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Effects of metformin treatment on serum levels of C-reactive protein and interleukin-6 in women with polycystic ovary syndrome: a meta-analysis
A PRISMA-compliant article

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Abstract

Background: Metformin is effective for the treatment of polycystic ovary syndrome (PCOS), but conflicting results regarding its impact on serum levels of C-reactive protein (CRP) and interleukin-6 (IL-6) in women with PCOS have been reported. To provide high-quality evidence about the effect of treatment with metformin on CRP and IL-6 in PCOS, relevant studies that assessed the serum levels of CRP and IL-6 in women with PCOS receiving metformin treatment were reviewed and analyzed.

Methods: A literature search was conducted in the Science Citation Index, PubMed, Embase, and Cochrane Library databases, and personal contact was made with the authors. Random-effects model was used to estimate the standardized mean differences (SMDs) with 95% confidence intervals (95% CIs). To ensure synthesis of the best available evidence, subgroup analysis, sensitivity analysis, meta-regression analysis, and publication bias were performed.

Results: Of 216 studies identified, 20 were included in the meta-analysis (7 prospective, nonrandomized studies, and 13 randomized control trials). Data suggest that serum levels of CRP were decreased after metformin treatment in PCOS patients with an SMD (95% CI) of −0.86 [−1.24 to −0.48] and $P = .000$ (random-effects). However, significant heterogeneity was detected across studies ($I^2 = 84.6\%$ and $P = .000$). Unfortunately, the sources of heterogeneity were not found by subgroup analysis and meta-regression analysis. Serum IL-6 concentrations were not significantly changed after metformin treatment in PCOS with an SMD (95% CI) of −0.48 [−1.26 to 0.31] and $P > .05$ (random-effects). Significant heterogeneity was also detected across studies ($I^2 = 90.9\%$ and $P = .000$). The subgroup analysis suggested that treatment-related reductions in serum IL-6 levels were significantly correlated with BMI, whereas the sources of heterogeneity were not found. In addition, we noticed that metformin treatment could decrease BMI in the CRP and IL-6 related studies (SMD = −0.45, 95% CI: −0.68 to −0.23; SMD = −0.44, 95% CI: −0.73 to −0.16).

Conclusion: This meta-analysis showed a significant decrease of serum CRP levels, especially in obese women, but no significant changes in IL-6 levels after metformin treatment in women with PCOS. In general, the data support that early metformin therapy may ameliorate the state of chronic inflammation in women with PCOS. Considering the obvious heterogeneity reported in the literature, further well-designed investigations with larger samples are needed to ascertain the long-term effects of metformin on chronic inflammation in PCOS.

Abbreviations: BMI = body mass index, CIs = confidence intervals, CRP = C-reactive protein, EE-CA = ethinyl estradiol cyproterone acetate, IL-6 = interleukin-6, IRS-2 = insulin receptor substrate-2, NIH = National Institute of Health, PCOS = polycystic ovary syndrome, RCTs = randomized clinical trials, SD = standard deviation, SMD = standard mean differences.

Keywords: C-reactive protein, interleukin-6, meta-analysis, metformin, polycystic ovary syndrome

1. Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder in women that is characterized by hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology.[1] It is frequently accompanied by insulin resistance and obesity, resulting in a high risk for type 2 diabetes and cardiovascular disease.[2] It is now clear that PCOS is a proinflammatory state, and emerging data suggest that chronic low-grade inflammation seems to play an essential role in the pathogenesis of PCOS, and might be the precursor to the development of insulin resistance and metabolic consequences.[3–5] Significant elevations in serum levels of inflammatory factors including C-reactive protein (CRP) and interleukin-6 (IL-6) were observed in women with PCOS compared with the control women.[6–8] IL-6 may be an early low-grade chronic inflammatory marker in PCOS,[9] a finding that is consistent with other evidence that IL-6 may be a key mediator of low-grade chronic inflammation in PCOS.[10] CRP, secreted in response to cytokines, may not only be a maker of inflammatory disease but also amplify the inflammation process by further activation of monocytes and endothelial cells.[11] A meta-analysis revealed that CRP is the most reliable circulating marker of chronic low-grade inflammation in PCOS.[12]

There are accumulated evidences that insulin resistance played a crucial role in the pathogenesis of PCOS.[13–15] Chronic low-grade inflammation induces insulin resistance both directly and indirectly via activation of serine kinase and mobilization of free fatty acids.[16,17] Moreover, metformin not only improves chronic inflammation through the improvement of metabolic parameters such as hyperglycemia and insulin resistance but also has a direct anti-inflammatory action. Recent studies have suggested that metformin has a direct anti-inflammatory action by inhibition of nuclear factor κB via adenosine monophosphate-activated protein kinase dependent and independent pathways.[18,19] Thus, metformin has been widely used in women with PCOS to improve insulin sensitivity and induce ovulation.

However, the impact of metformin on serum inflammation makers (CRP and IL-6) in PCOS remains controversial. Some studies reported positive results,[9,20–25] whereas others found no significant changes in CRP and IL-6 levels after metformin administration.[26–38] The aim of the present study was to systematically review the literature and to meta-analyze the best evidence on the effects of metformin treatment on serum levels of CRP and IL-6 in women with PCOS.

2. Methods

2.1. Search strategy

The following databases were searched for prospective studies and randomized clinical trials (RCTs) published until October 2016: Science Citation Index, PubMed, Embase, and Cochrane Library. No limits were set on publication date or language. The following search terms were included:

(1) [(C-reactive protein OR CRP) AND metformin] AND (PCOS OR polycystic ovary syndrome)
(2) [(interleukin-6 OR IL-6) AND metformin] AND (PCOS OR polycystic ovary syndrome)

2.2. Inclusion and exclusion criteria

Studies that met the following criteria were included: measuring serum CRP and IL-6 levels in women with PCOS receiving metformin therapy in prospective studies and RCTs; reporting CRP and IL-6 means and standard deviation (SD) or sufficient information that can calculate CRP and IL-6 means and SD; Rotterdam[39] or National Institute of Health (NIH) criteria[40] were used as diagnosis criteria of PCOS.

Studies were excluded if they were publications from letters, reviews, animal studies, case reports, and studies without controls. If the studies based on the same study population published more than one article, we chose the most recent published date or the one with the largest sample size.

2.3. Data extraction and quality assessment

Information from each study was extracted independently by 2 researchers (Wang and Zhu) by using a standardized data extraction form. The general characteristics, body mass index (BMI), and outcomes (both the total CRP/IL-6 means and SDs before and after metformin administration) of each study were recorded, where available, and double-checked. Where appropriate, the data set was completed through communication with the authors. Disagreements were resolved by consensus (Xu). The quality of the information accessed in RCTs was classified as low, unclear, or high by evaluating the following 7 components: random sequence generation, allocation concealment, blinding of outcome assessment, blinding of participants and personnel, incomplete outcome data, selective outcome reporting, and "other bias" according to the guidelines of the Cochrane Handbook for Systematic Reviews of Interventions.

