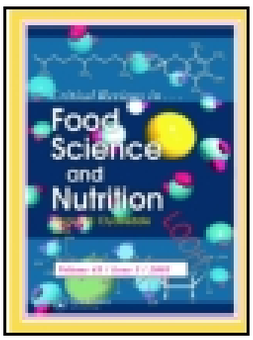

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REVIEW



# Effects of probiotics, prebiotics, and synbiotics on polycystic ovary syndrome: a systematic review and meta-analysis

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**ABSTRACT**

This meta-analysis of randomized controlled trials (RCTs) was performed to summarize the effects of probiotics, prebiotics, and synbiotics on insulin resistance (IR), lipid profiles, anthropometric indices, and C-reactive protein (CRP) level for polycystic ovary syndrome (PCOS). We searched 8 databases from their inception until 1st October, 2020. The effect sizes were expressed as standardized mean difference (SMD) with 95% confidence intervals (95% CI). Subgroup analyses were undertaken for further identification of effects of probiotics, prebiotics, and synbiotics, based on the following aspects: (1) type of intervention (probiotics, prebiotics, or synbiotics); (2) study duration ($\geq$ 12 weeks or $<$ 12 weeks); (3) number of probiotic strains (multi strains or single strain); (4) probiotic dose ($\geq 2 \times 10^8$ colony-forming units [CFU] or $< 2 \times 10^8$ CFU). A total of 17 eligible RCTs with 1049 participants were included. Results showed that probiotic, prebiotic, and synbiotic intake decreased fasting plasma glucose (SMD, −1.35; 95% CI, −2.22 to −0.49; $p = 0.002$), fasting insulin (SMD, −0.68; 95% CI, −1.08 to −0.27; $p = 0.001$), homeostatic model of assessment for IR (SMD, −0.73; 95% CI, −1.15 to −0.31; $p = 0.001$), triglycerides (SMD, −0.85; 95% CI, −1.59 to −0.11; $p = 0.024$), total cholesterol (SMD, −1.09; 95% CI, −1.98 to −0.21; $p = 0.015$), low-density lipoprotein cholesterol (SMD, −0.84; 95% CI, −1.64 to −0.03; $p = 0.041$), very-low-density lipoprotein cholesterol (SMD, −0.44; 95% CI, −0.70 to −0.18; $p = 0.001$), and increased quantitative insulin sensitivity check index (SMD, 2.00; 95% CI, − 0.79 to 3.22; $p = 0.001$). However, probiotic, prebiotic, and synbiotic supplements did not affect anthropometric indices, high-density lipoprotein cholesterol, and CRP levels. Subgroup analysis showed that probiotic or prebiotic might be the optimal choice for ameliorating IR or lipid profiles, respectively. Additionally, the effect was positively related to courses and therapeutical dose. Overall, the meta-analysis demonstrates that probiotic, prebiotic, or synbiotic administration is an effective and safe intervention for modifying IR and lipid profiles.

**KEYWORDS**

Meta-analysis; polycystic ovary syndrome synbiotic; prebiotic; probiotic

## Introduction

Polycystic ovary syndrome (PCOS) is one of the most common gynecologic endocrine disorders, generally considered as a major cause of infertility (Fauser et al. 2012; Teede, Deeks, and Moran 2010). The syndrome as defined in 2003 by the Rotterdam Consensus statement (PCOS GREA 2004) was characterized by the presence of at least two of three classical features of PCOS: menstrual irregularity (oligomenorrhoea or amenorrhea), hyperandrogenism (acne, hirsutism), and enlarged "polycystic" ovaries on pelvic ultrasound. The incidence ranges from 6% to 21% (Azziz et al. 2016), depends on the studied population and diagnostic criteria. Besides, PCOS has been linked to higher risks of metabolic disorders, including insulin resistance (IR), glucose intolerance, Diabetes Mellitus type 2, dyslipidemia, and cardiovascular diseases (Gunning et al. 2020; Macut et al. 2017).

The exact pathophysiology behind PCOS is unknown presently, although genetic, neuroendocrine and metabolic causes have been suggested (Goodarzi et al. 2011; Coutinho and Kauffman 2019). It is believed that no single pathological process can account for all cases of PCOS since the disorder is somewhat heterogeneous (Patel 2018). However, recent studies suggest that the gut microbiota may play a role in PCOS (Qi et al. 2019), obesity (Seganfredo et al. 2017), metabolic syndrome, and diabetes mellitus (Vallianou, Stratigou, and Tsagarakis 2018). Altered microbiota composition features in PCOS progression, which is related to IR and chronic inflammation (Jiao et al. 2018; Scheithauer et al. 2016). With the enhancing understanding of intestinal microbiota in the pathogenesis of PCOS, the use of microbiota-targeted agents (such as probiotics, prebiotics, and synbiotics) in treating PCOS have been discussed recently.

A probiotic, as defined by the WHO (2002), is a "live microorganism which, when administered in adequate amounts, confers a health benefit to the host" (Yamashiro 2017). Probiotics can improve metabolic profiles in PCOS

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by antagonizing the growth of pathogenic microorganism, increasing intestinal mucus layer production, reducing intestinal permeability, and modulation of the gastrointestinal immune system (Meng et al. 2019; Abenavoli et al. 2019). Prebiotics are defined as nonliving indigestible fibers that may stimulate the growth and activity of beneficial microorganisms in the gut (Gibson et al. 2017). Prebiotics can improve host metabolism, reduce pro-inflammatory markers, and ameliorate blood lipid profiles by the proliferation of health-promoting bacteria such as *Bifidobacteria*, *Lactobacilli* and increasing the production of short-chain fatty acids (SCFAs) (Holscher 2017). Synbiotics are defined as food or products that contain probiotics and prebiotics (Pandey, Naik, and Vakil 2015). Synbiotics effectively regulate glycemia (Jumpertz et al. 2011) and serum lipids (Ferrarese et al. 2018). Moreover, synbiotic preparations may exert their beneficial effects on body weight (BW) through the gut-brain axis by activating host satiety pathways and affecting the host's appetite (Breton et al. 2016). However, current evidence is inconclusive on the effects of pre-, pro-, and synbiotic supplementation in the management of PCOS.

A previous systematic review concluded that probiotic and synbiotic consumption might improve glucose homeostasis parameters, hormone, and inflammation (Hadi et al. 2020). Consistently, a recent meta-analysis has also indicated that probiotic and synbiotic reduce glucose, body mass index (BMI), hormonal and inflammatory parameters in PCOS (Cozzolino et al. 2020). However, other meta-analysis showed conflicting results (Shamasbi, Ghanbari-Homayi, and Mirghafourvand 2020; Tabrizi et al. 2019; Heshmati et al. 2019). To the best of our knowledge, no meta-analysis has simultaneously assessed pro-, pre-, and synbiotic administration effects on metabolic parameters in PCOS. Hence, the objective of this review was to review available RCTs systematically and to evaluate the overall efficacy of pro-, pre-, and synbiotic supplementation on metabolic parameters: e.g., IR, lipid profiles, anthropometric measures: e.g., BW, BMI, and C-reactive protein (CRP).

## Methods

This systematic review was conducted and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement guidelines (Moher et al. 2015) and has been registered in the international prospective register of systematic reviews (registration no. CRD42020200508).

### Data source and search strategy

The systematic literature search was performed in 8 databases: Cochrane Central Register of Controlled Trials (CENTRAL), PubMed, EMBASE, Web of Science, Chinese Biomedical Database (CBM), China National Knowledge Infrastructure (CNKI), VIP information database and Wanfang Data from their inception until 1st October, 2020. We also checked reference lists and conference proceedings manually to obtain additional data. No restrictions were imposed on language or publication date. Two review authors (Y.L.L. and G.C.X.) judged the eligibility of articles and any nonagreement was resolved by the corresponding author (Y.T.). Details of the search strategy in PubMed are as follows:

#1 Probiotics[MeSH] OR Prebiotics[MeSH] OR Synbiotics[MeSH] OR Inulin [Title/Abstract] OR Resistant Dextrin[Title/Abstract] OR Microbiota[Title/Abstract] OR Microbiotas[Title/Abstract] OR Microbiome[Title/Abstract] OR Microbiomes[Title/Abstract] OR Gut microflora[Title/Abstract] OR Human Microbiome[Title/Abstract] OR Human Microbiomes[Title/Abstract] OR Microbiomes, Human[Title/Abstract] OR Microbiome, Human[Title/Abstract] OR Bacteroides[Title/Abstract] OR Bacteroidetes[Title/Abstract] OR Bifidobacterium[Title/Abstract] OR Eubacterium[Title/Abstract] OR Clostridium[Title/Abstract] OR Lactobacillus[Title/Abstract] OR Fusobacterium[Title/Abstract] OR Firmicutes [Title/Abstract]

#2 Polycystic Ovary Syndrome[MeSH] OR Ovary Syndrome, Polycystic[Title/Abstract] OR Syndrome, Polycystic Ovary[Title/Abstract] OR Stein-Leventhal Syndrome[Title/Abstract] OR Stein Leventhal Syndrome [Title/Abstract] OR Syndrome, Stein-Leventhal[Title/Abstract] OR Sclerocystic Ovarian Degeneration[Title/Abstract] OR Ovarian Degeneration, Sclerocystic[Title/Abstract] OR Sclerocystic Ovary Syndrome[Title/Abstract] OR Polycystic Ovarian Syndrome[Title/Abstract] OR Ovarian Syndrome, Polycystic[Title/Abstract] OR Polycystic Ovary Syndrome[Title/Abstract] OR Sclerocystic Ovaries [Title/Abstract] OR Ovary, Sclerocystic[Title/Abstract] OR Sclerocystic Ovary[Title/Abstract]

#3 random*[tw]

#4 (#1 AND #2 AND #3)

### Study selection criteria

Studies were included in the systematic review if they met the following criteria: (1) Study designs: RCT; (2) Participants: women with a definite diagnosis of PCOS; (3) Intervention: probiotics, prebiotics, synbiotics intake separately or in combination with other drugs, compared with placebo; (4) Outcomes: outcomes included anthropometric indices, serum metabolic profiles or inflammatory markers, at least one of following statistics: fasting plasma glucose (FPG), fasting insulin (FINS), homeostatic model of assessment for insulin resistance (HOMA-IR), quantitative insulin sensitivity check index (QUICKI), triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), very-low-density lipoprotein cholesterol (VLDL-c), BW, BMI, waist circumference (WC), hip circumference (HC), and CRP. Exclusion criteria were as follows: (1) quasi-randomized trials, cohort or case-control studies, reviews, meta-analysis, case reports, animal or cell experiments; (2) studies without explicit inclusion and exclusion criteria; (3) studies with unavailable data and unreported target outcomes.

