ejtahed et al., 2012	C: 51.0 T: 50.9	60 (23M:37F)	4×10^9	6	Yogurt	P, DB	Conventional yogurts	C: 29.14 T: 28.95	Usual	I	Iran	C: 75.42 T: 76.18
Sabico et al., 2017	C: 46.6 T: 48.0	78 (40M:38F)	10^{10}	12	Powder	SC, DB	Maize starch and maltodextrins	C: 30.1 T: 29.4	NR	I	Saudi Arabia	C: 79.5 T: 75.6
Ostadrahimi et al., 2015	35 to 65	60 (32M:28F)	4.6×10^{10}	8	Fermented milk	NR, DB	Conventional fermented milk (dough)	C: 27.47 T: 28.89	Usual	NR	Iran	C: 74.92 T: 77.46
Mobini et al., 2017	C: 65 TA: 66 TB: 64	44 (34M:10F)	10^8 or 10^{10}	12	Powder	P, TB	Placebo	C: 30.7 TA: 30.6 TB: 32.3	Usual	A	Sweden	C: 93.5 TA: 93.1 TB: 101.4
Feizollahzadeh et al., 2016	C: 53.6 T: 56.90	40 (19M:21F)	10^7	8	Soy milk	P, DB	Conventional soy milk	C: 26.58 T: 26.68	Usual	A	Iran	C: 71.61 T: 70.84
Sabico et al., 2017	C: 46.6 T: 48.0	61 (26M:35F)	4×10^{10}	24	Powder	P, DB	Maize starch and maltodextrins	C: 30.1 T: 29.4	NR	A	Saudi Arabia	NR
Junko et al., 2017	C: 65.0 T: 64.0	68 (49M:19F)	4×10^{10}	16	Fermented milk	P, DB	Not receive a probiotic intervention	C: 23.9 T: 24.2	Restrict calorie intake	A-I	Japan	NR
Hove et al., 2015	C: 60.6 T: 58.5	41	NR	12	Yogurt	P, DB	Artificially acidified milk	C: 27.7 T: 29.2	Usual	A-I	Denmark	C: 85.2 T: 93.2
Shakeri et al., 2014	C: 53.1 TA: 52.3 TB: 52.3	78	1.2×10^{10}	8	Breads	P, TB	Control bread	C: 30.6 TA: 29.5 TB: 30.9	Usual	A-I	Iran	C: 76.9 TA: 74.4 TB: 80.8
Ebrahimi et al., 2016	C: 64.0 T: 64.2	60	6×10^9	12	Capsules	P, db	Placebos (starch)	C: 29.6 T: 32.3	Usual	A	Iran	C: 74.3 T: 79.2
Mohamadshahi et al., 2014	C: 49 T: 53	44	1.1×10^9	8	Yogurt	P, NR	Conventional yogurts	C: 29.22 T: 28.36	Usual	A	Iran	C: 79.33 T: 74.66
Kobyliak et al., 2018	C: 57.18 T: 52.23	53	10^{12}	8	“Symbiter”	SCP, DB	Placebos	C: 35.65 T: 34.70	NECP	I	Ukraine	C: 96.95 T: 99.32
Ebrahimi et al., 2014	C: 53.4 TA: 51.3 TB: 52	81 (15M:66F)	1.2×10^{10}	8	Breads	P, TB	Control bread	C: 30.5 TA: 29.8 TB: 30	Usual	A	Iran	C: 76.8 TA: 80.6 TB: 75.1
Hosseinzadeh et al., 2014	C: 45.7 T: 46.8	84 (21M:63F)	1.8g	12	Tablets	P, DB	Placebos	C: 29.9 T: 30.0	NR	A	Iran	NR
Judiono et al., 2014	NR	NR	2×10^9	4	Milk	P, TB	NR	NR	A standard diet	I	Indonesian	NR
Razmpoosh et al., 2018	C: 61.3 T: 58.6	60 (33M:27F)	4.9×10^{10}	6	Capsules	P, DB	Fructo-oligosaccharide and magnesium stearate	C: 27.1 T: 27.7	NR	NR	Iran	C: 73.8 T: 75.2
Chaiyasut et al., 2021	C:58.9 T:54.8	72	10^{11}	12	Sachet	P, DB	Corn starch	C:30.0 T:29.0	Usual	A	Thailand	C: 68.2 T: 69.1
Toejing et al., 2021	C:61.8 T:63.5	36 (8M; 28F)	5×10^{10}	12	Sachet	P, DB	Corn starch	C23.1 T:23.2	Usual	A	Thailand	NR
Jiang et al., 2020	C:27.5 T:26.4	76 (27M,49F)	9.7×10^9	12	Sachet	P, DB	Starch	C:27.5 T:26.4	Usual	I	China	NR
Madempudi et al., 2019	C:50.6 T:54.1	79	3×10^{10}	12	Capsules	P, DB	Maltodextrin	NR	Usual	I	India	C: 67.6 T: 67.0
Kanazawa et al., 2021	C:55.9 T:61.1	86	6.5×10^8	24	Powder	P, DB	Placebos	C:29.1 T:29.5	Usual	I	Japan	NR

