

# Effect of probiotics on metabolic profiles in type 2 diabetes mellitus

## A meta-analysis of randomized, controlled trials

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### Abstract

Type 2 diabetes mellitus (T<sub>2</sub>DM) is a prevalent metabolic disease which is imposing heavy burden on global health and economy. Recent studies indicate gut microbiota play important role on the pathogenesis and metabolic disturbance of T<sub>2</sub>DM. As an effective mean of regulating gut microbiota, probiotics are live micro-organisms that are believed to provide a specific health benefit on the host. Whether probiotic supplementation could improve metabolic profiles by modifying gut microbiota in T<sub>2</sub>DM or not is still in controversy.

The aim of the study is to assess the effect of probiotic supplementation on metabolic profiles in T<sub>2</sub>DM.

We searched PubMed, EMBASE, and Cochrane Library up to 12 April 2016. Two review authors independently assessed study eligibility, extracted data, and evaluated risk of bias of included studies. Data were pooled by using the random-effect model and expressed as standardized mean difference (SMD) with 95% confidence interval (CI). Heterogeneity was assessed and quantified ( $I^2$ ).

A total of 12 randomized controlled trials (RCTs) were included. Lipid profiles (n=508) and fasting blood glucose (FBG) (n=520) were reported in 9 trials; the homeostasis model of assessment for insulin resistance index (HOMA-IR) (n=368) and glycosylated hemoglobin (HbA1c) (n=380) were reported in 6 trials. Probiotics could alleviate FBG (SMD -0.61 mmol/L, 95% CI [-0.92, -0.30],  $P=0.0001$ ). Probiotics could increase high-density lipoprotein-cholesterol (HDL-C) (SMD 0.42 mmol/L, 95% CI [0.08, 0.76],  $P=0.01$ ). There were no significant differences in low-density lipoprotein-cholesterol (LDL-C), total cholesterol (TC), triglyceride (TG), HbA1c and HOMA-IR between the treatment group and the control group.

Probiotics may improve glycemic control and lipid metabolism in T<sub>2</sub>DM. Application of probiotic agents might become a new method for glucose management in T<sub>2</sub>DM.

**Abbreviations:** CI = confidence interval, CNS = central nervous system, FBG = fasting blood glucose, GIP = glucose-dependent insulinotropic peptide, GLP = glucagon-like peptide, HbA1c = glycosylated hemoglobin, HDL-C = high-density lipoprotein-cholesterol, HOMA-IR = homeostasis model of assessment for insulin resistance index, LDL-C = low-density lipoprotein-cholesterol, LPS = lipopolysaccharides, MS = metabolism syndrome, NF- $\kappa$ B = nuclear factor kappa B, PYY = peptide YY, RCTs = randomized controlled trials, SMD = standardized mean difference, T<sub>2</sub>DM = type 2 diabetes mellitus, TC = total cholesterol, TG = triglyceride, TLR = toll-like receptor.

### 1. Introduction

T<sub>2</sub>DM is a common metabolic disorder in the world, which is characterized by hyperglycemia due to insulin resistance and

relative insulin deficiency. Over the past decades, the incidence of T<sub>2</sub>DM increased rapidly. According to the recent study, 592 million people worldwide will suffer from diabetes by the year 2035.<sup>[1]</sup> Abnormal metabolic profiles in T<sub>2</sub>DM could lead to several severe complications, such as cardiovascular disease, diabetic retinopathy, neuropathy, and nephropathy.<sup>[2]</sup> Diabetes is exerting heavy burden on global health and becoming one of the main causes of death around the world.<sup>[3]</sup>

Recent studies indicate gut microbiota play important role on the pathogenesis and metabolic disturbance of T<sub>2</sub>DM. Gut microbiota consisting of at least 10<sup>14</sup> bacteria of different species is virtually viewed as a complex whole ecosystem.<sup>[4]</sup> The genome of the entire gut microbiota named as “microbiome” exceeds the human nuclear genome by at least 100 times.<sup>[5]</sup> There is increasing evidence that gut microbiota play important role on energy homeostasis through the microbe-gut-brain axis.<sup>[6]</sup> Changes in gut microbiota could alter enteroendocrine signals sent to the Central Nervous System (CNS). The gut hormones such as glucose-dependent insulinotropic peptide (GIP), glucagon-like peptide (GLP), peptide YY (PYY) could affect  $\beta$ -cell function, insulin secretion, and regulate energy homeostasis through insulinotropic, satiety properties.<sup>[7]</sup> Disturbance of the normal gut microflora by having too much high fat and fructose food could induce systemic, low grade chronic

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CFL and XL contributed equally to this study.