### Data extraction

Two reviewers performed data extraction independently. Data were cross-checked to minimize potential errors, and disagreements were handled through discussion with the corresponding author. The following information was extracted from included trials: (1) first author's name; (2) study publication year; (3) study country; (4) participants' characteristics, including sample size, baseline age, BMI of intervention and control groups, and criteria used to define PCOS; (5) study design and duration; (6) characteristics of interventions, including type, dosage, form, and the number of probiotic strains. (7) changes in outcomes of IR, lipid profiles, anthropometric indices, and CRP level, including FPG, FINS, HOMA-IR, QUICKI, TG, TC, HDL-c, LDL-c, VLDL-c, BW, BMI, WC, HC, and CRP.

### Assessment of study quality

Two authors independently assessed the methodological quality of eligible trials via a Cochrane Collaboration tool (Higgins et al. 2019). Studies were evaluated as low, unclear risk, or high bias based on the following domains: selection bias, performance bias, detection bias, attrition bias, reporting bias and other bias. All discrepancies and disagreements were resolved by consensus or discussion with the third investigator (Y.T.).

### Data synthesis and statistics

All related statistical analysis was performed using STATA software version 12.0 (Stata Corp., College Station, TX) and RevMan V.5.4 software (Cochrane Collaboration, Oxford, UK). The pooled effect sizes were considered as standardized mean differences (SMDs) with 95% confidence intervals (95% CI). If more than one time point for outcomes was reported, we took data from the last time point for analyses. Heterogeneity between studies was estimated using the Cochrane Q test and the I-squared ($I^2$) statistic (degree of heterogeneity). In each analysis, heterogeneity was presented as low ($I^2 \leq 40\%$), moderate ($40\% < I^2 \leq 70\%$), or high ($I^2 > 70\%$) (Schünemann et al. 2008). The random-effects method was performed for calculating summary effect measures since clinical heterogeneity was inevitable. $p < 0.05$ represented statistical significance. We carried out a pre-planned subgroup analysis based on the type of intervention (probiotics, prebiotics, or synbiotics), study duration ($\geq$ 12 weeks or $<$ 12 weeks), number of probiotic strains (multi strains or single strain), and probiotic dose ($\geq 2 \times 10^8$ colony-forming units [CFU] or $< 2 \times 10^8$ CFU). Sensitivity analysis was performed by omitting one study in each turn to detect any significant changes in obtained results. Egger's test and funnel plots were generated to investigate the potential publication bias when more than ten trials were included in the analysis.

## Results

### Characteristics of included studies

The preliminary search identified a total of 1173 studies. After removing 330 duplicated studies, 843 records were assessed by screening titles and abstracts. Among them, 767 items were excluded due to apparent ineligibility, such as meta-analysis, reviews, case reports, and animal or cell experiments. Seventy-six articles were selected for full-text revision, and 59 of these were excluded for following reasons: (1) Not RCTs (n = 17); (2) Participants without PCOS (n = 14); (3) Not meet inclusion demands (n = 15); (4) No placebo-controlled (n = 8); (5) Inappropriate outcomes reported (n = 2); (6) Unsuitable for meta-analysis (n = 3). Finally, 17 RCTs (1049 participants) were eligible for meta-analysis. Details of the selection process are shown in a PRISMA flow diagram (Figure 1).

The summarized characteristics of 17 RCTs (1049 women with PCOS) are shown in Table 1 (Ahmadi et al. 2017; Shoaei et al. 2015; Esmaeilinezhad et al. 2019; Zahra et al. 2019; Ghanei et al. 2018; Shamasbi et al. 2018; Sevda et al. 2018; Jamilian et al. 2018; Karamali et al. 2018; Karimi et al. 2020; Karimi et al. 2018; Nasri et al. 2018; Ostadmohammadi et al. 2019; Samimi et al. 2018; Shabani et al. 2018; Zhu, Pengbin, and Yaqiong 2020; Chen, Minghui, and Channi 2018). In all studies, PCOS diagnosis was based on the Rotterdam criteria (Eshre TR GASP 2004). All of the included studies were randomized, parallel-group, and placebo-controlled trials between 2015 and 2020. All clinical trials adopted the blind method, of which 11 were double-blind (Ahmadi et al. 2017; Shoaei et al. 2015; Ghanei et al. 2018; Jamilian et al. 2018; Karamali et al. 2018; Karimi et al. 2020; Karimi et al. 2018; Nasri et al. 2018; Ostadmohammadi et al. 2019; Samimi et al. 2018; Shabani et al. 2018) and 4 were triple-blind (Esmaeilinezhad et al. 2019; Zahra et al. 2019; Shamasbi et al. 2018; Sevda et al. 2018) except for studies conducted by Zhu, Pengbin, and Yaqiong (2020) and Chen, Minghui, and Channi (2018).

Interventions included RCTs evaluated different forms of pro-, pre-, or synbiotics. Nine trials studied the efficacy of probiotics (Ahmadi et al. 2017; Shoaei et al. 2015; Ghanei et al. 2018; Jamilian et al. 2018; Karamali et al. 2018; Ostadmohammadi et al. 2019; Shabani et al. 2018; Zhu, Pengbin, and Yaqiong 2020; Chen, Minghui, and Channi 2018). Two investigated the efficacy of prebiotics (Shamasbi et al. 2018; Sevda et al. 2018). Six assessed the efficacy of synbiotics (Esmaeilinezhad et al. 2019; Zahra et al. 2019; Karimi et al. 2020; Karimi et al. 2018; Nasri et al. 2018; Samimi et al. 2018). Pro-, pre-, and synbiotics were administered in different forms, including capsules (Ahmadi et al. 2017; Shoaei et al. 2015; Ghanei et al. 2018; Jamilian et al. 2018; Karamali et al. 2018; Karimi et al. 2020; Karimi et al. 2018; Nasri et al. 2018; Ostadmohammadi et al. 2019; Samimi et al. 2018) tablets (Shabani et al. 2018; Zhu, Pengbin, and Yaqiong 2020), liquid preparations (Esmaeilinezhad et al. 2019; Zahra et al. 2019; Shamasbi et al. 2018; Sevda et al. 2018), and powder (Chen, Minghui, and Channi 2018). The intervention duration also differed among trials, ranging from 8 weeks to 3 months.

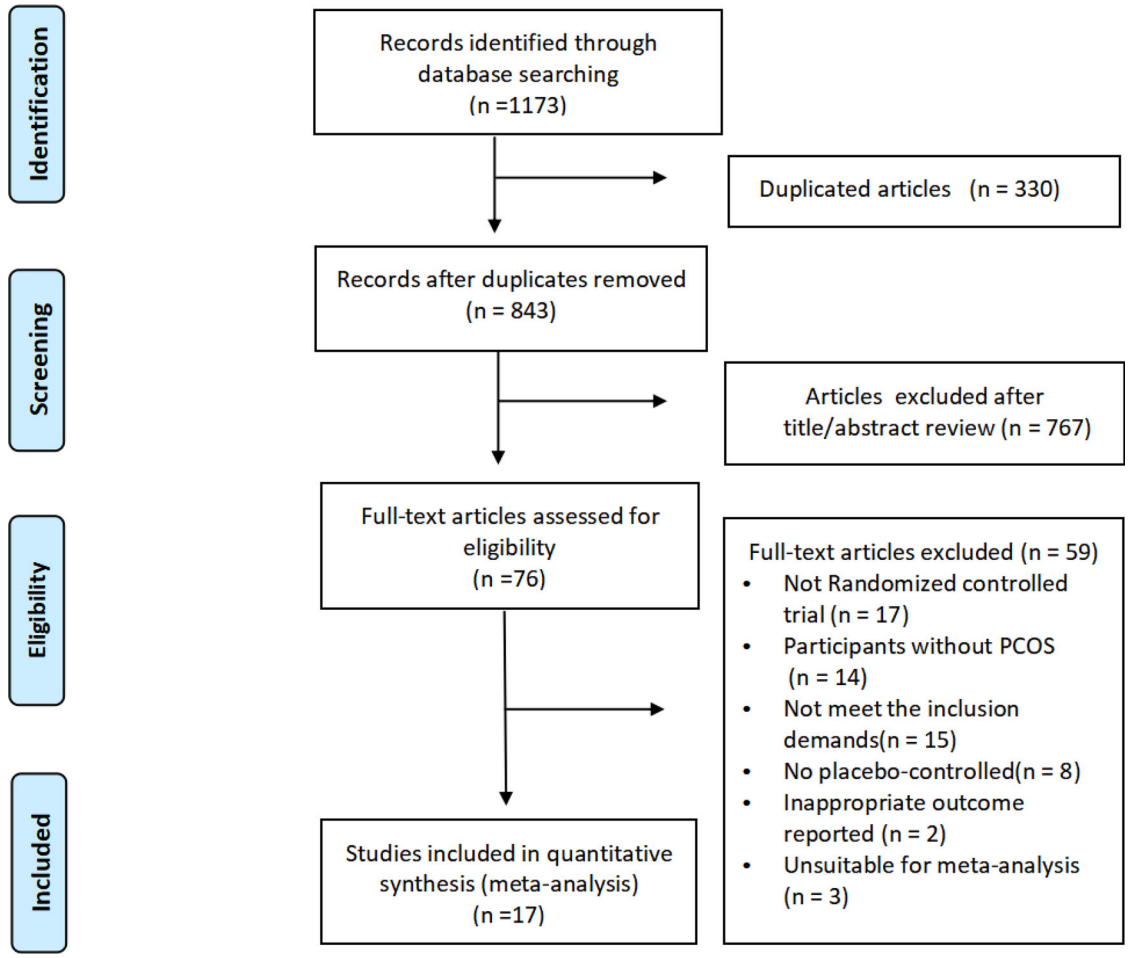


**Figure 1.** PRISMA flow diagram of study selection process.

### *Risk of bias and quality assessment of individual studies*

Figure 2 summarizes the risk of bias of included trials based on different quality domains using the Cochrane Collaboration tool. Adequate randomized sequence generation was reported in all included trials. All the trials except 2 (Zhu, Pengbin, and Yaqiong 2020; Chen, Minghui, and Channi 2018) had a low risk of bias in allocation concealment. Participants and personnel were blind in all studies, apart from 2 (Zhu, Pengbin, and Yaqiong 2020; Chen, Minghui, and Channi 2018). Outcome assessors were blind in 4 studies using triple-blind (Esmaeilinezhad et al. 2019; Zahra et al. 2019; Shamasbi et al. 2018; Sevda et al. 2018). All trials except 2 (Zhu, Pengbin, and Yaqiong 2020; Chen, Minghui, and Channi 2018) were preregistered in a clinical trial registry, which might have efficiently controlled reporting bias. Besides, all of the trials showed a low risk of bias based on incomplete outcomes data.