A, agency; A-I, agency–industry; *B., Bifidobacterium*; Ba, baseline; Cn, control; Cr, crossover; DB, double blind; Int, Intervention; *L., Lactobacillus*; *S., Streptococcus*; NCEP, National Cholesterol Education Program; NR, not report; FG, fasting blood glucose; HOMA-IR, homeostasis model of assessment–insulin resistance; HOMAB, homoeostatic model assessment-beta cell function; P, parallel; SB, single blind; SC, single center; T, treatment; TA, treatment A; TB, treatment B. * Means values ± SDs presented.

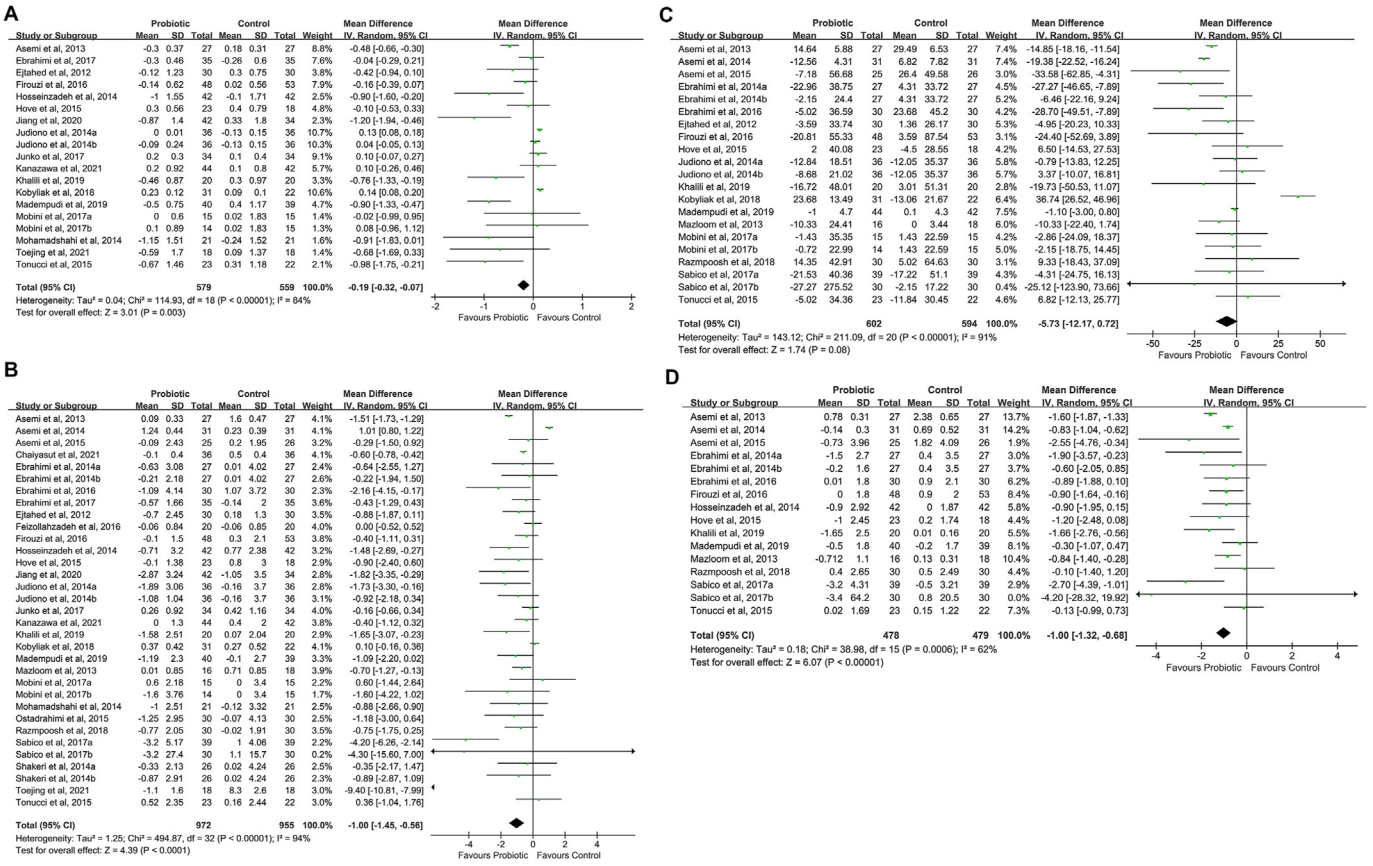

Fig. 2. Forest plot of the meta-analysis on the effect of probiotic supplementation on (A)HbA_{1c}, (B) fasting glucose, (C) fasting insulin, and (D) HOMA-IR. Diamond represents the pooled effect estimate for overall. Standardized mean difference were calculated with the inverse-variance method in a random-effect model. Interstudy heterogeneity is quantified by I^2 with significance $P < 0.05$.

3.8. Publication bias

Supplementary Fig. S5 shows the funnel plots for HbA_{1c}, fasting glucose, fasting insulin, and HOMA-IR. Visual inspection of the funnel plots indicated no asymmetry in the fasting glucose, insulin, and HOMA-IR data and mild asymmetry in the HbA_{1c} data. The results of Egger's and Begg's tests were not significant for the evidence of small-study effects. The trim-and-fill analyses performed for HbA_{1c} identified three additional trials that were imputed to adjust for funnel plot asymmetry. After imputing the trials, fasting insulin results showed an adjusted MD of −0.15 pmol/L (95% CI: −0.30, 0.00; $P = 0.04$), suggesting evidence of small-study effects (Supplementary Fig. S6).

3.9. Grading of the evidence

The summary of the GRADE assessment for each outcome is shown in Supplementary Table S7. The effect estimates of HbA_{1c} and fasting glucose were graded as low quality due to downgrades for serious inconsistency and imprecision. The effect estimates of fasting insulin were graded as moderate quality based on downgrades for serious imprecision (Supplementary Table S7).

3.10. Adverse events

Thirteen studies provided information about adverse events, and they reported no serious adverse events. Most of the observed adverse effects were minor gastric disturbances [41,51]. In one trial, a participant withdrew due to abdominal discomfort [51]. In another trial, one patient complained of short-term diarrhea and nausea, and two other patients had mild abdominal pain [41].