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inflammation, and cause metabolic diseases such as obesity and T<sub>2</sub>DM.<sup>[8]</sup> It is reported that gut microbiota between adults with T<sub>2</sub>DM and nondiabetic adults is quite different.<sup>[9]</sup> The content of *Bifidobacteria* decreased, whereas *Enterococci* and *Escherichia coli* increased significantly.<sup>[10]</sup> The gut microbiota could influence inflammatory pathway and energy metabolism of host, in other words, alteration of the gut microbiota could affect glucose, lipid metabolism, and insulin action. One of the most effective methods of maintaining the balance of the intestinal microbiota is the use of probiotics, which is defined as live micro-organisms that bring specific health benefit to the host when administered in adequate amounts.<sup>[11]</sup> Probiotics were well known for their health benefits in improving immune system function and preventing diarrhea.<sup>[12]</sup> Effect of probiotics on metabolic profiles had been evaluated in hyperlipidemic patients and healthy adults previously.<sup>[13,14]</sup> Recently, more and more studies showed that probiotics could change the gut flora, improve total cholesterol, and low-density lipoprotein cholesterol levels,<sup>[15-17]</sup> and reduce blood glucose level and insulin resistance.<sup>[18,19]</sup> Probiotics particularly *Lactobacilli* and *bifidobacteria* might become prospective biotherapeutics for T<sub>2</sub>DM by improving the altered gut microbial composition.<sup>[20]</sup>

Therefore, in this study, we included all the randomized controlled trials and combined the data to evaluate the effect of probiotics on blood lipids, glucose, HbA1c, and insulin sensitivity in T<sub>2</sub>DM.

## 2. Materials and methods

We conducted a systematic review of the literature using a prespecified research protocol. The methods for identifying, selecting, evaluating, and synthesizing the evidence are described below. As this is a systematic review and meta-analysis of previously published studies, ethical approval and informed consent are not required.

### 2.1. Literature search

Relevant studies were searched from electronic databases, including PubMed, EMBASE, and Cochrane Library up to 12 April 2016. Search terms included “dyslipidemia,” “hyperlipidemia,” “hyperlipemia,” “hyperglycemia,” “insulin resistance,” “Metabolic Syndrome X,” “metabolic syndrome,” “metabolic X syndrome,” “metabolic X-syndrome,” “gut microbiota,” “probiotics,” “Lactobacillus,” “Bifidobacterium,” “Saccharomyces,” “Streptococcus,” “Enterococcus,” “intestinal microbia,” “diabetes,” “diabetes mellitus,” “diabetic,” “Diabetes Mellitus, Type 2,” “T<sub>2</sub>DM,” “DM,” “random\*,” “randomized controlled trial.” In addition, the references of retrieved articles were further hand-scanned to add potential eligible studies. In searching the literatures and presenting the results, the guidelines provided in the Preferred Reporting Items for Systematic Reviews and Meta-Analysis: The PRISMA Statement were followed.<sup>[21]</sup> The methodology of this systematic review is registered at the International Prospective Register for Systematic Review with the registration number CRD42015019851.

### 2.2. Study selection

All eligible studies should meet the following criteria: the studies must be human randomized, controlled trials. The patients must be with T<sub>2</sub>DM. The intervention was probiotic agent, and placebo was applied as comparison to the intervention. The

studies had accessible full articles in English. Articles reported at least one of the outcomes: FBG, HDL-C, LDL-C, TC, TG, HbA1c and HOMA-IR.

### 2.3. Quality assessment

Two reviewers independently assessed the methodological quality of the included studies using the Cochrane Collaboration Risk of Bias tool,<sup>[22]</sup> which consisted of 7 domains: sequence generation, allocation concealment, blind method of participants and personnel, blind method of outcome assessors, incomplete outcome data, selective reporting, and other sources of bias. Discrepancies were resolved through consensus or by third-party adjudication.

### 2.4. Data extraction

Two authors (CFL and XL) individually conducted an initial screening of studies based on the titles. Then they carried out a review of abstracts and an examination of the full text according to the eligibility criteria. All of these items, including the first