### *Findings from the meta-analysis*

#### *Effects on insulin resistance indices*

*FPG.* Effects of pro-, pre-, and synbiotic supplementation on FPG was assessed in 8 RCTs (496 participants). The result revealed a significant decline in the FPG level after pro-, pre-, and synbiotic intake in comparison with controls (SMD, −1.35; 95% CI, −2.22 to −0.49; $p = 0.002$) (Figure 3A). The heterogeneity was high among studies ($I^2 = 94.6\%$, $p = 0.000$). Therefore, sub-analyses were performed to investigate the possible sources of it. Subgroup analyses suggested that the finding did not alter among subgroups: the intervention of pro- or prebiotic administration, studies duration $\geq$ 12 weeks, and probiotic dose $\geq 2 \times 108$ CFU (Table 2 and Figure S1.1–1.3).

*FINS.* FINS was assessed in 7 studies with 434 participants (cases = 216 and controls = 218). Pooled results from random-effects model indicated that the FINS level was lower after pro- and synbiotic supplementation compared with the placebo (SMD, −0.68; 95% CI, −1.08 to −0.27; $p = 0.001$; $I^2 = 76.7\%$) (Figure 3B). The conclusion remained unchanged in all subgroups of study duration and probiotic dose (Table 2 and Figure S2.2–2.3). Notably, stratification of the study duration removed the heterogeneity. On the other hand, subgroup analysis of intervention showed a greater reduction in FINS after the probiotic intake, while there was no evidence of difference after synbiotic intake (Table 2 and Figure S2.1).

*HOMA-IR.* Seven trials (434 participants) reported the effect of pro- and synbiotic supplementation on HOMA-IR. HOMA-IR after consumption of pro- and symbiotic was

**Table 1.** Characteristics of included randomized controlled trials.

| Author year (ref) | Country | Diagnostic criteria | Sample size (case/placebo) | Intervention arm | Control arm | Duration (week or month) | Probiotic strain | Probiotic dose (cfu) | Outcomes |
|---|---|---|---|---|---|---|---|---|---|
| Ahmadi et al. (2017) | Iran | Rotterdam | 30/30 | Probiotic | Placebo (starch) | 12w | 1,2,9 | $2 \times 10^9$ | a,b,c,d,e,f,g,h,i,j,k |
| Chen, Minghui, and Channi (2018) | China | Rotterdam | 27/28 | Probiotic | Placebo | 12w | 1,9,10 | $1.0 \times 10^7$ | a,b,c,e,f,g,h,i,j,k |
| Esmaeilinezhad et al. (2019) | Iran | Rotterdam | 23/23 | Synbiotic | Placebo | 8w | 8,11,12 | $2 \times 10^8$ | a,b,c,d,j,k,l,m |
| Ghanei et al. (2018) | Iran | Rotterdam | 30/30 | Probiotic | Placebo (maltodextrin) | 12w | 1,3,4,5 | $1 \times 10^9$ | j,k,l,n |
| Jamilian et al. (2018) | Iran | Rotterdam | 30/30 | Probiotic | Placebo | 12w | 1,4,6,9 | $2 \times 10^9$ | j,k,n |
| Karamali et al. (2018) | Iran | Rotterdam | 30/30 | Probiotic | Placebo (starch) | 12w | 1,2,9 | $2 \times 10^9$ | j,k,n |
| Karimi et al. (2018) | Iran | Rotterdam | 44/44 | Synbiotic | Placebo (starch and maltodextrin) | 12w | 1,2,7,8,9,13 | $10^8$, $10^9$, $10^{10}$ | a,b,c,d,n |
| Karimi et al. (2020) | Iran | Rotterdam | 44/44 | Synbiotic | Placebo (starch and maltodextrin) | 12w | 1,2,7,8,9,13 | $10^8$, $10^9$, $10^{10}$ | e,f,g,h,j,k,l,m |
| Nasri et al. (2018) | Iran | Rotterdam | 30/30 | Synbiotic | Placebo | 12w | 1,2,9 | $2 \times 10^9$ | j,k,n |
| Ostadmohammadi et al. (2019) | Iran | Rotterdam | 30/30 | Probiotic | Placebo (starch) | 12w | 1,4,6,9 | $2 \times 10^9$ | j,k,n |
| Samimi et al. (2018) | Iran | Rotterdam | 30/30 | Synbiotic | Placebo (starch) | 12w | 1,2,9 | $2 \times 10^9$ | a,b,c,d,e,f,g,h,i,j,k |
| Sevda et al. (2018) | Iran | Rotterdam | 31/31 | Prebiotic | Placebo (maltodextrin) | 3m | NA | NA | j,k,l,m |
| Shabani et al. (2018) | Iran | Rotterdam | 30/30 | Probiotic | Placebo | 12w | 1,4,6,9 | $2 \times 10^9$ | a,b,c,d,e,f,g,h,i,j,k |
| Shamasbi et al. (2018) | Iran | Rotterdam | 31/31 | Prebiotic | Placebo (maltodextrin) | 3m | NA | NA | a,e,f,g,h,n |
| Shoaei et al. (2015) | Iran | Rotterdam | 32/33 | Probiotic | Placebo (starch and maltodextrin) | 8w | 1,2,7,8,9,13 | $10^8$, $10^9$, $10^{10}$ | a,b,c,d,n |
| Zahra et al. (2019) | Iran | Rotterdam | 22/21 | Synbiotic | Placebo | 8w | 8,11,12 | $2 \times 10^8$ | e,f,g,h,n |
| Zhu, Xia Pengbin, and Yaqiong (2020) | China | Rotterdam | 30/30 | Probiotic | Placebo | 12w | 1 | $5 \times 10^6$ | k,l,m |

Probiotic strain: 1- Lactobacillus acidophilus, 2-Lactobacillus casei, 3-Lactobacillus Plantarum, 4-Lactobacillus Fermentum, 5-Lactobacillus Gasseri, 6-Lactobacillus reuteri, 7-Lactobacillus bulgaricus, 8-Lactobacillus rhamnosus, 9-Bifidobacterium bifidum, 10-Enterococcus, 11-bacillus koagolans, 12-bacillus indicous, 13-Streptococcus thermophilus.
Outcomes: a = FPG, b = FINS, c = HOMA-IR, d = QUICKI, e = triglycerides, f = Total cholesterol, g = HDL, h = LDL, i = VLDL, j = Weight, k = BMI, l = WC, m = HC, n = CRP.
NA, not applicable.

reduced significantly (SMD, −0.73; 95% CI, −1.15 to −0.31; $p = 0.001$) (Figure 3C) while the heterogeneity remained in a high level ($I^2 = 78.1\%$, $p = 0.000$). According to subgroup analyses based on study duration and probiotic dose, the pooled result remained consistent in all subgroups (Table 2 and Figure S3.2–3.3). Moreover, probiotic was more favorable in HOMA-IR compared with synbiotic supplementation (Table 2 and Figure S3.1).

*QUICKI.* Six trials containing 379 subjects (189 of them in the treatment group, other 190 in the control group) were used to analyze the effects of pro- and synbiotics on QUICKI. The level of QUICKI in pro- and synbiotics group was higher than that in placebo group (SMD, 2.00; 95% CI, − 0.79 to 3.22; $p = 0.001$; $I^2 = 96.1\%$) (Figure 3D). Stratified analyses revealed that the higher QUICKI level favors consuming probiotics and intervention $\geq$ 12 weeks' subgroups compared with synbiotics and $<$ 12 weeks' subgroups (Table 2 and Figure S4.1–4.2).

### Effects on biomarkers of lipid profiles

*TG.* In total, 7 studies (428 participants) mentioned a change in TG level. Pro-, pre-, and synbiotic supplementation were found to have reductive effects on TG (SMD, −0.85; 95% CI, −1.59 to −0.11; $p = 0.024$) (Figure 4A). The heterogeneity was high among related studies ($I^2 = 92.2\%$, $p = 0.000$). Subgroup analyses showed that the inference was identical in groups of taking pro-, prebiotic and study duration $\geq$ 12 weeks (Table 2 and Figure S5.1–5.2). In the probiotic dose subgroup, the effects of taking pro-, pre-, and synbiotic on the TG level remained decrease in all subgroups and have reduced the heterogeneity to 22% (Table 2 and Figure S5.3).

*TC.* A meta-analysis of 7 trials (428 individuals), found that the TC decrease greater after pro-, pre-, and synbiotic supplementation in comparison with the placebo (SMD, −1.09; 95% CI, −1.98 to −0.21; $p = 0.015$; $I^2 = 94.3\%$) (Figure 4B). Further subgroup analysis presented that the finding remained unchanged in studies with prebiotic supplementation, duration $\geq$ 12 weeks, and probiotic dose $\geq 2 \times 10^8$ CFU (Table 2 and Figure S6.1–6.3).

*HDL-c.* In the pooled meta-analysis including 7 RCTs (428 women), we found no effect of pre-, pro-, and synbiotic supplements on HDL-c when compared with the placebo (SMD, 0.53; 95% CI, −0.33 to 1.39; $p = 0.228$; $I^2 = 94.3\%$) (Figure 5A). Subgroup analysis showed that the pooled effect did not change in all subgroups according to the number of probiotic dose (Table 2 and Figure S7.3). However, in