2.4. Statistical analysis

Standard mean differences (SMDs) and 95% confidence intervals (95% CIs) of the CRP and IL-6 levels were calculated for all eligible studies and combined by using appropriate fixed or random effects models. The statistical heterogeneity was assessed using the X^2-based Q-statistic and the I^2-statistic. P value less than .05 for Q-statistic or I^2 larger than 50% was considered as having significant heterogeneity, which existed between the studies. The random-effects model was applied if heterogeneity was detected; otherwise, the fixed-effects model was used. Subsequently, to identify the sources of heterogeneity, subgroup analysis was carried out, including age, country, BMI, therapy duration, and dose. Furthermore, restricted maximum likelihood-based random effects meta-regression analysis was performed to evaluate the aforementioned potential heterogeneity factors. Univariate meta-regression analysis was carried out first, after which the variables that were significant at the 0.1 level were entered into the multivariable model. A sensitivity analyses was performed to evaluate the stability of the meta-analysis results. Potential publication bias was investigated by using the Begg test. All statistical analyses were performed using Stata (version12; StataCorp, College Station, TX).

2.5. Ethical approval

The ethical approval was not necessary for the reason that our study was a meta-analysis belonging to secondary analysis.

3. Results

3.1. Flow chart of study selection

Figure 1 provides details of the study selection. The primary search identified 216 articles. After title and abstract screening, 30 potentially eligible studies were retrieved for full text review.
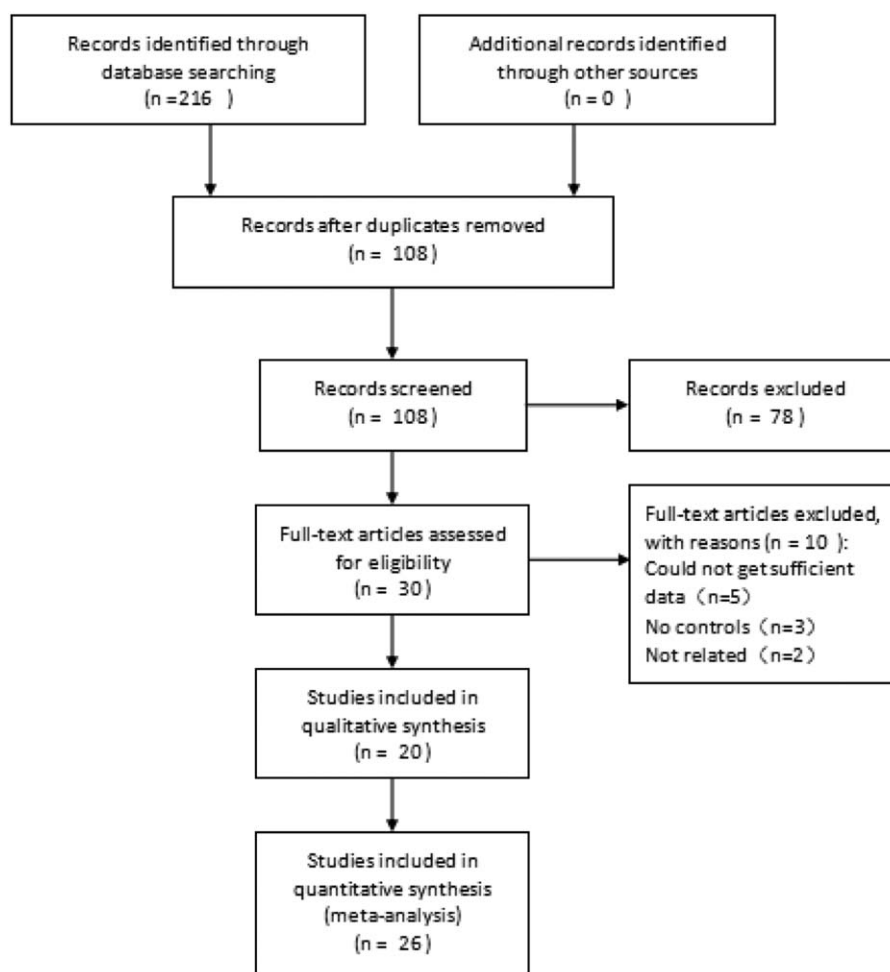
Figure 1. The search flow diagram.

Of these 30 articles, 10 were excluded: 5 did not offer sufficient data, 3 did not meet the inclusion criteria, and 2 measured other proteins in women with PCOS receiving metformin therapy. Therefore, 20 studies were included in the meta-analysis: 7 prospective, nonrandomized studies comparing the use of metformin in PCOS women and healthy controls[9,21,22,25,27,28,36] and 13 RCTs [3 studies comparing metformin with ethinyl estradiol cyproterone acetate (EE-CA) in PCOS women, 3 comparing metformin with rosiglitazone in PCOS women, 2 comparing metformin with rimonabant in PCOS women, 1 comparing metformin with flutamide levonorgestrel ethinyl estradiol in PCOS women, 1 comparing metformin with exenatide in PCOS women, 1 comparing metformin with rosuvastatin in PCOS women, 1 comparing metformin with hypocaloric diet in PCOS women, and 1 comparing metformin with placebo in PCOS women].[20,23,24,26,29–35,37,38]

3.2. Characteristics of included studies

Table 1 summarizes the characteristics of the 20 studies included in the meta-analysis. Seven studies focused on eastern women[9,23,29,32,33,35,37] and 13 on western women. Sixteen of these studies employed Rotterdam criteria for the diagnosis of PCOS and 4 used NIH criteria.[29,30,33,37] One study (comparing metformin vs rosiglitazone) did not report the mean age for each group separately, which included young women,[32] and 7 included PCOS women over 28 years old.[20,21,24,28,31,37,38] Study duration ranged from 3 to 6 months. Two 6-month studies also reported 3-month data.[20,33] Study dose ranged from 1000 to 2550 mg per day.