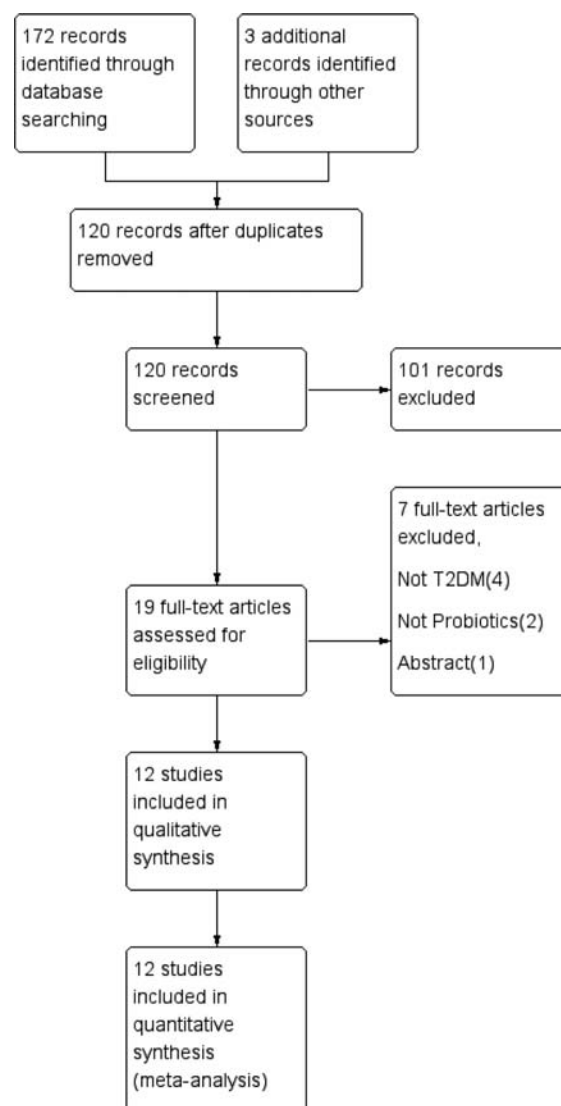


Figure 1. Flowchart of the study selection.

author's name, year of publication, country, study design, demographic characteristics of the participants, sample size of intervention and control groups, dosage of probiotic agent, duration of treatment, results on metabolic profiles, were collected. The data were compared and the disagreements were resolved by a third author (HLC).

### 2.5. Statistical analysis

Review Manager 5.3 (Cochrane Collaboration, Oxford, England) and STATA version 12.0 (Stata Corp., College Station, TX) were used for data analysis. SMD of FBG, HDL-C, LDL-C, TC, TG, HbA1c, and HOMA-IR were pooled, respectively. Heterogeneity was evaluated through the  $I^2$ -test. Substantial heterogeneity existed when  $I^2$  exceeds 50% or  $P < 0.05$ , the random-effect

model was used; otherwise, the fixed-effect model was applied. To evaluate the influence of each individual study on the combined results, leave-one-out sensitivity analysis was carried out. Potential publication bias was assessed by Egger's test.

## 3. Results

### 3.1. Literature search and study selection

The flowchart showing the detailed process of study selection is presented in Fig. 1.

### 3.2. Characteristics and quality of included studies

Twelve trials, with 714 participants, were included in the final meta-analysis and systematic review. The included studies were

**Table 1**  
Characteristics of included studies.

Study	Location	Design	Participants, age (no. of intervention/ no. of control)	Probiotic source, duration, wk	Probiotic dose, CFU	Side effect
Ejtahed et al <sup>[27]</sup>	Tabriz, Iran	DB, PC, P	T <sub>2</sub> DM 30–60 (30/30)	Yogurt 6	<i>L. acidophilus</i> La <sup>5</sup> ( $4.14 \times 10^6$ CFU/g) <i>B. lactis</i> Bb12 ( $3.61 \times 10^6$ CFU/g)	No
Ejtahed et al <sup>[28]</sup>	Tabriz, Iran	DB, PC, P	T <sub>2</sub> DM 30–60 (30/30)	Yogurt 6	<i>L. acidophilus</i> La5 ( $7.23-1.85 \times 10^6$ CFU/g) <i>B. lactis</i> Bb12 ( $6.04-1.79 \times 10^6$ CFU/g)	No
Hosseinzadeh et al <sup>[29]</sup>	Tehran, Iran	DB, PC, P	T <sub>2</sub> DM 35–55 (42/42)	Tablets 12	<i>Brewer's yeast</i> (1800 mg/d)	No
Hosseinzadeh et al <sup>[23]</sup>	Tehran, Iran	DB, PC, P	T <sub>2</sub> DM 35–55 (42/42)	Tablets 12	<i>Brewer's yeast</i> (1800 mg/d)	No
Asemi et al <sup>[30]</sup>	Kashan, Iran	DB, PC, P	T <sub>2</sub> DM* 35–70 (27/27)	Capsules 8	<i>L. acidophilus</i> ( $2 \times 10^9$ CFU), <i>L. casei</i> ( $7 \times 10^9$ CFU), <i>L. rhamnosus</i> ( $1.5 \times 10^9$ CFU), <i>L. bulgaricus</i> ( $2 \times 10^8$ CFU), <i>B. breve</i> ( $2 \times 10^{10}$ CFU), <i>B. longum</i> ( $7 \times 10^9$ CFU), <i>S. thermophilus</i> ( $1.5 \times 10^9$ CFU)	No
Mazloom et al <sup>[34]</sup>	Shiraz, Iran	SB, PC, P	T <sub>2</sub> DM 25–65 (21/21)	Capsules 6	<i>L. acidophilus</i> , <i>L. bulgaricus</i> , <i>L. bifidum</i> , <i>L. casei</i>	No
Shakeri et al <sup>[31]</sup>	Kashan, Iran	DB, PC, P	T <sub>2</sub> DM* 35–70 (26/26)	Bread 8	<i>L. sporogenes</i> ( $1 \times 10^8$ CFU)	No
Tajadadi-Ebrahimi et al <sup>[24]</sup>	Kashan, Iran	DB, PC, P	T <sub>2</sub> DM* 35–70 (27/27)	Bread 8	<i>L. sporogenes</i> ( $1 \times 10^8$ CFU)	No
Mohamadshahi et al <sup>[32]</sup>	Ahvaz, Iran	DB, PC, P	T <sub>2</sub> DM 30–60 (21/21)	Yogurt 12	<i>L. acidophilus</i> <i>B. lactis</i> ( $3.7 \times 10^6$ CFU/mg)	No
Hove et al <sup>[25]</sup>	Denmark	DB, PC	T <sub>2</sub> DM 40–70 (23/18)	Yogurt 12	<i>Lactobacillus</i> <i>L. helveticus</i>	No
Bayat et al <sup>[33]</sup>	Isfahan, Iran	PC, P	T <sub>2</sub> DM 25–75 (20/20)	Yogurt 8	(No data in the article)	No
Firouzi et al <sup>[26]</sup>	Kuala Lumpur, Malaysia	DB, PC, P	T <sub>2</sub> DM 30–70 (48/53)	Sachet 12	Genus <i>Lactobacillus</i> , <i>Firmicutes</i> phyla; genus <i>Bifidobacterium</i> and <i>Actinobacteria</i> phyla ( $3 \times 10^{10}$ CFU)	No