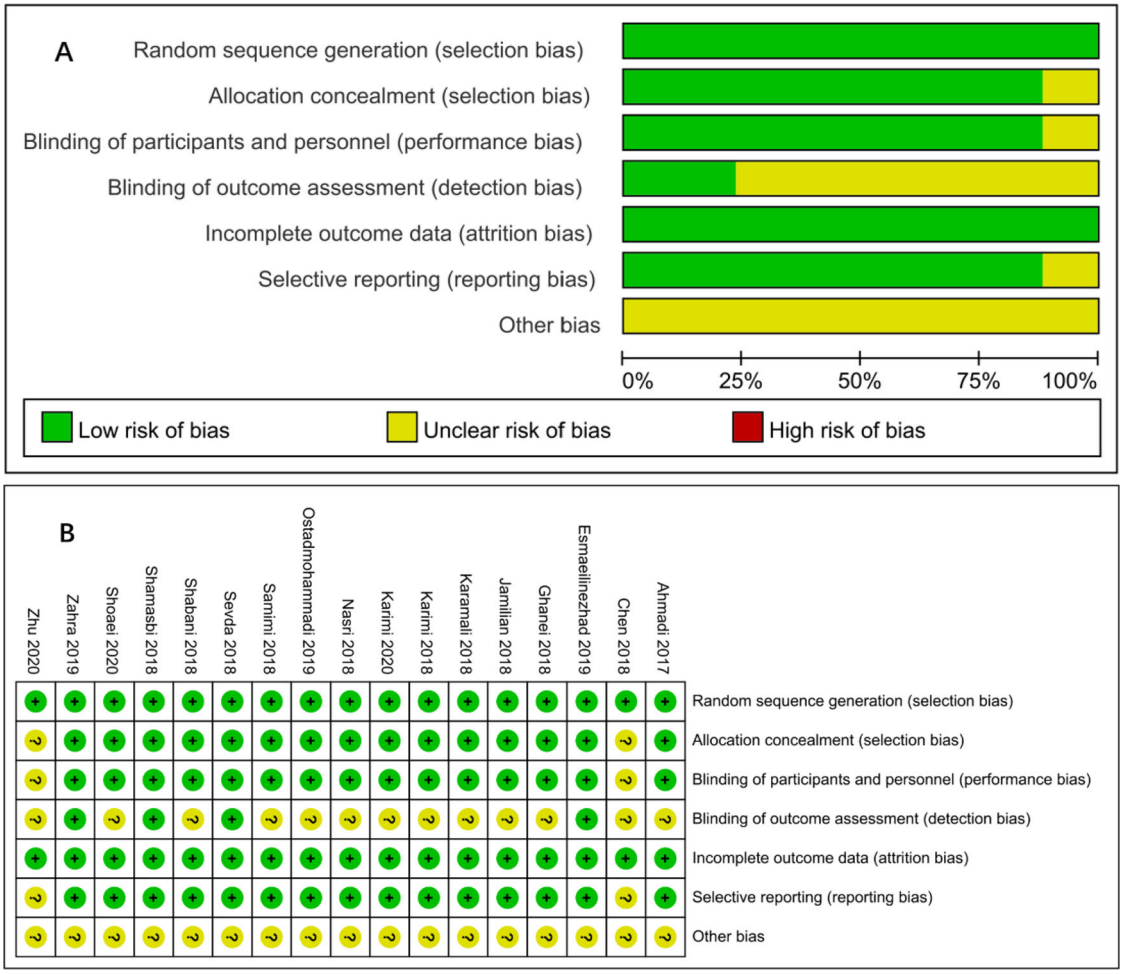


**Figure 2.** Risk of bias graph (A) and risk of bias summary (B) for included RCTs.

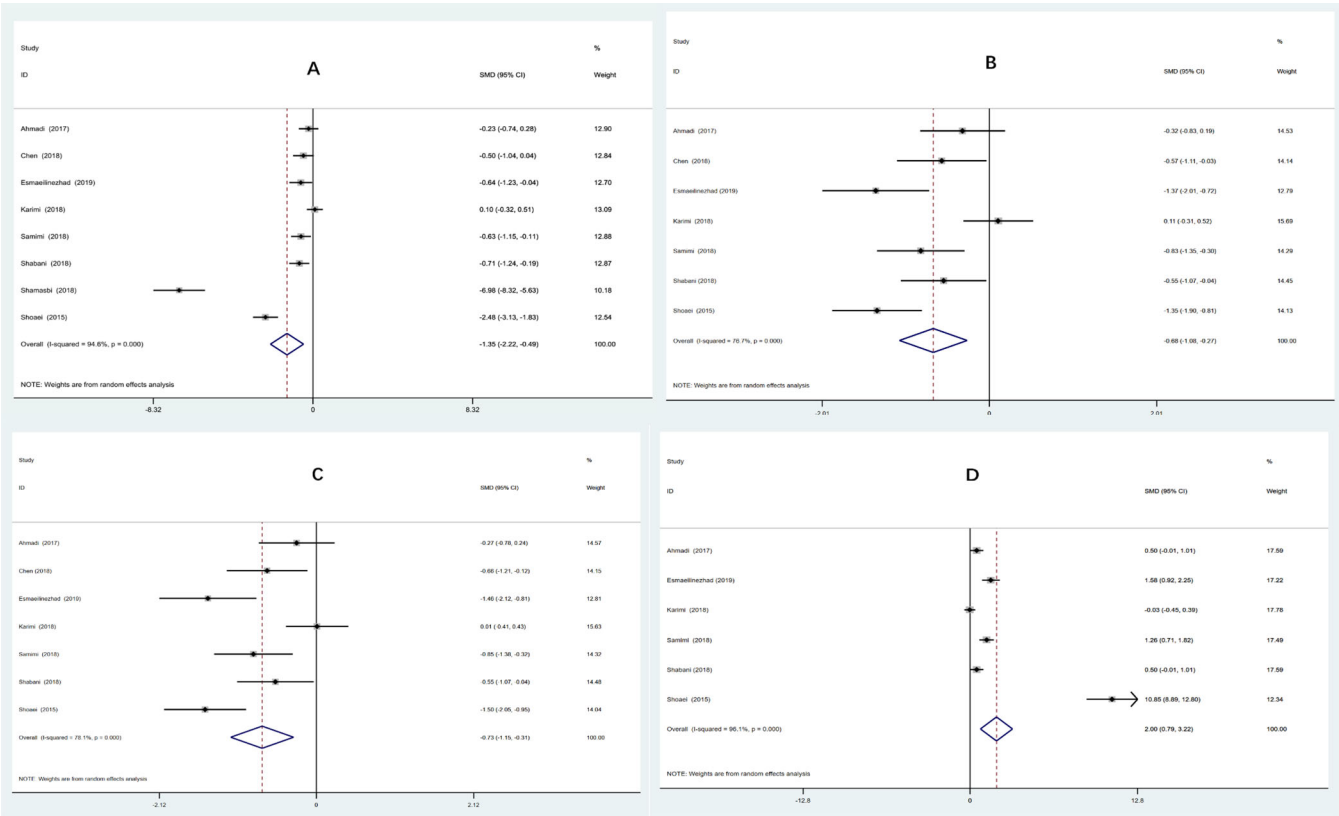


**Figure 3.** Forest plots of the effect of probiotic, prebiotic, and synbiotic supplementation on FPG (A), FINS (B), HOMA-IR (C), and QUICKI (D).

**Table 2.** The effects of probiotic, prebiotic or synbiotic spplementation on insulin resistance indices and lipid profiles based on subgroup analysis.

| Variable | Subgrouped by | No. of trials | No. of participants | SMD | 95% CI | P | I2(%) | P for heterogeneity |
|---|---|---|---|---|---|---|---|---|
| FPG | Type of intervention | | | | | | | |
| | Probiotic | 4 | 240 | −0.96 | −1.86, −0.07 | 0.035 | 90.50% | 0.000 |
| | Synbiotic | 3 | 194 | −0.36 | −0.87, 0.15 | 0.169 | 67.70% | 0.045 |
| | Prebiotic | 1 | 62 | −6.98 | −8.32, −5.63 | 0.000 | NA | NA |
| | Study duration | | | | | | | |
| | ≥ 12 weeks | 6 | 385 | −1.29 | −2.29, −0.29 | 0.012 | 94.90% | 0.000 |
| | < 12 weeks | 2 | 111 | −1.55 | −3.35, 0.25 | 0.092 | 94.00% | 0.000 |
| | Probiotic dose | | | | | | | |
| | ≥ $2 \times 10^8$CFU | 6 | 379 | −0.74 | −1.38, −0.10 | 0.024 | 88.80% | 0.000 |
| | < $2 \times 10^8$CFU | 1 | 55 | −0.50 | −1.04, 0.04 | 0.068 | NA | NA |
| FINS | Type of intervention | | | | | | | |
| | Probiotic | 4 | 240 | −0.70 | −1.13, −0.26 | 0.002 | 63.60% | 0.041 |
| | Synbiotic | 3 | 194 | −0.67 | −1.54, 0.20 | 0.130 | 87.90% | 0.000 |
| | Study duration | | | | | | | |
| | ≥ 12 weeks | 5 | 323 | −0.41 | −0.74, −0.08 | 0.016 | 55.00% | 0.064 |
| | < 12 weeks | 2 | 111 | −1.36 | −1.77, −0.95 | 0.000 | 0.00% | 0.973 |
| | Probiotic dose | | | | | | | |
| | ≥ $2 \times 10^8$CFU | 6 | 379 | −0.70 | −1.18, −0.22 | 0.004 | 80.60% | 0.000 |
| | < $2 \times 10^8$CFU | 1 | 55 | −0.57 | −1.11, −0.03 | 0.037 | NA | NA |
| HOMA-IR | Type of intervention | | | | | | | |
| | Probiotic | 4 | 240 | −0.74 | −1.25, −0.23 | 0.005 | 73.10% | 0.011 |
| | Synbiotic | 3 | 194 | −0.74 | −1.59, 0.11 | 0.088 | 87.20% | 0.000 |
| | Study duration | | | | | | | |
| | ≥ 12 weeks | 5 | 323 | −0.44 | −0.75, −0.13 | 0.006 | 49.50% | 0.094 |
| | < 12 weeks | 2 | 111 | −1.48 | −1.91, −1.06 | 0.000 | 0.00% | 0.931 |
| | Probiotic dose | | | | | | | |
| | ≥ $2 \times 10^8$CFU | 6 | 379 | −0.75 | −1.25, −0.25 | 0.003 | 81.80% | 0.000 |
| | < $2 \times 10^8$CFU | 1 | 55 | −0.66 | −1.21, −0.12 | 0.017 | NA | NA |
| QUICKI | Type of intervention | | | | | | | |
| | Probiotic | 3 | 185 | 3.65 | 0.71, 6.58 | 0.015 | 98.10% | 0.000 |
| | Synbiotic | 3 | 194 | 0.92 | −0.12, 1.96 | 0.084 | 91.00% | 0.000 |
| | Study duration | | | | | | | |
| | ≥ 12 weeks | 4 | 268 | 0.54 | 0.02, 1.06 | 0.044 | 77.50% | 0.004 |
| | < 12 weeks | 2 | 111 | 6.17 | −2.91, 15.25 | 0.183 | 98.70% | 0.000 |
| TG | Type of intervention | | | | | | | |
| | Probiotic | 3 | 175 | −0.50 | −0.80, −0.20 | 0.001 | 0.00% | 0.929 |
| | Synbiotic | 3 | 191 | −0.14 | −0.47, 0.20 | 0.427 | 25.20% | 0.263 |
| | Prebiotic | 1 | 62 | −4.41 | −5.35, −3.48 | 0.000 | NA | NA |
| | Study duration | | | | | | | |
| | ≥ 12 weeks | 6 | 385 | −0.94 | −1.80, −0.08 | 0.031 | 93.50% | 0.000 |
| | < 12 weeks | 1 | 43 | −0.37 | −0.97, 0.23 | 0.229 | NA | NA |
| | Probiotic dose | | | | | | | |
| | ≥ $2 \times 10^8$ CFU | 5 | 373 | −0.27 | −0.52, −0.01 | 0.040 | 22.00% | 0.274 |
| | < $2 \times 10^8$CFU | 1 | 55 | −0.55 | −1.09, −0.01 | 0.045 | NA | NA |
| TC | Type of intervention | | | | | | | |
| | Probiotic | 3 | 175 | −0.26 | −0.85, 0.32 | 0.378 | 73.50% | 0.023 |
| | Synbiotic | 3 | 191 | −0.28 | −0.56, 0.01 | 0.059 | 0.00% | 0.526 |
| | Prebiotic | 1 | 62 | −7.52 | −8.95, −6.08 | 0.000 | NA | NA |
| | Study duration | | | | | | | |
| | ≥ 12 weeks | 6 | 385 | −1.22 | −2.26, −0.19 | 0.020 | 95.20% | 0.000 |
| | < 12 weeks | 1 | 43 | −0.50 | −1.11, 0.11 | 0.106 | NA | NA |
| | Probiotic dose | | | | | | | |
| | ≥ 2 × 108 CFU | 5 | 373 | −0.38 | −0.61, −0.16 | 0.001 | 0.00% | 0.566 |
| | < 2 × 108CFU | 1 | 55 | 0.33 | −0.20, 0.87 | 0.220 | NA | NA |
| HDL-c | Type of intervention | | | | | | | |
| | Probiotic | 3 | 175 | −0.17 | −0.98, 0.63 | 0.672 | 85.90% | 0.001 |
| | Synbiotic | 3 | 191 | 0.09 | −0.48, 0.65 | 0.762 | 72.70% | 0.026 |
| | Prebiotic | 1 | 62 | 4.28 | 3.37, 5.20 | 0.000 | NA | NA |
| | Study duration | | | | | | | |
| | ≥ 12 weeks | 6 | 385 | 0.50 | −0.48, 1.49 | 0.315 | 95.10% | 0.000 |
| | < 12 weeks | 1 | 43 | 0.72 | 0.10, 1.34 | 0.022 | NA | NA |
| | Probiotic dose | | | | | | | |
| | ≥ $2 \times 10^8$ CFU | 5 | 373 | −0.11 | −0.62, 0.40 | 0.675 | 80.10% | 0.000 |
| | < $2 \times 10^8$CFU | 1 | 55 | 0.32 | −0.21, 0.85 | 0.241 | NA | NA |
| LDL-c | Type of intervention | | | | | | | |
| | Probiotic | 3 | 175 | −0.13 | −0.42, 0.17 | 0.404 | 0.00% | 0.438 |
| | Synbiotic | 3 | 191 | −0.22 | −0.51, 0.06 | 0.127 | 0.00% | 0.500 |
| | Prebiotic | 1 | 62 | −5.57 | −6.69, −4.46 | 0.000 | NA | NA |
| | Study duration | | | | | | | |
| | ≥ 12 weeks | 6 | 385 | −0.91 | −1.84, 0.03 | 0.057 | 94.40% | 0.000 |
| | < 12 weeks | 1 | 43 | −0.54 | −1.15, 0.07 | 0.081 | NA | NA |
| | Probiotic dose | | | | | | | |
| | ≥ $2 \times 10^8$CFU | 5 | 373 | −0.22 | −0.44, 0.00 | 0.053 | 0.00% | 0.694 |
| | < $2 \times 10^8$CFU | 1 | 55 | 0.07 | −0.45, 0.60 | 0.784 | NA | NA |