The 3 RCTs included in the meta-analysis, comparing metformin versus EE-CA in PCOS women, lasted 3 to 6 months. Two studies used the same dosages for both drugs[20,33] and 1 used a higher dosage of metformin.[34] The sample size ranged from 10 to 35 women for both the PCOS group treated with metformin and the PCOS group treated with EE-CA. Three studies compared metformin with rosiglitazone in PCOS women and used the same dosages for rosiglitazone.[24,29,32] Two studies compared metformin with rimonabant in overweight/obese women with PCOS for 3 months reporting CRP and IL-6 parameters, respectively.[31,38] The rest RCTs compared metformin with flutamide levonorgestrel ethinyl estradiol or exenatide or rosuvastatin or hypocaloric diet or placebo.[23,26,30,35,37]

The quality assessment of randomized trials included in our study is summarized in Table 2. Randomization was performed according to a computer-generated random list or by means of a randomly generated number pattern in a majority of the trials.[24,26,29–31,34,35,37,38] Most randomized trials included in our study were characterized by a low risk of incomplete outcome data and selective outcome reporting.[20,23,24,26,29,30,32,33,35,37]

Table 1

Characteristics of the 20 studies in the meta-analysis.

Ref.	Country	PCOS criteria	Age (MG vs CG) Mean ± SD	Size MG /CG	Types of studies and intervention	Doses, mg/d	Therapy, mo
Morin-Papunen et al[20]	Finland	Rotterdam	29.5 ± 1.1 (all participants)	11/14	RCT comparing the use of metformin with ethinyl estradiol + cyproterone acetate in overweight/obese PCOS women	Metformin: 1000 mg/d ethinyl estradiol + cyproterone acetate: 35 μg+2 mg (21 d/mo followed a 7-d free)	3
Morin-Papunen et al[20]	Finland	Rotterdam	29.5 ± 1.1 (all participants)	8/10	RCT comparing the use of metformin with ethinyl estradiol + cyproterone acetate in overweight/obese PCOS women	Metformin: 2000 mg/d ethinyl estradiol + cyproterone acetate: 35 μg+2 mg (21 d/mo followed a 7-d free)	6
Mohlig et al[21]	Germany	Rotterdam	28.9 ± 0.8 vs 32.0 ± 2.3	9/9	Prospective study comparing the use of metformin in obese PCOS women and obese healthy controls	Metformin: 2550 mg/d	6
Diamanti-Kandarakis et al[22]	Greece	Rotterdam	24.3 ± 0.6 vs 25.9 ± 0.6	22/45	Prospective study comparing the use of metformin in obese PCOS women and obese healthy controls	Metformin was incremented stepwise to maximum 850 mg twice daily	6
Elkind-Hirsch et al[26]	American	Rotterdam	27.7 ± 1.3 vs 28.2 ± 1.1	14/14	RCT comparing the use of metformin with exenatide in overweight/obese PCOS women	Metformin was incremented stepwise to maximum 1000 mg twice daily; Exenatide was incremented stepwise to maximum 10 μg twice daily	6
Heutling et al[27]	Germany	Rotterdam	27.8 ± 4.7 vs 27.8 ± 5.6	21/39	Prospective study comparing the use of metformin in obese PCOS women and obese healthy controls	Metformin was incremented stepwise to maximum 850 mg twice daily	6
Jakubowska et al[28]	Poland	Rotterdam	28.2 ± 6.3 vs 31.6 ± 7.4	29/29	Prospective study comparing the use of metformin in obese PCOS women and obese healthy controls	Metformin: 1000 mg/d	6
Jensterle et al[29]	Slovenia	NIH	23.1 ± 3.7 vs 25.0 ± 4.9	15/11	RCT comparing the use of metformin with rosiglitazone in overweight/obese PCOS women	Metformin: 1700 mg/d Rosiglitazone: 4 mg/d	6
Hoeger et al[30]	American	NIH	14.7 ± 1.6 vs 15.8 ± 1.6	16/16	RCT comparing the use of metformin with placebo in overweight/obese PCOS women	Metformin was incremented stepwise to maximum 1700 mg/d	6
Sathyapalan et al[31]	UK	Rotterdam	29.8 ± 1.8 vs 27.4 ± 1.5	10/10	RCT comparing the use of metformin with rimonabant in overweight/obese PCOS women	Metformin: 1500 mg/d rimonaban: 20 mg/d	3
Cetinkalp et al[32]	Turkey	Rotterdam	NA	47/14	RCT comparing the use of metformin with rosiglitazone in overweight PCOS women	Metformin: 2000 mg/d Rosiglitazone: 4 mg/d	4
Aghamohammadzadeh et al[33]	Iran	NIH	24.9 ± 11.0 vs 22.0 ± 5.2	30/35	RCT comparing the use of metformin with ethinyl estradiol + cyproterone acetate in overweight PCOS women	Metformin: 1000 mg/d ethinyl estradiol +cyproterone acetate: 35 μg+2 mg (21 d/mo followed a 7-d free)	3
Aghamohammadzadeh et al[33]	Iran	NIH	24.9 ± 11.0 vs 22.0 ± 5.2	30/35	RCT comparing the use of metformin with ethinyl estradiol + cyproterone acetate in overweight PCOS women	Metformin: 1000 mg/d ethinyl estradiol +cyproterone acetate: 35 μg+2 mg (21 d/mo followed a 7-d free)	6
Luque-Ramírez and Escobar-Morreale [34]	Spain	Rotterdam	25.0 ± 7.0 vs 23.0 ± 6.0	12/15	RCT comparing the use of metformin with ethinyl estradiol +cyproterone acetate in overweight/obese PCOS women	Metformin: 1700 mg/d ethinyl estradiol +cyproterone acetate: 35 μg+2 mg (21 d/mo followed a 7-d free)	6
Lin et al[9]	China	Rotterdam	27.7 ± 0.5 vs 32.0 ± 0.6	97/10	Prospective study comparing the use of metformin in obese PCOS women and healthy controls	Metformin: 1500 mg/d	3
Celik and Acbay[23]	Turkey	Rotterdam	25.9 ± 5.7 vs 27.6 ± 5.9	20/18	RCT comparing the use of metformin with metformin +rosuvastatin in obese PCOS women	Metformin: 2000 mg/d Metformin +Rosuvastatin: 2000 mg/day+10 mg/d	3
Esfahanian et al[35]	Iran	Rotterdam	21.9 ± 9.3 vs 20 ± 4.6	17/13			3

(continued)

Table 1

(continued).