4. Discussion

This meta-analysis quantified the effects of probiotics on indices of glycemic homeostasis in 33 RCT comparisons that included 1927 T2D patients. Pooled estimates indicated that a median probiotic dose of ~10^{10} cfu/day for a median duration of 8 weeks led to an absolute reduction of 0.19% in the HbA_{1c} level, 1.00 mmol/L in the fasting glucose levels, 5.73 pmol/L in the fasting insulin levels, and 1.00 in HOMA-IR. Our analyses suggest that probiotic supplementation does not induce a clinically meaningful reduction in the HbA_{1c} level, as the reduction was not found to meet the FDA-established threshold of clinical significance (≥0.3%) for new anti-hyperglycemic drug development [24]. Although subgroup analysis showed that probiotics in the capsule/tablet form induced a greater reduction in the HbA_{1c} level that met the established threshold (≥0.3%), this subgroup effect was analyzed only in four of the included trials and showed high heterogeneity across studies (Supplementary Table S2). The reductions in fasting glucose and fasting insulin levels met the thresholds for clinical significance [25]. Our results are similar to those of another meta-analysis of 15 RCTs [16], which reported a change of −0.24% (95% CI [−0.44, −0.04]) in the HbA_{1c} level, − 0.44 mmol/L (95% CI [−0.74, −0.15]) in the fasting glucose levels, and −1.07 (95% CI [−1.58, −0.56]) in the HOMA-IR of T2D patients with high BMI following probiotic intake, supporting that probiotic supplementation does not induce clinically meaningful reductions in the

HbA_{1c} level. Previous two RCT study [47,50] revealed that oral supplementation of a mixture of *Lactobacillus paracasei, Bifidobacterium longum* and *Bifidobacterium breve* or *L. paracasei* HII0 for 12 weeks did not improve glycemic homeostasis, which are conflicting with the results of previous meta-analysis [52–54]. Another three RCT trail [46,48,49] demonstrated that probiotics reduced glycosylated hemoglobin (HbA1c), a biomarker that has been rarely analyzed in previous meta-analysis studies in patients. Therefore, we think these five references provide updated information and should be considered in our present meta-analysis.

Our meta-analysis suggested that the improvement in the glycemic homeostasis profile of T2D patients depends on a variety of factors including treatment characteristics (i.e., probiotic dose and number of probiotic strains) and patient's characteristics (i.e., BMI and age).

Our subgroup analyses demonstrated greater reductions in the fasting glucose levels of patients receiving multi-strain probiotics. Consistent with our finding, Chapman et al. reported that multi-strain probiotic mixtures had more health benefits than their single strain components [55]. The underlying mechanism may be cooperative interactions between different probiotic strains. This may be due to interact with one another as functional groups to improve host glycemic parameters [4]. Our results indicated that in addition to the number of probiotic strains, the types of strain (e.g., *Bifidobacterium*, *Lactobacillus*, and yeast) determine the effectiveness of probiotics. Specifically, Brewer's yeast significantly reduced the fasting glucose levels, whereas *Lactobacillus casei* and *Lactobacillus sporogenes* had no effects when administered as single strain formulas.

Further, our results indicated a dose–response effect of probiotics on the fasting glucose levels and HOMA-IR-specifically, greater reductions in these measures were found with higher probiotic doses. Survival of probiotic strains through the gastrointestinal environment, which includes acids, bile salts, and various digestive enzymes, to reaching colon is considered a key requirement of probiotics, which can be affected by host gastrointestinal environment. After oral supplement, probiotic encounters acids, bile salts, and various digestive enzymes when pass through the gastrointestinal tract, and eventually reach the colon [56]. As some probiotic strains have a low survival rate, high probiotic doses may ensure the survival of sufficient numbers of live strains during the gastrointestinal transit. Supporting these analyses, probiotic administration in capsule/tablet showed a more significant effect. However, those studies included in our meta-analysis did detect the abundance of live bacteria in the colon after administration.