(*continued*)

**Table 2.** Continued.

| Variable | Subgrouped by | No. of trials | No. of participants | SMD | 95% CI | P | I2(%) | P for heterogeneity |
|---|---|---|---|---|---|---|---|---|
| VLDL-c | Type of intervention | | | | | | | |
| | Probiotic | 3 | 175 | −0.48 | −0.78, −0.18 | 0.002 | 0.00% | 0.955 |
| | Synbiotic | 1 | 60 | −0.32 | −0.83, 0.19 | 0.215 | NA | NA |
| | Probiotic dose | | | | | | | |
| | $\geq 2 \times 10^8$CFU | 3 | 180 | −0.42 | −0.72, −0.13 | 0.005 | 0.00% | 0.850 |
| | $< 2 \times 10^8$CFU | 1 | 55 | −0.48 | −1.02, 0.05 | 0.078 | NA | NA |

Abbreviations: FPG, fasting plasma glucose; FINS, fasting insulin; HOMA-IR, homeostatic model of assessment for insulin resistance; QUICKI, quantitative insulin sensitivity check index; TG, triglycerides; TC, total cholesterol; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; VLDL-c, very-low-density lipoprotein cholesterol; SMD, standardized mean difference; NA, not applicable.

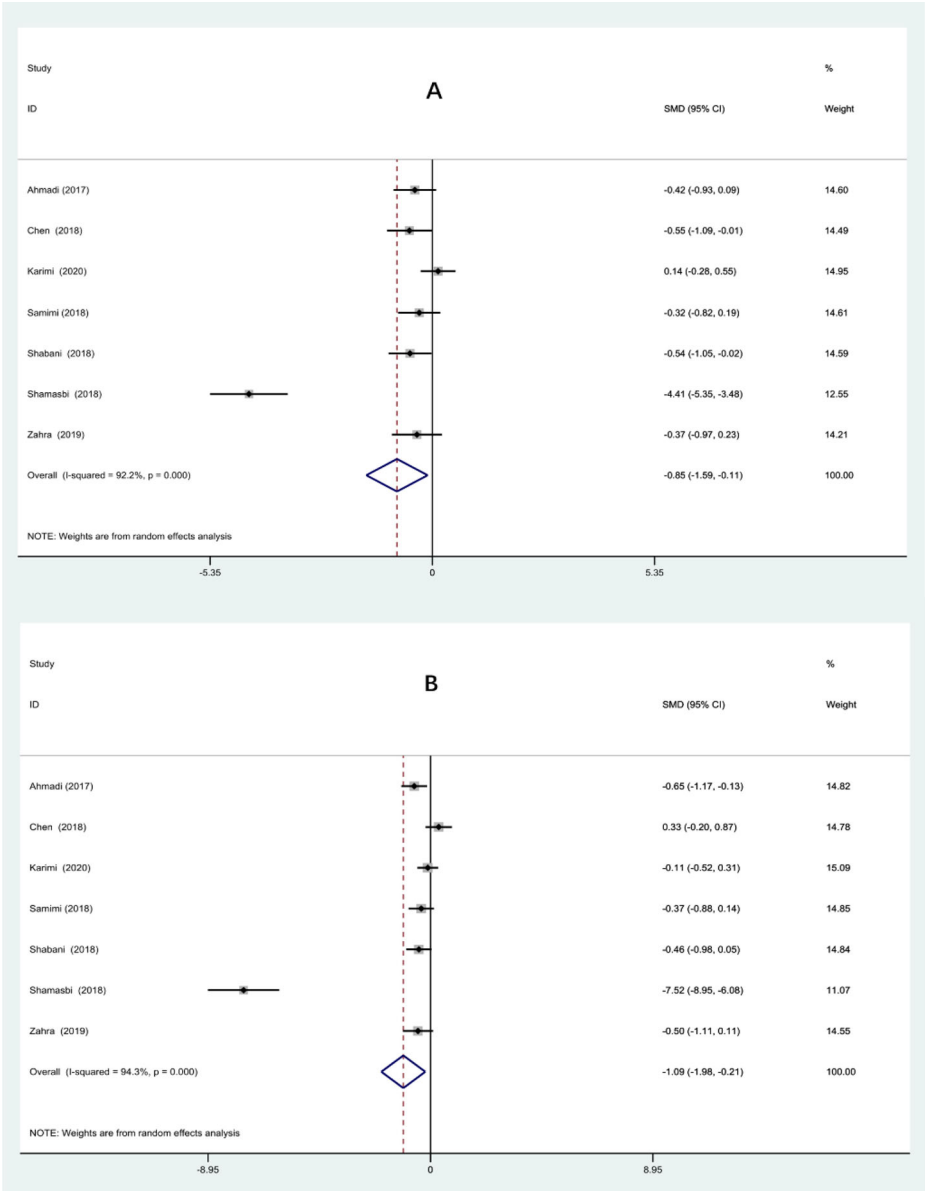


**Figure 4.** Forest plots of the effect of probiotic, prebiotic, and synbiotic supplementation on TG (A) and TC (B).

subgroups of intervention and study duration, there was a significant increase of HDL-c with prebiotic supplements and duration < 12 weeks (Table 2 and Figure S7.1–7.2).

*LDL-c.* The pooled analysis of 7 studies containing 428 participants showed that pre-, pro-, and synbiotic supplementation were superior in reducing LDL-c than placebo (SMD, −0.84; 95% CI, −1.64 to −0.03; $p = 0.041$) (Figure 5B). The heterogeneity among related studies was high ($I^2 = 93.4\%$, $p = 0$). The subgroup analysis based on the type of intervention showed that the LDL-c level decreased in the group with taking prebiotic and interstudy heterogeneity was removed ($I^2 = 0\%$) (Table 2 and Figure S8.1). On the contrary, there was no statistical difference in pro- and