CFU = colony-forming unit, DB = double blind, P = parallel, PC = placebo control, SB = single blind, T<sub>2</sub>DM = type 2 diabetes mellitus, X = cross-over.

\* Criteria of the American Diabetes Association.

	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)	Other bias
Asemi 2013	?	?	?	?	+	?	?
Bayat 2016	?	?	?	?	?	?	?
Ejtahed 2011	?	+	+	?	+	?	?
Ejtahed 2012	?	+	+	?	+	?	?
Firouzi 2016	+	+	+	+	+	?	?
Hosseinzadeh 2013	+	+	?	?	+	?	?
Hosseinzadeh 2013a	?	?	?	?	+	?	?
Hove 2015	+	+	+	+	+	?	?
Mazloom 2013	?	?	-	-	-	?	?
Mohamadshahi 2014	?	?	?	?	?	?	?
Shakeri 2014	?	?	?	?	+	?	?
Tajadadi-Ebrahimi 2014	+	+	+	+	+	?	?

Figure 2. The methodological quality of included studies.

all randomized, controlled trials. Four studies reported sequence generation using either computer-generated numbers or a table of random numbers and mentioned allocation concealment<sup>[23–26]</sup>; 5 studies reported a double-blind design,<sup>[24–28]</sup> and 6 studies did not report the blinding process,<sup>[23,29–33]</sup> whereas 1 study reported single-blind<sup>[34]</sup>; there was no attrition bias of all the studies. The duration of intervention ranged from 6 to 12 weeks. Nine trials reported data for lipid profiles (n=508) and FBG (n=520), six for HOMA-IR (n=368) and HbA1c (n=380). Patients with antidiabetic medications did not change their medicine and dietary habit during the study. Probiotic species and dose used varied between studies. Four studies used a single species of probiotics, whereas the other studies used multispecies. The characteristics of included studies are presented in Table 1. The methodological quality of included studies is showed in Fig. 2.

### 3.3. Pooled effects of probiotics on blood lipid, glucose, and insulin sensitivity in T2DM

Nine studies, with 520 participants, reported the effect of probiotics on FBG. Results of this meta-analysis showed that the probiotic agent alleviated FBG (SMD -0.61 mmol/L, 95% CI [-0.92, -0.30], P=0.0001). The forest plot of this effect is presented in Fig. 3. A high level of statistical heterogeneity was observed for the meta-analysis of FBG (I<sup>2</sup>=66%; P=0.003), so the random-effect model was used. Sensitive analysis revealed that the study Asemi 2013 may be the source of statistical heterogeneity in the meta-analysis. When this outlier study Asemi 2013 was removed, there was no evidence of heterogeneity in the 8 remaining studies on FBG. Also, sensitivity analysis highlighted the significant effect of those studies on the total effect. For trials that included multispecies probiotics revealed significant reduction of FBG, whereas the mild effect was observed in the single species of probiotic. Probiotic use for ≤8 weeks showed a significant reduction on FBG. However, mild effect was observed in Probiotic use for >8 weeks. Subgroup analysis is presented in Table 2.