Ref.	Country	PCOS criteria	Age (MG vs CG) Mean±SD	Size MG /CG	Types of studies and intervention	Doses, mg/d	Therapy, mo
					RCT comparing the use of metformin with hypocaloric diet in overweight/obese PCOS women	Metformin was incremented stepwise to maximum 2000 mg/d	
Mohiyiddeen et al[24]	UK	Rotterdam	30.0±0.9 vs 29.0±1.0	17/18	RCT comparing the use of metformin with rosiglitazone in overweight PCOS women	Metformin: 1000 mg/d Rosiglitazone: 4 mg/d	3
Victor et al[36]	Spain	Rotterdam	25.2±7.3 vs 26.2±4.4	35/41	Prospective study comparing the use of metformin in obese PCOS women and obese healthy controls	Metformin was incremented stepwise to maximum 1500 mg/d	3
Mehrabian et al[37]	Iran	NIH	29.2±8.3 vs 29.0±7.7	34/34	RCT comparing the use of metformin with flutamide +levonorgestrel+ethinyl estradiol in obese PCOS women	Metformin: 1000 mg/d flutamide +levonorgestrel+ethinyl estradio: 62.5 mg/d+0.15 mg/d+0.03 mg/d	6
Fruzzetti et al[25]	Italy	Rotterdam	18.0±1.0 vs 20.0±1.0	18/17	Prospective study comparing the use of metformin in obese PCOS women and healthy controls	Metformin: 1700 mg/d	6
Sathyapalan et al[38]	UK	Rotterdam	NA	10/10	RCT comparing the use of metformin with rimonabant in overweight/obese PCOS women	Metformin: 1500 mg/d rimonaban: 20 mg/d	3

CG = control group, MG = metformin group, NA = not available, NIH = National Institute of Health, RCT = randomized controlled trial, SD = standard deviation.

Eight randomized trials included in our study were characterized by a high risk of blinding of participants and personnel and outcome assessment.[20,23,24,26,29,33,35,37] Moreover, all randomized trials were with an unclear risk of other bias. In conclusion, the quality of these studies was low to moderate.

3.3. CRP
3.3.1. Selection of studies. The characteristics of the included studies for the CRP levels are provided in Table 3.

3.3.2. Pooled analysis. Seventeen studies, including 403 women, were eligible for the meta-analysis. The I^2 value was 84.6%, indicating high heterogeneity among the included studies. Therefore, we used the random-effects model to combine effect

size. The meta-analysis revealed that serum CRP concentrations were decreased after metformin treatment in PCOS with an SMD (95% CI) of −0.86 [−1.24 to −0.48] and $P=.000$ (Fig. 2). We also performed a meta-analysis regarding the impact of metformin administration on BMI in patients with PCOS. The result showed that the total SMD for BMI was −0.45 units (95% CI, −0.68 to −0.23, $P<.05$) (Fig. 3). These results suggested that metformin therapy was associated with a significant decrease in BMI in patients with PCOS.

3.3.3. Subgroup analysis and meta-regression analysis. Subgroup analysis was performed according to age (<28, ≥28 years, or not available), country (Eastern or Western), BMI (<30 or ≥30 kg/m²), therapy duration (≤3 or >3 months), and dose

Table 2

The risk of bias of randomized trials.

Ref.	Random sequence generation	Allocation concealment	Blinding of outcome assessment	Blinding of participants and personnel	Incomplete outcome data	Selective outcome reporting	Other bias
Morin-Papunen et al[20]	Unclear	Yes	Unclear	Unclear	Yes	Yes	Unclear
Elkind-Hirsch et al[26]	Yes	Yes	No	No	Yes	Yes	Unclear
Jensterle et al[29]	Yes	Yes	Unclear	Unclear	Yes	Yes	Unclear
Hoeger et al[30]	Yes	Yes	Yes	Yes	Yes	Yes	Unclear
Sathyapalan et al[31]	Yes	Yes	Yes	Yes	Yes	No	Unclear
Cetinka 2009[32]	Unclear	Yes	Yes	Yes	Yes	Yes	Unclear
Aghamohammadzadeh et al[33]	Unclear	Yes	Unclear	Unclear	Yes	Yes	Unclear
Luque-Ramírez and Escobar-Morreale [34]	Yes	Yes	Yes	Yes	Unclear	Yes	Unclear
Celik and Acbay [23]	Unclear	No	Unclear	Unclear	Yes	Yes	Unclear
Esfahanian et al[35]	Yes	Yes	No	No	Yes	Yes	Unclear
Mohiyiddeen et al[24]	Yes	Yes	Unclear	Unclear	Yes	Yes	Unclear
Mehrabian et al[37]	Yes	Yes	No	No	Yes	Yes	Unclear
Sathyapalan et al[38]	Yes	Yes	Yes	Yes	Yes	No	Unclear

Yes, a low risk of bias; No, high risk of bias; Unclear, unclear or unknown risk of bias.

Table 3

Characteristics of the 17 included studies on CRP.

Ref.	Country	Age, y	Size	BMI		CRP		Therapy, mo	Doses, mg/d
				Pre-Met	Post-Met	Pre-Met	Post-Met		
Morin-Papunen et al[20]	Western	≥28	11	28.7±1.5	28.2±1.6	3.08±0.70 mg/L	2.08±0.54 mg/L	≤3	<2000
Morin-Papunen et al[20]	Western	≥28	8	28.7±1.5	26.0±1.7	3.08±0.70 mg/L	1.52±0.26 mg/L	>3	≥2000
Mohlig et al[21]	Western	≥28	9	31.6±1.3	29.6±1.0	3.34±0.82 mg/L	1.92±0.29 mg/L	>3	≥2000
Diamanti-Kandarakis et al[22]	Western	<28	22	30.7±1.4	28.8±2.1	1.92±0.60 mg/dL	0.52±0.26 mg/dL	>3	<2000
Elkind-Hirsch et al[26]	Western	<28	14	43.3±2.0	42.3±2.0	6.50±1.70 mg/L	5.04±1.90 mg/L	>3	≥2000
Heutling et al[27]	Western	<28	21	32.8±6.1	31.4±5.9	4.00±3.00 mg/L	4.00±2.20 mg/L	>3	<2000
Jakubowska et al[28]	Western	≥28	29	35.3±5.0	33.5±4.8	3.53±3.64 mg/L	3.86±4.27 mg/L	>3	<2000
Jensterle et al[29]	Eastern	<28	15	29.6±6.9	29.0±6.8	1.80±7.22 mg/dL	1.92±6.18 mg/dL	>3	<2000
Hoeger et al[30]	Western	<28	16	35.0±8.2	35.7±8.6	3.60±2.70 mg/L	2.80±2.00 mg/L	>3	≥2000
Sathyapalan et al[31]	Western	≥28	10	35.7±1.4	35.1±1.5	4.20±1.70 mg/L	4.00±1.30 mg/L	≤3	<2000
Cetinkalp et al[32]	Eastern	NA	47	25.8±6.1	24.8±5.3	1.00±1.86 mg/dL	0.28±0.30 mg/dL	≤3	≥2000
Aghamohammadzadeh et al[33]	Eastern	<28	30	26.5±5.7	25.8±6.4	8.89±4.50 mg/L	8.27±3.50 mg/L	≤3	<2000
Aghamohammadzadeh et al[33]	Eastern	<28	30	26.5±5.7	24.5±4.1	8.89±4.50 mg/L	8.39±2.90 mg/L	>3	<2000
Celik and Acbay [23]	Eastern	<28	20	30.2±6.5	27.9±5.7	0.72±0.40 mg/L	0.30±0.20 mg/L	≤3	≥2000
Esfahanian et al[35]	Eastern	<28	17	33.1±2.7	32.3±3.2	5.20±2.50 mg/dL	3.70±1.90 mg/dL	≤3	≥2000
Mohiyiddeen et al[24]	Western	≥28	17	31.2±1.1	29.1±1.0	4.11±1.09 mg/L	1.98±0.55 mg/L	≤3	<2000
Victor et al[36]	Western	<28	35	30.9±8.9	30.8±8.8	4.15±3.07 mg/L	3.69±3.02 mg/L	≤3	<2000
Mehrabian et al[37]	Eastern	≥28	34	29.8±4.2	29.5±4.2	1.49±0.43 mg/L	1.45±0.47 mg/L	>3	<2000
Fruzzetti et al[25]	Western	<28	18	26.8±6.3	25.8±5.6	5.60±1.50 mg/L	3.90±1.30 mg/L	>3	<2000