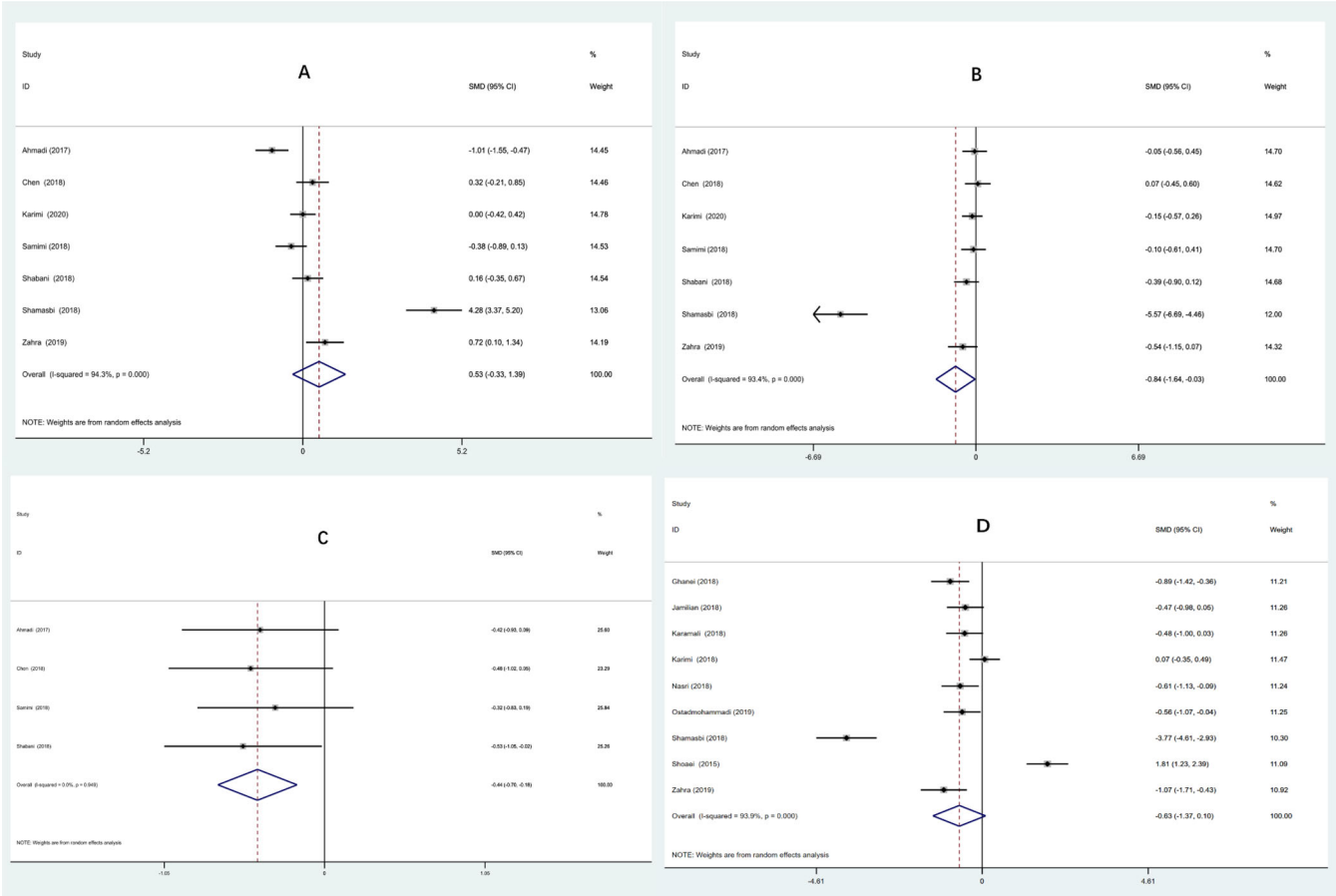


**Figure 5.** Forest plots of the effect of probiotic, prebiotic, and synbiotic supplementation on HDL-c (A), LDL-c (B), VLDL-c (C), and CRP (D).

synbiotic groups. Moreover, in the study duration and probiotic dose subgroups, there was no clear difference in LDL-c (Table 2 and Figure S8.2–8.3). Analyses of intergroup heterogeneity showed that the type of intervention and probiotic dose were the source of heterogeneity.

*VLDL-c.* When we combined data from 4 studies (235 participants), a reduction of VLDL-c was observed after pro-, pre-, and synbiotic supplementation (SMD, −0.44; 95% CI, −0.70 to −0.18; $p = 0.001$) without heterogeneity ($I^2 = 0.0\%$, $p = 0.949$) (Figure 5C). Stratified analyses revealed that VLDL-c maintained a decrease in categories of taking probiotic and dose $\geq 2 \times 10^8$ CFU (Table 2 and Figure S9.1–9.2).

### Effects on anthropometric indices

*BW.* Data were extracted from 12 studies with 731 patients. We found no apparent difference of BW following administration of pro-, pre-, and synbiotic (SMD, −0.11; 95% CI, −0.34 to 0.13; $p = 0.379$), and there was moderate heterogeneity among studies ($I^2 = 61.5\%$, $p = 0.003$) (Figure 6A). However, the subgroup analysis revealed a significant decrease of the BW among related trials of prebiotic intervention and studies duration < 12 weeks (Table 3 and Figure S10.1 − 10.2). No effective modifications were observed in the probiotic dose subgroup (Table 3 and Figure S10.3).

*BMI.* Combining data from 13 studies (791 participants) explored the influence of pro-, pre-, and synbiotics on BMI, no difference was shown (SMD, −0.10; 95% CI, −0.30 to 0.09; $p = 0.295$; $I^2 = 47.3\%$) (Figure 6B). The finding of pro-, pre-, and synbiotic effects on BMI did not change in subgroups of the number of probiotic strains and probiotic dose. Otherwise, in the categorical analysis based on the type of intervention and study duration, trials with prebiotic intervention and duration < 12 weeks significantly had decreased BMI (Table 3 and Figure S11.1–11.4).

*WC.* Five trials (316 participants) reported this outcome. Compared with the placebo, pro-, pre-, and synbiotic supplementation did not present any decreasing influence on WC measurements (SMD, 0.37; 95% CI, −0.78 to 1.53; $p = 0.525$; $I^2 = 95.5$) (Figure 6C). Further subgroup analyses indicated that the pooled analysis did not change in subgroups of the number of probotic strains and probiotic doses (Table 3 and Figure S12.3–12.4). Nevertheless, subgroup analysis showed a significant reduction in the WC was found in studies with prebiotic supplementation and studies duration < 12 weeks (Table 3 and Figure S12.1–12.2).

*HC.* In the pooled analysis of 4 studies, there was no clear difference between pro-, pre-, synbiotic and the placebo in HC (SMD, −0.25; 95% CI, −0.78 to 0.27; $p = 0.344$) with a high level of heterogeneity ($I^2 = 76.9\%$) (Figure 6D). The inference was the same after subgroup analysis based on the

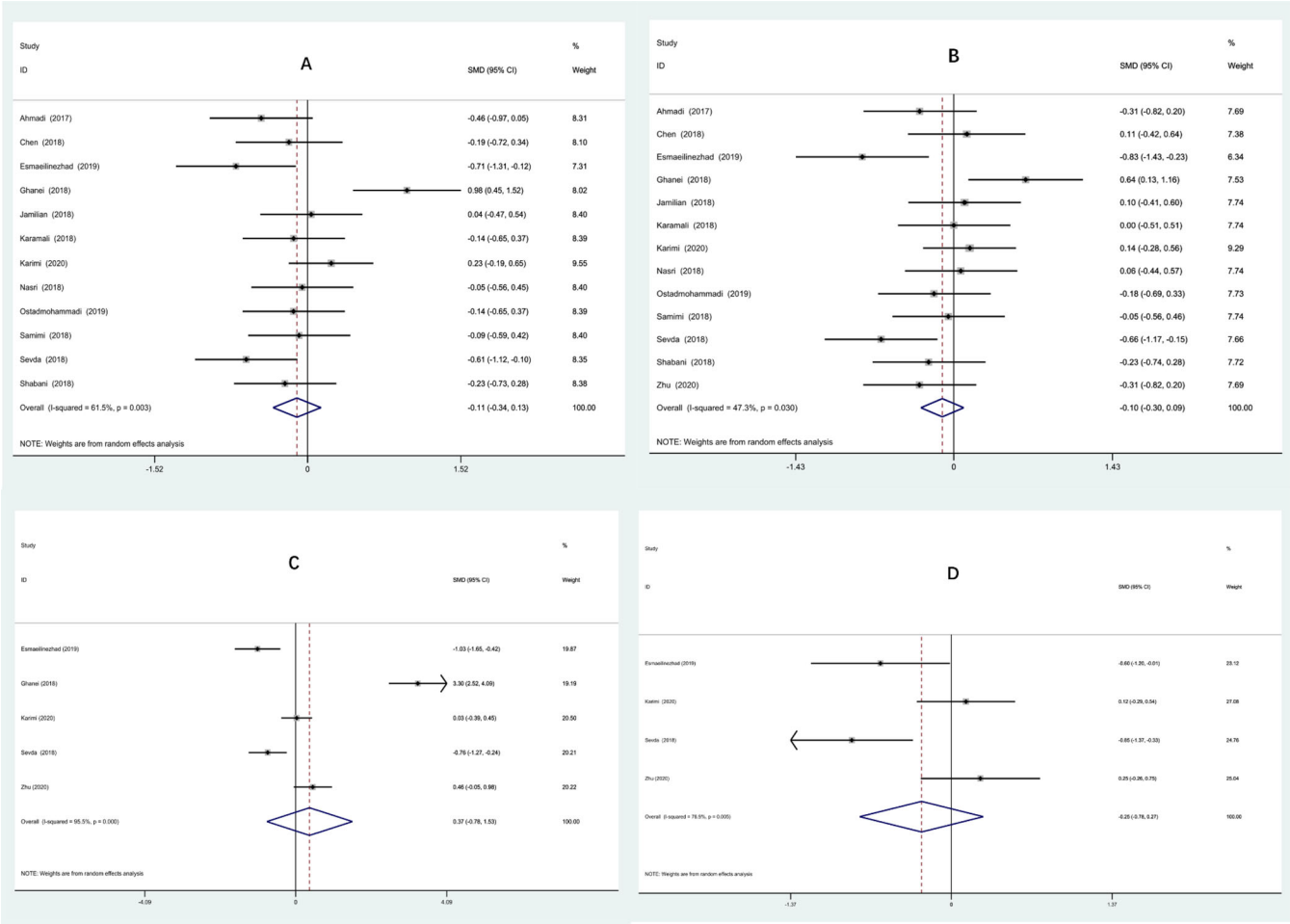


**Figure 6.** Forest plots of the effect of probiotic, prebiotic, and synbiotic supplementation on BW (A), BMI (B), WC (C), and HC (D).

number of probiotic strains and probiotic dose (Table 3 and Figure S13.3–13.4). However, in subgroups of the type of intervention and study duration, a greater HC level decrease with taking prebiotic and study duration < 12 weeks was found (Table 3 and Figure S13.1–13.2).

### *Effects on CRP level*

*CRP.* Combining data from 9 studies containing 558 patients investigated pro-, pre-, and synbiotic effects on CRP level. CRP showed no statistical difference (SMD, −0.63; 95% CI, −1.37 to 0.10; $p = 0.089$) (Figure 5D). Subgroup analysis showed a significant reduction of CRP level following the intake of prebiotic and studies duration ≥ 12 weeks (Table 3 and Figure S14.1–14.2).

### *Sensitivity analysis and publication bias*

Sensitivity analyses made no material difference to SMDs of FPG, FINS, HOMA-IR, QUICKI, TG, TC, HDL-c, VLDL-c, BMI, WC, and HC (Figure S1–7, 9, 11–13). Results of sensitivity analysis showed that the majority of conclusions were stable and were not affected by the quality of the included trials. However, sensitivity analysis conducted by the exclusion of one study (Ghanei et al. 2018) (Figure S10) showed a significant decrease of BW (SMD, −0.19; 95% CI, −0.35 to −0.02; p = 0.027; $I^2 = 15.3$). For LDL-c, we found no statistical difference of pooled SMD (SMD, −0.18; 95% CI, −0.38 to 0.03; p = 0.093; $I^2 = 0$) (Figure S8) after excluding Shamasbi et al. (2018). A similar result was also found in the analysis of CRP after removing Shoaei et al. (2015) (SMD, −0.92; 95% CI, −1.51 to −0.32; $p = 0.002$; $I^2 = 89.7\%$) (Figure S14).