Nine studies reported the effect of probiotics on lipid profiles among 508 subjects. Probiotic agent increased HDL-C (SMD 0.42 mmol/L, 95% CI [0.08, 0.76], P=0.01). The forest plot of this effect is presented in Fig. 4A. Significant evidence of interstudy heterogeneity was observed (I<sup>2</sup>=71%; P=0.0005), so the random-effect model was used. These heterogeneities may be due to design difference among the studies, including the difference of probiotic agent and duration of intervention. As

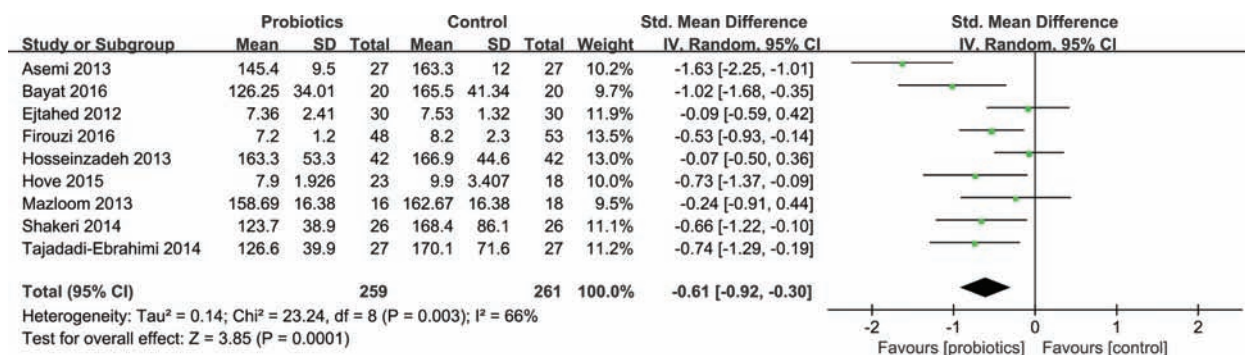


Figure 3. Forest plot of randomized controlled trials comparing the effect of probiotics on FBG with placebo in T<sub>2</sub>DM. Weighted mean differences (95% CIs) for FBG are shown. Pooled estimates calculated by the random-effect method. The squares indicate the effect of probiotics in a particular study. The horizontal lines represent 95% confidence intervals (CIs). The diamond indicates the pooled effect. CI = confidence interval, FBG = fasting blood glucose, IV = inverse variance, T<sub>2</sub>DM = type 2 diabetes mellitus.



**Table 2****Subgroup analyses of effect of probiotics on FBG and HDL-C.**

Subgroup	FBG (mmol/L)						HDL-C (mmol/L)					
	Number of studies	Number of participants	<i>P</i>	Mean difference	95%CI	<i>P</i>	Number of studies	Number of participants	<i>P</i>	Mean difference	95%CI	<i>P</i>
Number of strains of probiotics												
Single strain of probiotics	3	190	56	-0.46	-0.9, -0.02	0.04	2	136	84	0.74	-0.14, 1.63	0.10
Multiple strains of probiotics	6	330	71	-0.69	-1.12, -0.26	0.002	6	332	65	0.26	-0.12, 0.64	0.18
Duration of probiotics administration												
Probiotic use for >8 weeks	3	226	46	-0.41	-0.78, -0.04	0.03	4	268	84	0.43	0.21, 1.07	0.19
Probiotic use for ≤8 weeks	6	294	70	-0.72	-1.16, -0.27	0.002	5	240	55	0.41	0.02, 0.80	0.04

CI = confidence interval, FBG = fasting blood glucose, HDL-C = high-density lipoprotein-cholesterol.

shown in Figs. 4B–D, 5, and 6, there were no significant differences in LDL-C, TC, TG, HbA1c, and HOMA-IR between treatment group and control group. Sensitivity analysis was performed by removing the trials one by one to evaluate the reliability of the pooled mean difference, results remained consistent after removing the trials. No significant effects were observed in single species and multispecies. Probiotic use for ≤8 weeks revealed mild increase of HDL-C. However, there is no significant effect on HDL-C in probiotic use for >8 weeks. Subgroup analysis is presented in Table 2.

### 3.4. Publication bias

The publication bias of this meta-analysis was assessed using funnel plot and Egger's test. As shown in Fig. 7A and B, no evidence of significant publication was found by inspection of statistical test (Egger's test,  $P=0.161$ ).

## 4. Discussion

T<sub>2</sub>DM is a metabolic disease which is characterized by hyperglycemia,<sup>[35]</sup> insulin resistance, and associated with metabolic disturbance of blood lipid.<sup>[36]</sup> It brings not only severe pain to patients, but also a heavy burden to the family and society. In recent years, individual studies have reported that probiotics has varied effects on metabolic disturbance in T<sub>2</sub>DM. So we systematically analyze these studies and assess the effect of probiotics on metabolic profiles in T<sub>2</sub>DM.