BMI = body mass index, CRP = C-reactive protein, NA = not available, Post-Met = post-metformin, Pre-Met = pre-metformin.
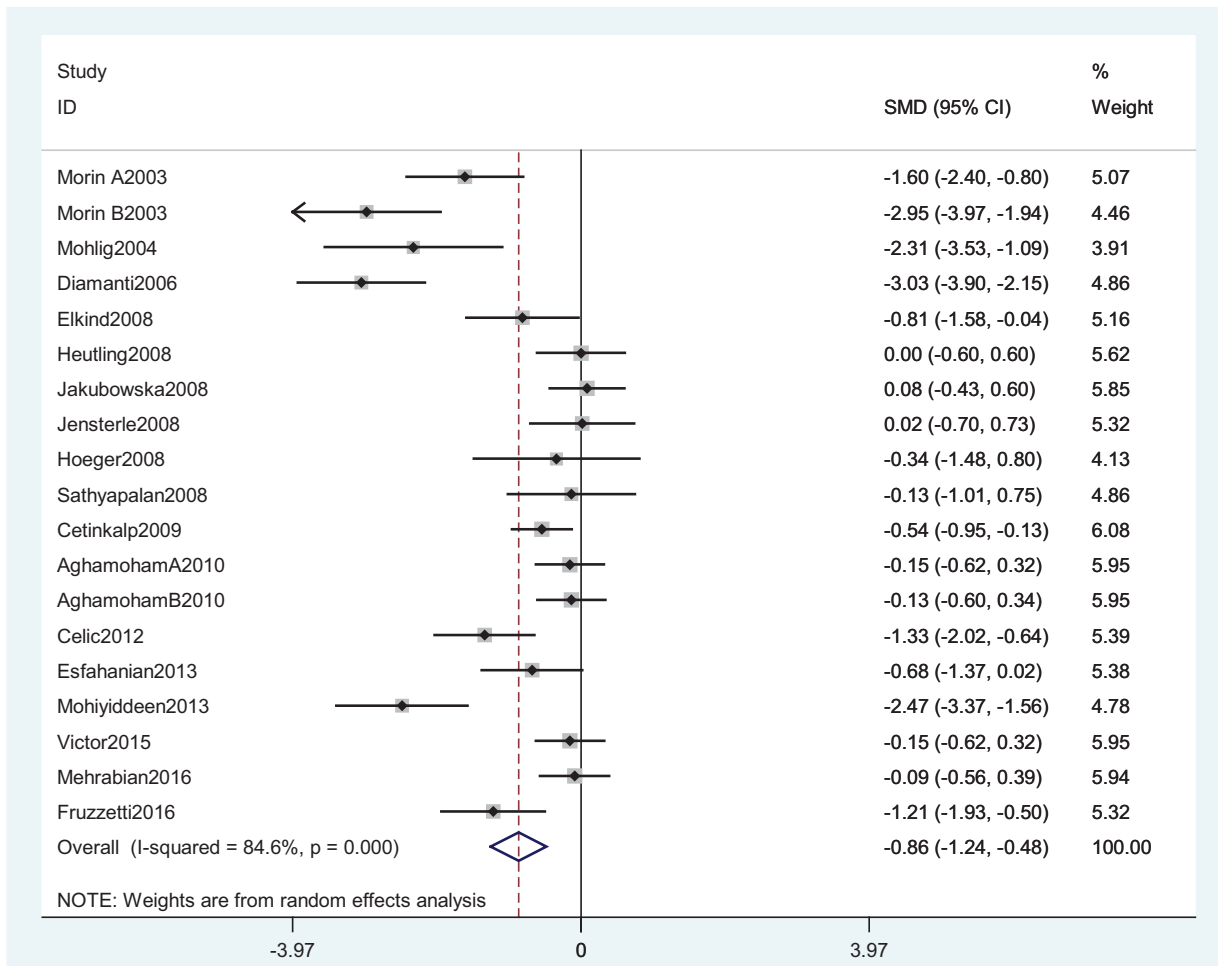