Evaluation of publication bias by visual inspection of the funnel plot (figures S15.1 and S15.3) and Egger's test demonstrated no evidence for publication bias in the meta-analysis of pro-, pre-, and synbiotics on BW (p = 0.321) and BMI (p = 0.150) (figures S15.2 and S15.4). Publication bias was not assessed in all outcomes because all of the analyses (except BW and BMI) were < 10 studies. The Cochrane handbook recommends that ≥ 10 studies are necessary to achieve clear conclusions regarding publication bias (Higgins et al. 2019).

## Discussion

This systematic review and meta-analysis is the first report of the effects of pro-, pre-, and synbiotic intake on IR, lipid profiles, anthropometric indices, and CRP in PCOS. In this review, supplementation with pro-, pre-, and synbiotic can significantly ameliorate 1): IR, assessed by decreased HOMA-IR, FPG, FINS and increased QUICKI; 2): lipid profiles, evidenced by decreased TG, TC, LDL-c, and VLDL-c

**Table 3.** The effects of probiotic, prebiotic or synbiotic spplementation on anthropometric indices and C-reactive protein based on subgroup analysis.

| Variable | Subgrouped by | No. of trials | No. of participants | SMD | 95% CI | P | I2(%) | P for heterogeneity |
|---|---|---|---|---|---|---|---|---|
| BW | Type of intervention | | | | | | | |
| | Probiotic | 7 | 415 | −0.02 | −0.36, 0.31 | 0.892 | 66.20% | 0.007 |
| | Synbiotic | 4 | 254 | −0.12 | −0.49, 0.25 | 0.534 | 53.50% | 0.092 |
| | Prebiotic | 1 | 62 | −0.61 | −1.12, −0.10 | 0.019 | NA | NA |
| | Study duration | | | | | | | |
| | ≥ 12 weeks | 11 | 685 | −0.06 | −0.29, 0.18 | 0.626 | 58.70% | 0.007 |
| | < 12 weeks | 1 | 46 | −0.71 | −1.31, −0.12 | 0.019 | NA | NA |
| | Probiotic dose | | | | | | | |
| | $\geq 2 \times 10^8$CFU | 10 | 676 | −0.05 | −0.31, 0.21 | 0.717 | 62.50% | 0.004 |
| | $< 2 \times 10^8$CFU | 1 | 55 | −0.19 | −0.72, 0.34 | 0.492 | NA | NA |
| BMI | Type of intervention | | | | | | | |
| | Probiotic | 8 | 475 | −0.03 | −0.24, 0.19 | 0.823 | 31.80% | 0.174 |
| | Synbiotic | 4 | 254 | −0.13 | −0.53, 0.26 | 0.508 | 58.90% | 0.063 |
| | Prebiotic | 1 | 62 | −0.66 | −1.17, −0.15 | 0.012 | NA | NA |
| | Study duration | | | | | | | |
| | ≥ 12 weeks | 12 | 745 | −0.05 | −0.23, 0.12 | 0.553 | 34.30% | 0.115 |
| | < 12 weeks | 1 | 46 | −0.83 | −1.43, −0.23 | 0.007 | NA | NA |
| | Number of probiotic strains | | | | | | | |
| | Multi strain | 11 | 669 | −0.03 | −0.23, 0.16 | 0.743 | 39.70% | 0.412 |
| | Single strain | 1 | 60 | −0.31 | −0.82, 0.20 | 0.230 | NA | 0.199 |
| | Probiotic dose | | | | | | | |
| | ≥ 2 × 108 CFU | 10 | 614 | −0.05 | −0.26, 0.17 | 0.661 | 44.80% | 0.061 |
| | < 2 × 108CFU | 2 | 115 | −0.11 | −0.52, 0.31 | 0.620 | 22.40% | 0.256 |
| WC | Type of intervention | | | | | | | |
| | Probiotic | 2 | 120 | 1.87 | −0.91, 4.65 | 0.188 | 97.20% | 0.000 |
| | Synbiotic | 2 | 134 | −0.48 | −1.52, 0.56 | 0.368 | 87.20% | 0.005 |
| | Prebiotic | 1 | 62 | −0.76 | −1.27, −0.24 | 0.004 | NA | NA |
| | Study duration | | | | | | | |
| | ≥ 12 weeks | 4 | 270 | 0.73 | −0.60, 2.05 | 0.284 | 96.00% | 0.000 |
| | < 12 weeks | 1 | 46 | −1.03 | −1.65, −0.42 | 0.001 | NA | NA |
| | Number of probiotic strains | | | | | | | |
| | Multi strain | 3 | 194 | 0.75 | −1.35, 2.85 | 0.486 | 97.40% | 0.000 |
| | Single strain | 1 | 60 | 0.46 | −0.05, 0.98 | 0.077 | NA | NA |
| | Probiotic dose | | | | | | | |
| | $\geq 2 \times 10^8$ CFU | 3 | 194 | 0.75 | −1.35, 2.85 | 0.486 | 97.40% | 0.000 |
| | $< 2 \times 10^8$ CFU | 1 | 60 | 0.46 | −0.05, 0.98 | 0.077 | NA | NA |
| HC | Type of intervention | | | | | | | |
| | Synbiotic | 2 | 134 | −0.21 | −0.92, 0.50 | 0.565 | 74.20% | 0.049 |
| | Probiotic | 1 | 60 | 0.25 | −0.26, 0.75 | 0.341 | NA | NA |
| | Prebiotic | 1 | 62 | −0.85 | −1.37, −0.33 | 0.001 | NA | NA |
| | Study duration | | | | | | | |
| | ≥ 12 weeks | 3 | 210 | −0.15 | −0.80, 0.50 | 0.648 | 81.50% | 0.004 |
| | < 12 weeks | 1 | 46 | −0.60 | −1.20, −0.01 | 0.045 | NA | NA |
| | Number of probiotic strains | | | | | | | |
| | Multi strain | 2 | 134 | −0.21 | −0.92, 0.50 | 0.565 | 74.20% | 0.049 |
| | Single strain | 1 | 60 | 0.25 | −0.26, 0.75 | 0.341 | NA | NA |
| | Probiotic dose | | | | | | | |
| | $\geq 2 \times 10^8$ CFU | 2 | 134 | −0.21 | −0.92, 0.50 | 0.565 | 74.20% | 0.049 |
| | $< 2 \times 10^8$ CFU | 1 | 60 | 0.25 | −0.26, 0.75 | 0.341 | NA | NA |
| CRP | Type of intervention | | | | | | | |
| | Probiotic | 5 | 305 | −0.12 | −1.01, 0.77 | 0.785 | 92.90% | 0.000 |
| | Synbiotic | 3 | 191 | −0.50 | −1.16, 0.15 | 0.134 | 79.10% | 0.008 |
| | Prebiotic | 1 | 62 | −3.77 | −4.61, −2.93 | 0.000 | NA | NA |
| | Study duration | | | | | | | |
| | ≥ 12 weeks | 7 | 450 | −0.90 | −1.57, −0.24 | 0.008 | 91.00% | 0.000 |
| | < 12 weeks | 2 | 108 | 0.37 | −2.44, 3.19 | 0.795 | 97.60% | 0.000 |

*Abbreviations*: BW, body weight; BMI, body mass index; WC, waist circumference; HC, hip circumference; CRP, C-reactive protein; SMD, standardized mean difference; NA, not applicable.

in PCOS. However, there is no evidence of a clear difference between pro-, pre-, synbiotic and placebo in anthropometric indices and CRP.

### *Pro-, pre-, or synbiotic and insulin resistance*

Based on the evidence that IR is a common feature of PCOS, and it plays as a pathogen in this syndrome (Moghetti 2016). Moreover, IR is a determining factor in the pathophysiology of Diabetes Mellitus type 2 (Sacerdote et al. 2019). However, traditional IR treatments, such as insulin sensitizer, have gastrointestinal effects or cause vitamin B12 deficiency by long-term application (Viollet et al. 2012). However, lifestyle modification can deliver well-established benefits to women with PCOS in metabolism, physique, and psychology. Due to general (such as lack of time, fatigue, weather, and family matters) and PCOS-specific (social physique anxiety, appearance evaluation, depression) barriers (Trost et al. 2002; Kogure et al. 2020), most women refuse to change the current lifestyle. Therefore, optimal management strategies with safety and acceptability are required. Our meta-analysis shows that pro-, pre-, and synbiotic

supplements are effective in modifying insulin sensitivity in PCOS, which is consistent with the previous meta-analysis conducted by Cozzolino et al. (2020) and Hadi et al. (2020). Probiotics and prebiotics could be a promising approach to improving insulin sensitivity by modifying gut microbial community's composition, reducing gut permeability (leaky gut), intestinal endotoxin concentrations, and energy harvest (Gurung et al. 2020). The gut-brain axis, particularly the hypothalamic signals, is also known to play an important role in regulating whole-body metabolism (van de Wouw et al. 2017; Stanley et al. 2016). Emerging evidence has shown that hypothalamic energy signaling in terms of increasing expression of pro-opiomelanocortin was also modulated by prebiotic administration (Ahmadi et al. 2019).

### Pro-, pre-, or synbiotic and lipid profiles

Prevalence of cardiovascular disease (CVD) is high in atherogenic dyslipidemia, which is characterized by elevated TG, TC, VLDL-c, and LDL-c, and decreased HDL-c (Aguiar et al. 2015; Chapman et al. 2011). Intricate crosstalk links the gut microbiota and host lipid metabolism. Therefore, the gut microbiota has been targeted to treat diseases related to dyslipidemia (Schoeler and Caesar 2019). As for the lipid-lowering properties of pro-, pre-, and synbiotics in PCOS, our pooled findings are in line with the previous meta-analysis for patients with Diabetes Mellitus type 2 (Hendijani and Akbari 2018). However, the effects of intestinal microbiological preparations in improving lipoproteins are controversial. Several other meta-analysis have found probiotic or synbiotic intake is ineffective concerning TC and LDL-c in PCOS (Heshmati et al. 2019; Tabrizi et al. 2019; Cozzolino et al. 2020). Tabrizi et al. reported a decrease in TG, TC, VLDL-c and no increasing in LDL-c and HDL-c in subjects with diabetes after intake of synbiotic (Tabrizi et al. 2018). In another recent review, pro-, pre-, or synbiotic consumption has increased HDL-c in patients with diabetes (Bock et al. 2021). In contrast, we found no overall association between probiotics and TC, TG, LDL-c, and HDL-c in Diabetes Mellitus type 2 (Kasińska and Drzewoski 2015). These conflicting results can be due to different supplements' formulation, clinical heterogeneity, study design, and characteristics of studied populations. Pro-, pre-, or synbiotic might influence lipid profiles by improving the gut microflora (Ko et al. 2020), enhancing excretion of cholesterol by feces (Yoo and Kim 2016), modulation of metabolism of bile acids (Oberfroid et al. 2010), and increasing production of SCFAs via selective fermentation (Schoeler and Caesar 2019).