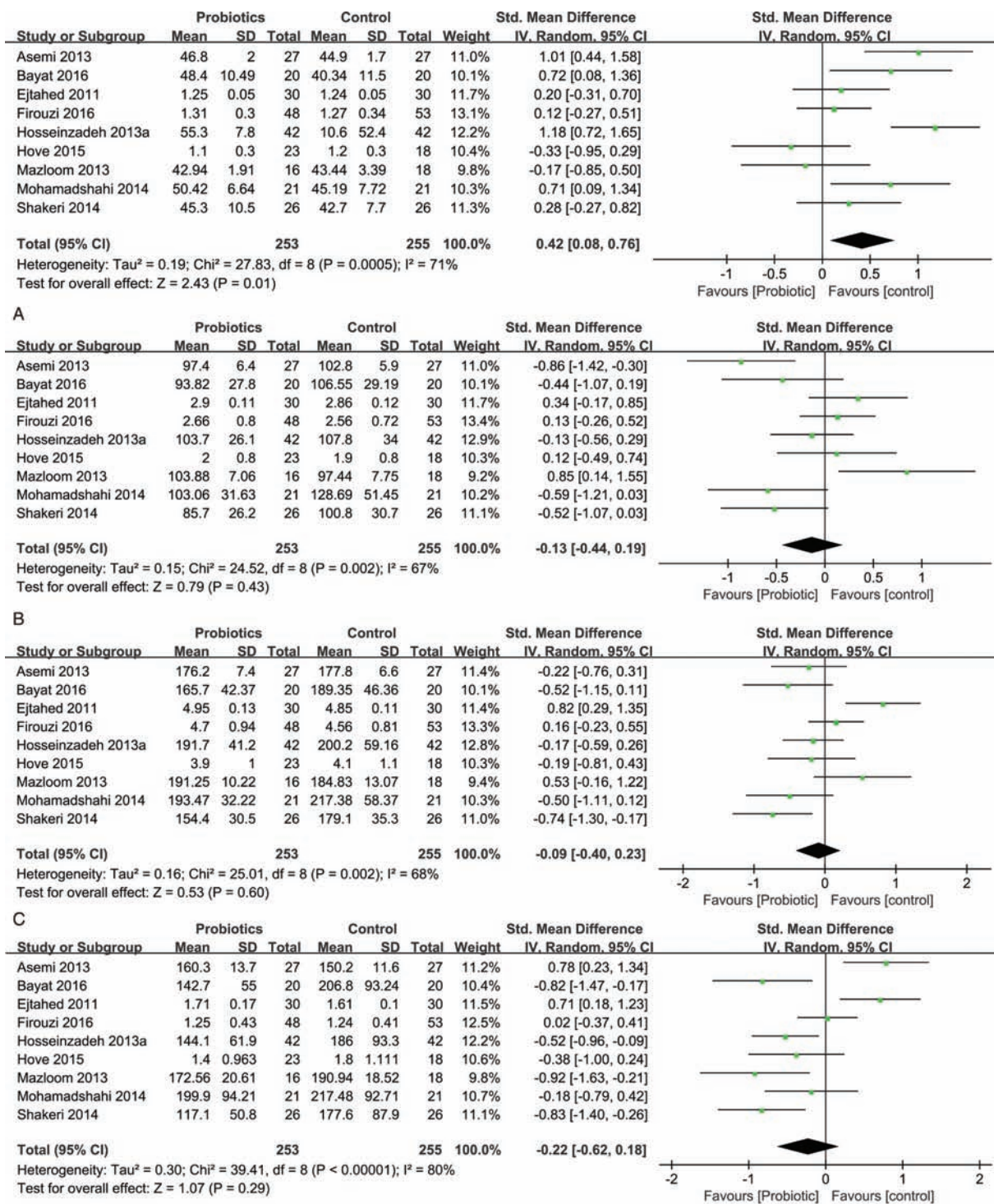
In our study, probiotics reduced FBG by 0.61 mmol/L, indicating a modest effect on glycemic control. Abnormal glucose metabolism in T<sub>2</sub>DM brings risks for many complications, such as nephropathy, retinopathy, and cardiovascular disease. However, even a small reduction in FBG could have important public health consequences. The beneficial effect of probiotics on glucose is not fully understood. The hypothesis that probiotics might be involved in maintenance of normal gut flora and glucose metabolism has received much attention. The gut microbial profiles show reductions in *Lactobacillus* spp and *Bifidobacterium* spp with increased plasma "Lipopolysaccharides" (LPS), which accelerate the apoptosis of pancreatic beta cells and cause the molecular onset of insulin resistance and hyperglycemia via "Nuclear Factor kappa B" (NF-κB).<sup>[37,38]</sup> Chronic systemic inflammation is common in T<sub>2</sub>DM and metabolism syndrome (MS) and is considered as a risk factor for arteriosclerosis and infarction.<sup>[39]</sup> Probiotics could affect the structure of gut flora, increase GLP-1 secretion from enteroendocrine L-cells to improve carbohydrate metabolism, and enhance insulin sensitivity of target cells.<sup>[40]</sup> Improving of intestinal epithelial integrity and permeability, regulation of immune

system,<sup>[41]</sup> and reduction of Toll-like receptor (TLR)-4 signaling are likely to illustrate the hypoglycemic effect of probiotics.<sup>[42]</sup> Probiotic dairy products demonstrate the beneficial effect on inflammatory factors by influencing the gut microbiota,<sup>[7]</sup> inhibition of ascorbic acid autoxidation, metal ion chelation, reduction activity and scavenging of superoxide anion free radicals, hydrogen peroxide.<sup>[43,44]</sup>