Figure 2. A meta-analysis of data about serum CRP levels in the women with PCOS before and after metformin treatment from 17 studies using a random-effect model. CI = confidence interval, CRP = C-reactive protein, PCOS = polycystic ovary syndrome, SMD = standard mean differences.
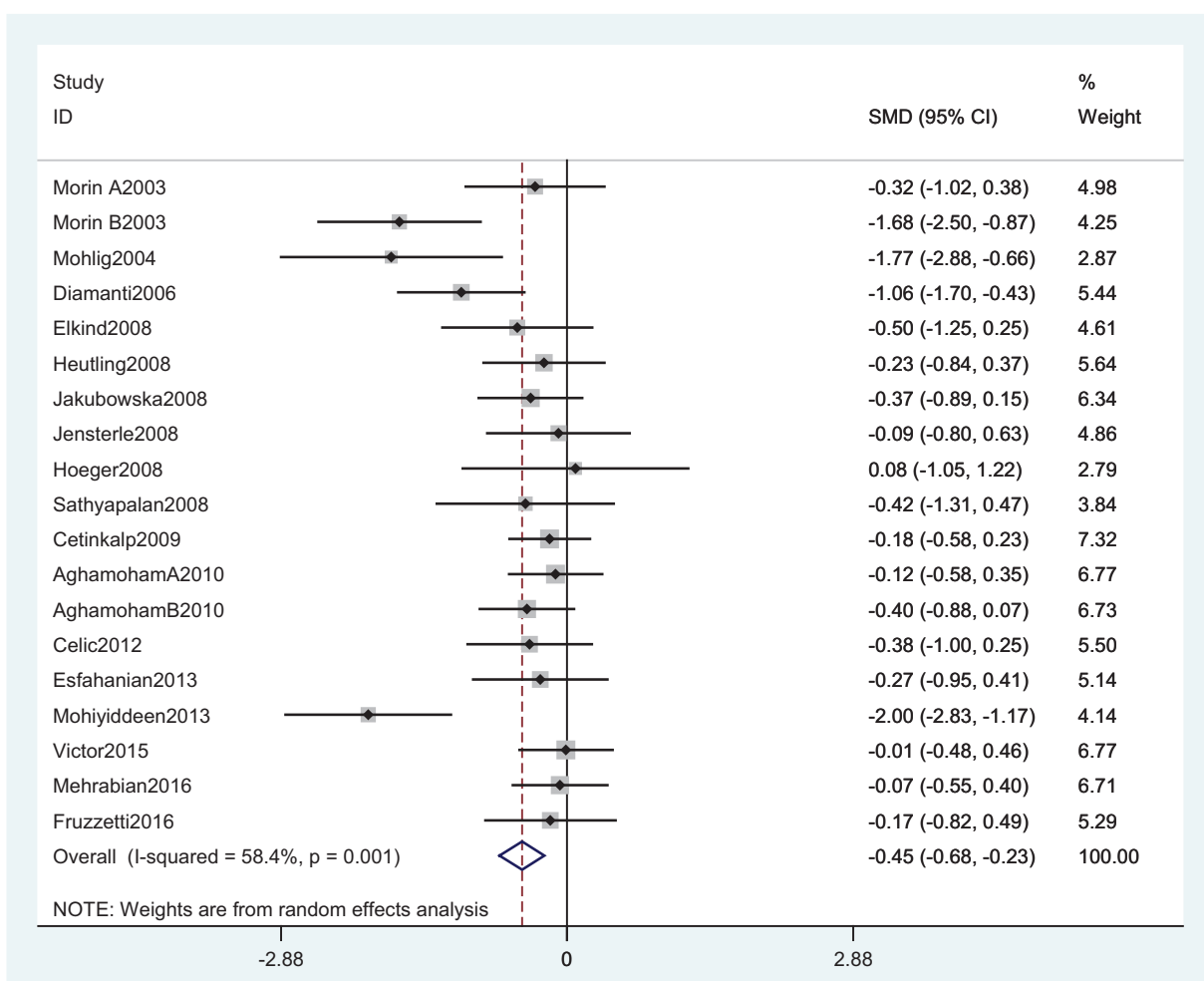
Figure 3. A meta-analysis of data about comparison of BMI before and after metformin treatment in the CRP-related studies using a random-effect model. BMI = body mass index, CI = confidence interval, CRP = C-reactive protein, SMD = standard mean differences.

(<2000 or ≥2000 mg/day). In the subgroup analysis, the overall pattern of pooled effect did not vary substantially by the potential sources of heterogeneity, including age, region, BMI, therapy duration, and dose (Table 4). Unfortunately, subgroup analysis showed obvious heterogeneity, and these variables were not found to be the main source of heterogeneity in all studies. To further investigate the impact of the characteristics mentioned above, we carried out a meta-regression analysis. A univariate

Table 4

Subgroup meta-analysis of the included studies for CRP.

Subgroup analysis	Number of studies	Random-effects SMD (95% CI)	I^2	P for heterogeneity
Age, y				
<28	11	−0.6 (−1.07 to −0.14)	81%	<.001
≥28	7	−1.29 (−2.2 to −0.38)	90.3%	<.001
NR	1	−0.86 (−1.24 to −0.48)	73%	.054
Country				
Eastern	7	−0.43 (−0.76 to −0.10)	58.3%	.035
Western	12	−1.10 (−1.70 to −0.50)	87.9%	<.001
BMI				
<30	8	−0.73 (−1.24 to −0.23)	83.6%	<.001
≥30	11	−0.97 (−1.56 to −0.37)	86.3%	<.001
Months				
≤3	8	−0.81 (−1.47 to −0.15)	83.5%	<.001
>3	11	−0.89 (−1.38 to −0.40)	86.2%	<.001
Dose, mg/d				
<2000	12	−0.48 (−0.65 to −0.31)	88.4%	.000
≥2000	7	−0.80 (−1.07 to −0.52)	51.9%	.065

BMI = body mass index, CRP = C-reactive protein, SMD = standard mean differences.

Table 5

Meta-regression analysis for the variables between studies.

	No. of studies	Coefficient	Standard error	T	P	95% CI
Age	18	−0.645	0.531	−1.21	.243	[−1.777 to 0.487]
Country	19	0.415	0.398	1.04	.312	[−0.424 to 1.254]
BMI	19	−0.192	0.488	−0.39	.699	[−1.222 to 0.838]
Months	19	−0.824	0.521	−0.16	.876	[−1.183 to 1.017]
Dose	19	−0.731	0.730	−1.00	.331	[−2.271 to 0.809]

BMI = body mass index, CI = confidence interval.

meta-regression analysis revealed that the regression coefficients of aforementioned variables were insignificant ($P > .1$) (Table 5) and multivariate meta-regression was not carried out further. Therefore, the source of heterogeneity still was not found.

3.3.4. Sensitivity analysis and publication bias. Sensitivity analysis revealed that removal of any study from the analysis did not subvert the result of the present pooled analysis (data not shown). Therefore, this pooled analysis outcome could be regarded with a higher degree of certainty. Furthermore, no publication bias was identified by Begg test (Fig. 4).

3.4. IL-6
3.4.1. Selection of studies. The characteristics of the included studies for the IL-6 levels are provided in Table 6.

3.4.2. Pooled analysis. Seven studies, including 192 women, were eligible for the meta-analysis. The I^2 value was 90.9%, indicating high heterogeneity among the included studies. Therefore, we used the random-effects model to combine effect size. The meta-analysis revealed that serum IL-6 concentrations were not significantly changed after metformin treatment in PCOS with an SMD (95% CI) of −0.48 [−1.26 to 0.31] and $P > .05$ (Fig. 5). We also performed a meta-analysis regarding the impact of metformin administration on BMI in patients with PCOS. The result showed that the total SMD for BMI was −0.44 units (95% CI, −0.73 to −0.16, $P < .05$) (Fig. 6). These results suggested that metformin therapy could decrease BMI in patients with PCOS.