Subgroup analysis showed that the effectiveness of probiotics on the management of glycemic profile is influenced by patients' BMI. In particular, probiotics are more effective in reducing the fasting insulin levels and HbA_{1c} levels of T2D patients with obesity, probably via altering the composition of their gut microbiota. The gut microbial balance in obese T2D patients is known to be disturbed [57], which has been demonstrated to be restored by probiotic administration [3,58,59]. Obesity increases the risk of metabolic disorders, such as insulin resistance, hyperglycemia, and hyperlipidemia. The mechanisms underlying the beneficial effects of probiotics on metabolic disorders present in obesity may include the modulation of immunological responses and altered production of intestine-derived metabolites [60–62].

Meta-regression analysis based on the mean age of the participants suggested that probiotic-induced improvements of the glucose homeostasis profile were more significant in older than in younger patients. With increasing age, the incidence of metabolic diseases such as metabolic syndrome, obesity, T2D and cardiovascular disease increases [63]. Aging is also associated with gut microbial disturbance, including reduction in the abundance of beneficial bacteria in the feces, such as *Bifidobacterium* [64,65]. The reduction of endogenous *Bifidobacterium* abundance in the host intestinal tract is beneficial for the colonization of exogenous *Bifidobacterium* due to low out-competition [66]. In addition, because the baseline fasting insulin levels in the elderly tend to be high, it is easier to observe the effects of probiotics on fasting insulin in this group.

This meta-analysis has several strengths. First, to the best of our knowledge, this study is one of the largest and most comprehensive meta-analyses of RCTs evaluating the effects of probiotics on T2D. Second, we used the GRADE approach to evaluate the evidence quality and certainty. Third, the included trials were from multiple countries, which makes our findings more generalizable and reduces potential confounders related to a single geographic location. However, this meta-analysis also has some limitations. First, some unexplainable heterogeneity in the results of fasting blood glucose levels and HOMA-IR ($I^2 > 50\%$ and $P < 0.10$) remained even after sensitivity analysis, subgroup analysis, and meta-regression analysis, which reduced the certainty of evidence. Second, we downgraded the certainty of evidence for the HbA_{1c}, fasting glucose, and fasting insulin results due to serious imprecision as the 95% CIs of their effect estimates overlapped the minimum clinically important difference required for clinical benefit. Third, only nine trials had a treatment duration of $\geq$12 weeks and eight trials had <8 weeks. The HbA_{1c} level reflects the blood glucose levels over the preceding 12 weeks; therefore, inclusion of shorter duration trials might have led to underestimation of the effect size. Fourth, our analysis revealed evidence of publication bias for many studies. However, we elected to not downgrade the certainty of evidence for HbA_{1c} for publication bias, because the results of Egger's and Begg's tests for HbA_{1c} were not significant, and the adjusted pooled effect estimate after the trim-and-fill analyses did not change the direction or significance of the findings, although visual inspection of funnel plots showed asymmetry for the HbA_{1c} data. After balancing these strengths and limitations, we graded the certainty of evidence as high for HOMA-IR, moderate for fasting insulin, and low for HbA_{1c} and fasting glucose.

5. Conclusion

In summary, compared with placebo, probiotic supplementation did not lead to clinically significant reductions in the HbA_{1c} levels of middle-aged T2D patients. The reductions in fasting glucose and fasting insulin levels were of marginal clinical significance. Additional high-quality RCTs are required to further improve the certainty of the estimates.

Funding

This work was supported by the Natural Science Foundation of Jiangsu Province [BK20200084]; The National Natural Science Foundation of China [No. 31972971 and U1903205]; and Collaborative Innovationcenter of Food Safety and Quality Control in Jiangsu Province.

Author contributions

C.C.Z., J.X.Z., W.C., and Q.X.Z. designed the research. C.W., and F.W.T. conducted the research. J.C.J., L.L.Y., and C.W. performed or assisted in performing the statistical analysis of the data, C.C.Z., J.C.J., S.J.L., L.L.Y., and H.Z. wrote the manuscript draft. C.C.Z., and Q.X.Z had primary responsibility for the final content. All authors contributed to the critical revision of the manuscript for important intellectual content and approved the final manuscript.

Conflict of interest

The authors declare that they have no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2021.11.037.

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