In our meta-analysis, probiotics could increase HDL-C by 0.42mmol/L, whereas there were no significant differences in LDL-C, TC and TG between treatment group and control group. Generally, an elevated HDL-C level is regarded as a protective factor reducing the risk of cardiovascular disease. Some studies observed significant effect on blood lipid profiles by using dairy products.<sup>[45–47]</sup> The beneficial effect of probiotics on blood lipid might be due to the inhibition of dietary cholesterol absorption and the suppression of bile acid reabsorption in the small intestine.<sup>[48]</sup> Fermentation of food-derived indigestible carbohydrates by probiotics could cause increased production of short-chain fatty acids, which decrease cholesterol concentrations either by inhibiting hepatic cholesterol synthesis or by redistributing cholesterol from plasma to the liver.<sup>[47]</sup> However, the composition of fermented milk is very complex, the exact role of certain substance should be further investigated. The protective effect of fermented milk on lipid profiles might be explained by the fatty acid and sphingolipids or other ingredient in dairy products rather than probiotics.<sup>[49]</sup> Moreover, the calcium and protein content of dairy products might play a role. It was reported that some probiotic-free dairy products could decrease serum lipid. Inverse association between the consumption of milk products and the LDL-C/HDL-C ratio was showed.<sup>[50]</sup>

Similar meta-analyses about the effect of probiotics on glucose control,<sup>[51]</sup> and insulin action,<sup>[52]</sup> and lipid profiles in diabetes<sup>[53]</sup> have been published recently; however, the major clinical endpoints and the characteristics of the participants of these meta-analyses are different from our study. In our meta-analysis, more metabolic outcomes are reported, which make it much more comprehensive. In addition, the latest randomized controlled trials are included in our paper, so the results are more powerful and reliable. The different findings between our study and others can be explained by the difference in probiotic strains, dosage and duration, the different fermentation method used. Unfortunately, it is not practical to analyze the effect of different probiotic species, dosage and treatment duration on metabolic profiles by subgroup analysis due to the lack or inadequate number of studies.

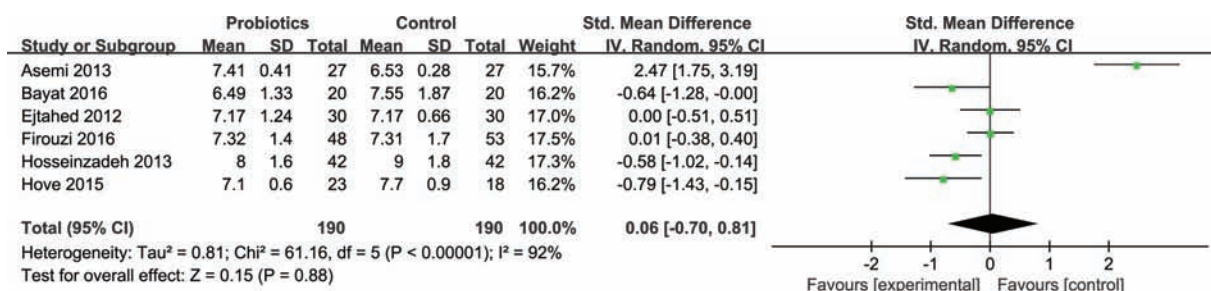
In order to minimize the risk of confounding factor, all the included studies were randomized controlled trials in this meta-analysis. By using the method of meta-analysis, we increased the