3.4.3. Subgroup analysis. Subgroup analysis was also performed according to age (<28, ≥28 years, or not available), country (Eastern or Western), BMI (<30 or ≥30 kg/m^2), therapy duration (≤3 or >3 months), and dose (<2000 or ≥2000 mg/day). In the subgroup analysis, the overall pattern of pooled effect did not vary substantially by the potential sources of heterogeneity, including age, region, BMI, therapy duration, and dose. Decreased levels of IL-6 after metformin treatment in PCOS were observed in the groups with BMI <30 [SMD (95% CI) of −1.99 (−2.33 to −1.65)]. However, there was no change of serum IL-6 concentrations in patients with BMI ≥30 after metformin treatment (Table 7). Unfortunately, subgroup analysis showed obvious heterogeneity, and these variables were not found to be the main source of heterogeneity in all studies.

4. Discussion

In the current meta-analysis, the results showed a significant decrease of serum CRP levels after metformin treatment in women with PCOS, especially in western obese women. In addition, we noticed that metformin treatment could decrease BMI in the CRP and IL-6 related studies. However, we found no significant changes in IL-6 levels during metformin treatment in the present study.

Similar to the findings of our meta-analysis, decreased levels of CRP have been observed in women with PCOS who were receiving 1000 to 2000 mg metformin daily for 6 months.[20,22,25] Insulin and glucose levels induced by activation of the AMP-kinase pathways have been shown to associate with CRP levels.[41,42] Decreased levels of CRP observed in women with PCOS after metformin administration may be the result of reduced insulin resistance and insulin levels. Elevated CRP levels are also associated with obesity in women with PCOS,[43] and serum CRP and IL-6 levels reduced after even moderate weight loss in obese subjects.[44] Interestingly, we observed a significant time and dose effect of metformin treatment on CRP levels in women with PCOS. Moreover, our study found that serum CRP levels decrease more in obese women with PCOS and their BMI reduced significantly after metformin treatment. In consistent with a study, it proved that higher dose metformin may be more efficient for BMI reduction in women with PCOS.[45] However, Chen et al[46] observed that CRP levels decreased more in the subgroup with a dose of <2000 mg/day than that of ≥2000 mg/day. The explanation could be that BMI, age, and clinical phenotypes influence the effect of metformin in PCOS. Thus, further investigations are needed to explore the optimal doses in PCOS women.

CRP is a classical marker of inflammation that is commonly used for cardiovascular disease risk stratification and improving cardiovascular risk prediction.[47,48] Large increases or sustained elevations in CRP over a 6-year period were associated with a
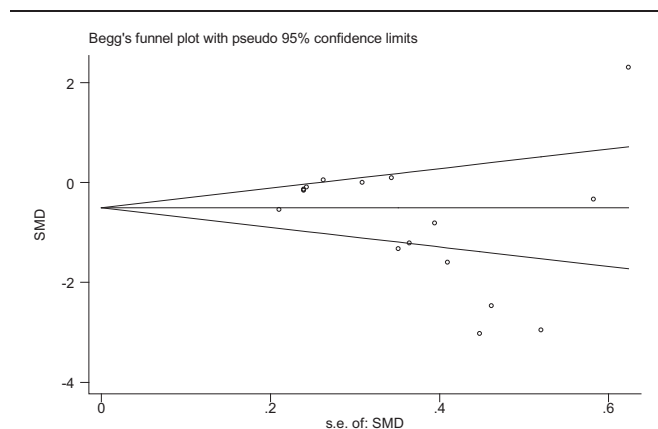

Figure 4. A funnel plot of studies evaluating the association between CRP and metformin in PCOS with $P = .149$ (Begg regression asymmetry test). CRP = C-reactive protein, PCOS = polycystic ovary syndrome, SMD = standard mean differences.

Table 6

Characteristics of the 7 included studies on IL-6.

Ref.	Country	Age, y	Size	BMI		IL-6, pg/mL		Therapy, mo	Doses, mg/d
				Pre-Met	Post-Met	Pre-Met	Post-Met		
Mohlig et al[21]	Western	≥28	9	31.6±1.3	29.6±1.0	1.91±0.31	1.72±0.30	>3	≥2000
Elkind-Hirsch et al[26]	Western	<28	14	43.3±2.0	42.3±2.0	4.50±1.00	4.40±0.90	>3	≥2000
Heutling et al[27]	Western	<28	21	32.8±6.1	31.4±5.9	1.85±1.21	2.11±1.00	>3	<2000
Jakubowska et al[28]	Western	≥28	29	35.3±5.1	33.5±4.8	34.32±8.28	29.92±9.04	>3	<2000
Luque-Ramírez and Escobar-Morreale [34]	Western	<28	12	30.0±7.0	28.0±6.0	1.00±0.90	0.70±0.90	>3	<2000
Lin et al[9]	Eastern	<28	97	24.3±0.5	NA	28.05±3.26	22.04±2.76	≤3	<2000
Sathyapalan et al[38]	Western	≥28	10	35.7±1.4	35.09±1.5	12.20±5.90	13.20±7.30	≤3	<2000

BMI = body mass index, IL-6 = interleukin-6, NA = not available, Post-Met = post-metformin, Pre-Met = pre-metformin.

subsequent increased risk of diabetes, cardiovascular events, and mortality.[49] On the basis of this meta-analysis and previous work, metformin treatment in women with PCOS was associated with a significant decrease in CRP levels. Therefore, it may be suspected that metformin administration is effective for preventing cardiovascular disease in women with PCOS.

In this meta-analysis, we found that serum IL-6 levels did not decrease significantly in women with PCOS after metformin treatment. In contrast with our results, 1 study[9] indicated that a significant change was observed in IL-6 concentration after treatment with 1500 mg of metformin daily for 3 months. Since the complexity of the factors that influence the effect of metformin on IL-6 levels, we could not identify the precise dose or duration of metformin therapy through our meta-analysis needed to maximize the decrease in IL-6 concentration in women

with PCOS. IL-6, a major proinflammatory cytokine in chronic inflammation, has been shown to be closely associated with insulin resistance.[50] Luque-Ramírez and Escobar-Morreale[31] pointed out that serum IL-6 levels decreased during treatment with metformin in parallel to amelioration of insulin resistance. Meanwhile, another study suggested that metformin treatment response on low-grade chronic inflammatory markers in women with PCOS was related to insulin receptor substrate-2 (IRS-2) polymorphism.[9] Therefore, our results could be explained by the differences in race and severity of insulin resistance. Unfortunately, subgroup analysis according to country revealed that IL-6 levels did not change significantly after metformin treatment in women with PCOS.