### Pro-, pre-, or synbiotic and anthropometric indices

Obesity is related to the infertility of PCOS and increases the risk for metabolic syndromes and clustering of cardiovascular risk factors in women (Insenser et al. 2018). In our meta-analysis, pro-, pre-, or synbiotic causes no significant changes in BW, BMI, WC, and HC compared with the placebo. While subgroup analyses indicated that anthropometric indices were reduced in trials with prebiotic supplementation and duration $< 12$ weeks. Consistent with our findings, the results of previous 2 systematic reviews (Hadi et al. 2020; Heshmati et al. 2019) showed that probiotic or synbiotic administration could not affect BW and BMI. Another study (Tabrizi et al. 2019) indicated that BMI and BW measurements were reduced in PCOS women who received probiotic supplements. Inconsistency results of Tabrizi et al. and the present review may be due to the difference of intervention methods and ethnic group differences. Our review included pro-, pre-, or synbiotic, while Tabrizi et al. only assessed the effects of probiotic supplements.

### Pro-, pre-, or synbiotic and CRP

Clinical evidence shows an inseparable association of chronic low-grade inflammation with hyperandrogenism and IR in PCOS (Shorakae et al. 2018). Long-term metabolic effects of PCOS may be partly attributed to additional effects of chronic low-grade inflammation (Shorakae et al. 2015). Moreover, dysbiosis of gut microbiota is closely related to the pathogenesis of PCOS (Liu et al. 2017). Ecological imbalance of gut microbiota leads to abnormal increasing of intestinal permeability. Intestinal-derived lipopolysaccharides (LPS) enters into the global circulation through the "intestine leaking" wall to induce systemic low-grade inflammation, leading to increasing testosterone production of ovaries and resulting in PCOS. (Lindheim et al. 2017; Zeng et al. 2019). Pro-, pre-, or synbiotic has been approved of alleviating PCOS symptoms via modulating gut microbiota, increasing proportions of *Bifidobacterium* and *Lactobacillus*, restoring the microbiota balance (Cozzolino et al. 2018), reducing intestinal permeability, and decreasing translocation of LPS from the intestine to the blood circulation (Xue et al. 2019). In our review, we also assessed the effects of pro-, pre-, or synbiotic consumption of CRP in PCOS. However, we failed to find any statistical differences between the intervention and control group. Our conclusion regarding the effects of CRP level is consistent with findings from the previous meta-analysis by Cozzolino et al. (2020), Shamasbi, Ghanbari-Homayi, and Mirghafourvand (2020), and Liao et al. (2018).

It is worthy mention that in the subgroup analysis of trials based on the type of intervention, we found that consumption of probiotic was statistically more effective in decreasing HOMA-IR, FPG, FINS, TG, VLDL-c and increasing QUICKI levels than synbiotics. Probiotics are preparations of microbial cells. They are generally safe for human consumption and act through microbiota modulation. The potential benefits of probiotics on improving glucose metabolism have been widely studied (Firouzi et al. 2017). Based on results obtained from animal models and humans (Andersson et al. 2010), probiotic interventions have been proposed as a potential strategy for preventing or treating PCOS.

In addition, the prebiotic intake has larger effects on anthropometric indices and TC, HDL-c, LDL-c compared

with probiotics and synbiotics intake in PCOS. Prebiotics are non-digestible food ingredients (polysaccharides) capable of stimulating growth and microbiota activity, especially *Lactobacilli* and *Bifidobacteria*, thereby providing health-promoting effects on host energy balance (Oberfroid et al. 2010). They also increase satiation by decrease the secretion of ghrelin, thereby reducing food intake (Oulangé et al. 2016). One study has observed 48 overweight adults with BMI $>$ 25 who ingested 21 g/day of oligofructose for 12 weeks and experienced a dramatic weight loss during the study accompanied by decreased ghrelin expression (Cani and Delzenne 2009). According to our subanalysis, anthropometric indices reduced by prebiotic supplementation showed a low quality of evidence (only one study was included to assess the effects of prebiotics on anthropometric indices). The result requires further robust investigation.

It should be mentioned that in the subgroup analysis of trials based on the duration of intervention, we found out that long-term ($\geq$ 12 weeks) intervention with pro-, pre-, and synbiotics was statistically more effective in decreasing FPG, TG, TC, CRP, VLDL-c and increasing QUICKI than short-term ($<$ 12 weeks) intervention. This is similar to previous reviews in which longer probiotic administration was beneficial for ameliorating IR (Tabrizi et al. 2019; Cozzolino et al. 2020).

However, in terms of improving anthropometric indices, the study duration $<$ 12 weeks may be more effective than duration $\geq$ 12 weeks. These findings of anthropometric indices should be interpreted suspiciously since only one study (Esmaeilinezhad et al. 2019) was included in the subgroup with study duration $<$ 12 weeks.

Another finding presents that the dose of probiotic ($\geq 2 \times 10^8$ CFU) has a larger effect on the FPG, TC, and VLDL-c than the dose $< 2 \times 10^8$ CFU. According to the FAO/WHO, probiotics are 'live microorganisms which, when administered in adequate amounts, confer a health benefit on the host (Araya et al. 2002). The requirement of minimal amounts differ among countries: products must contain at least $10^7$ CFU/g of probiotic bacteria in Japan, at least $10^8$ CFU/g probiotic bacteria in the USA and $10^9$ CFU/g probiotic bacteria in Canada. In general, $> 10^6 - 108$ CFU/g, or $> 10^8 - 1010$ CFU/d of viable cells are regarded efficacious (Homayoni Rad et al. 2012; Champagne et al. 2011).

This review has several strengths. First, it is the first systematic review and meta-analysis of RCTs that simultaneously evaluate pro-, pre-, and synbiotics on IR, lipid profiles, anthropometric indices, and CRP in PCOS. Additionally, diagnostic criteria of PCOS are clearly defined in all trials included in our analysis (diagnosed according to the Rotterdam criteria), which is highly homogeneous. Furthermore, all of the included trials except 2 (Zhu, Pengbin, and Yaqiong 2020; Chen, Minghui, and Channi 2018) were preregistered in a clinical trial registry, which might have controlled reporting bias efficiently. Moreover, we conducted the subgroup meta-analysis and assessment of the intervention type, study duration, number of probiotic strains, and probiotic dose.

However, the limitations of this meta-analysis should be taken into consideration. First, the heterogeneity between studies might stem from the type of intervention, study duration, strain numbers, probiotic dose, and other factors. Second, the limited inclusion of studies with relative smaller sample size in certain outcomes could influence type-2 statistical error. Finally, given that all of the studies included in our meta-analysis were conducted in Asian, results can only be applicable in the Asian population. They may increase the possibility of the selection bias. In this respect, more researches are needed in Eastern populations and other countries.

### Implications for practice

Accumulating evidence has highlighted a critical role of the gut microbiota and its potential action as a regulator of metabolic disorders in PCOS, such as IR and abnormal lipid metabolism. In the form of pro-, pre-, and synbiotics, microbial modulating therapies present a promising potentiality for healthcare practitioners, given to their low cost and innocuous nature. Although the evidence from this meta-analysis suggests that giving pro-, pre-, and synbiotic supplements have beneficial effects on IR and lipid metabolism, we are still far from providing guidelines for its clinical application due to the complex nature of gut microbiota.

### Implications for research

More well-designed studies are needed to confirm the effects of pro-, pre-, and synbiotics supplementation on PCOS. First, PCOS is a heterogeneous condition with different phenotypes. However, no included trials targeted on a specific phenotype, which made results difficult to be generalized. Future work should focus on the relationship between phenotypes and pro-, pre-, and synbiotics interventions, and it is essential to investigate effects accordingly. Second, the gut microbiota complexifies the process of the PCOS onset. Due to lack of information in most existing studies, effects of pro-, pre-, and synbiotics supplementation on composition and abundance of intestinal microflora were not investigated in the present meta-analysis. In this respect, more valid studies with sufficient follow-up investigation will be conducted necessarily. Third, the duration of most included trials were less than 3 months. Studies with longer follow-up periods will help to comprehensively unravel the effects of pro-, pre-, and synbiotics preparation in the long run.

## Conclusion

Based on this review, our results suggest that pro-, pre-, and synbiotic consumption has a beneficial effect on metabolic indicators in PCOS by reducing of HOMA-IR, FPG, FINS, TG, TC, LDL-c, VLDL-c, and increasing of QUICKI. However, we found this intervention has no statistically significant effect on anthropometric indices and CRP concentration. Further meta-analysis provides evidence for physicians to incorporate pro-, pre-, and synbiotic

preparation into management of PCOS. Nevertheless, results should be interpreted cautiously because of the heterogeneity among study. In addition, large-scale and well-designed RCTs on this topic are needed.

## Author's contributions

Y.L.L. and Y.T. conceived and designed the review. Y.L.L. drafted the paper. Y.L.L. and G.C.X. conducted the literature search and performed data extraction and quality assessment. Y.L.L. and J.Q.S. performed the statistical analysis. Y.T. critically revised the manuscript.

## Disclosure statement

The authors declare no conflicts of interests.

## Abbreviations

| | |
|---|---|
| **BMI** | body mass index |
| **BW** | body weight |
| **CFU** | colony-forming units |
| **CI** | confidence interval |
| **CRP** | C-reactive protein |
| **CVD** | cardiovascular disease |
| **FINS** | fasting insulin |
| **FPG** | fasting plasma glucose |
| **HDL-c** | high-density lipoprotein cholesterol |
| **HOMA-IR** | homeostatic model of assessment for insulin resistance |
| **IR** | insulin resistance |
| **LDL-c** | low-density lipoprotein cholesterol |
| **LPS** | lipopolysaccharides |
| **PCOS** | Polycystic ovary syndrome |
| **QUICKI** | quantitative insulin sensitivity check index |
| **RCT** | randomized controlled trial |
| **SCFAs** | short-chain fatty acids |
| **SMD** | standardized mean difference |
| **TC** | total cholesterol |
| **TG** | triglycerides |
| **VLDL-c** | very-low-density lipoprotein cholesterol |
| **WC** | waist circumference |

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