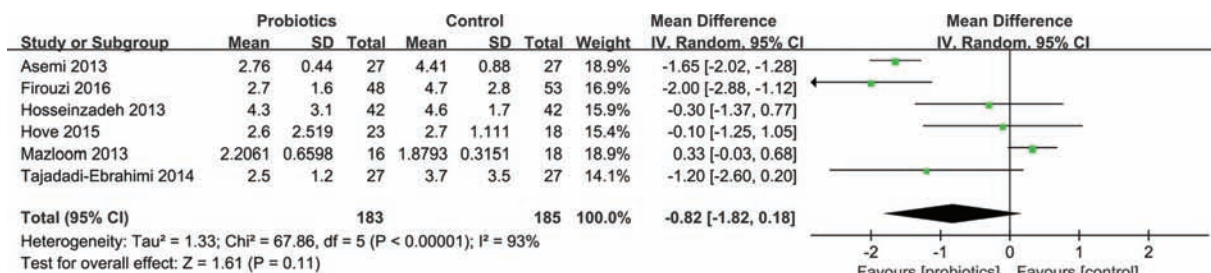
**Figure 4.** Forest plot of randomized controlled trials comparing the effect of probiotics on lipid profiles with placebo in T<sub>2</sub>DM. Weighted mean differences (95% CIs) for (A) HDL-C, (B) LDL-C, (C) TC, (D) TG are shown. Pooled estimates calculated by the random-effect method. The squares indicate the effect of probiotics in a particular study. The horizontal lines represent 95% confidence intervals (CIs). The diamond indicates the pooled effect. CI = confidence interval, HDL-C = high-density lipoprotein-cholesterol, LDL-C = low-density lipoprotein-cholesterol, IV = inverse variance, TG = triglyceride, TC = total cholesterol, T<sub>2</sub>DM = type 2 diabetes mellitus.

sample size and the statistical power. However, there are still some shortcomings. First, we only included studies published in English, causing the risk of publication bias. However, Egger's test showed no significant publication bias, indicating that the unpublished evidence did not affect the results of the meta-

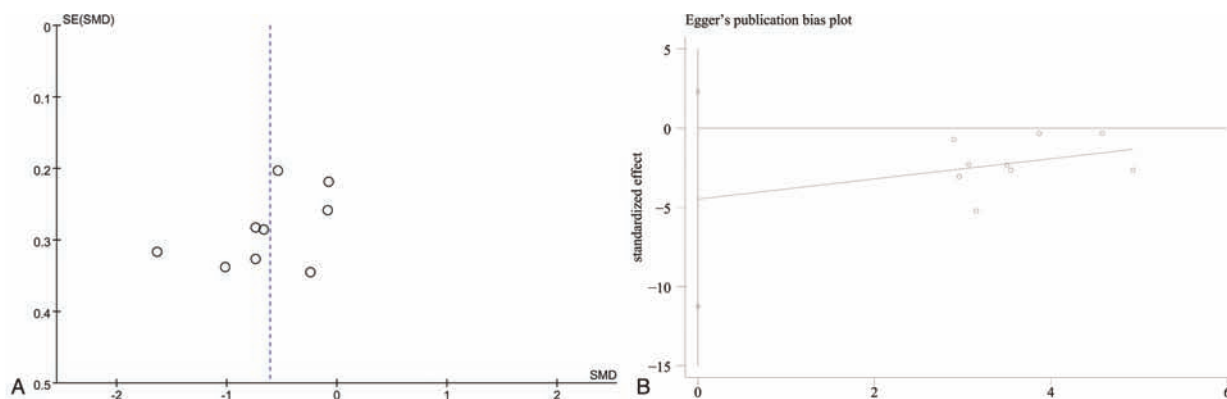
analysis. Second, the validity of meta-analysis depended on the quality of included studies. Although all trials selected were randomized and controlled, the quality of included study varied, for instance, the lack of double-blinding increased the risk of expectation bias.<sup>[34]</sup> Third, all trials had a relatively small sample



**Figure 5.** Forest plot of randomized controlled trials comparing the effect of probiotics on HbA1c with placebo in T<sub>2</sub>DM. Weighted mean differences (95% CIs) for HbA1c are shown. Pooled estimates calculated by the random-effect method. The squares indicate the effect of probiotics in a particular study. The horizontal lines represent 95% confidence intervals (CIs). The diamond indicates the pooled effect. CI = confidence interval, HbA1c = glycosylated hemoglobin, T<sub>2</sub>DM = type 2 diabetes mellitus.



**Figure 6.** Forest plot of randomized controlled trials comparing the effect of probiotics on HOMA-IR with placebo in T<sub>2</sub>DM. Weighted mean differences (95% CIs) for HOMA-IR are shown. Pooled estimates calculated by the random-effect method. The squares indicate the effect of probiotics in a particular study. The horizontal lines represent 95% confidence intervals (CIs). The diamond indicates the pooled effect. CI = confidence interval, HOMA-IR = homeostasis model of assessment for insulin resistance index, T<sub>2</sub>DM = type 2 diabetes mellitus.



**Figure 7.** Publication bias. (A) Funnel plots, (B) Egger's publication bias plot.

size and short treatment duration, so the validity of the results was limited. Due to short duration, whether the result of this meta-analysis could be translated into a long-term treatment effect or not is uncertain.

In conclusion, this meta-analysis of available RCTs suggests that probiotics has beneficial effect on FBG and HDL-C in T<sub>2</sub>DM. Probiotic agents might become a new method for management of T<sub>2</sub>DM. However, before they can be recommended for use in supportive treatment of T<sub>2</sub>DM, large-scale and long duration multicenter randomized controlled trials are still required.

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