We noticed that metformin treatment could reduce BMI in women with PCOS, and decreased IL-6 levels after metformin
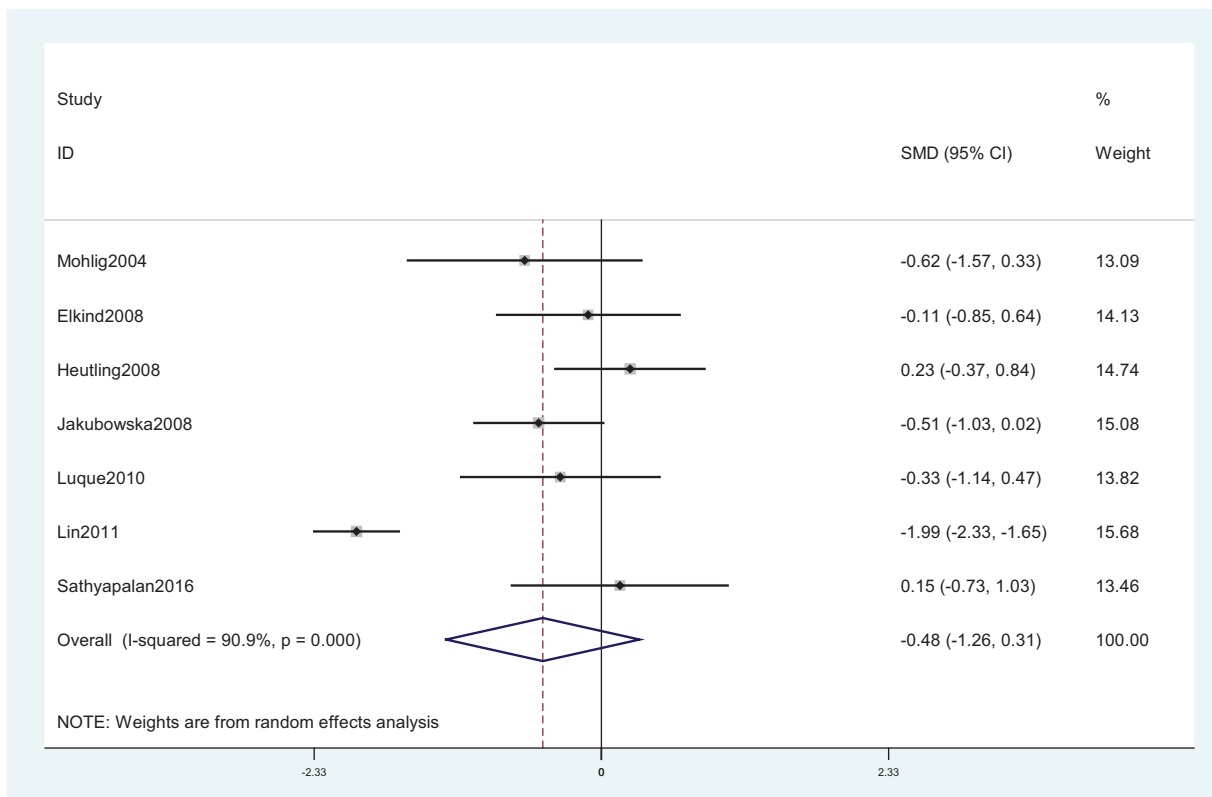

Figure 5. A meta-analysis of data about serum IL-6 levels in the women with PCOS before and after metformin treatment from 7 studies using a random-effect model. CI = confidence interval, IL-6 = interleukin-6, PCOS = polycystic ovary syndrome, SMD = standard mean differences.
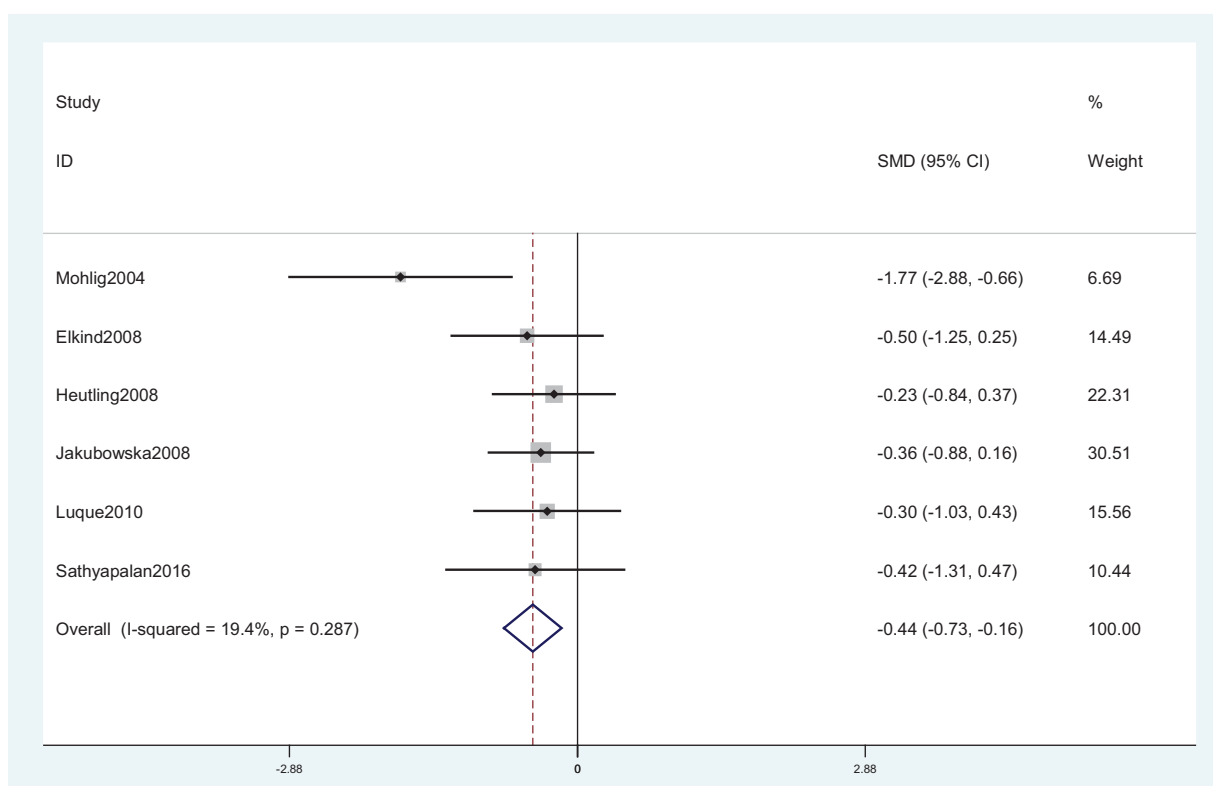
Figure 6. A meta-analysis of data about comparison of BMI before and after metformin treatment in the IL-6 related studies using a random-effect model. BMI = body mass index, CI = confidence interval, IL-6 = interleukin-6, SMD = standard mean differences.

treatment in PCOS were observed in the groups with BMI <30 in our study. Multiple regression analysis found that IL-6 levels correlated significantly with BMI of PCOS patients.[51] Futhermore, short-term metformin therapy facilitated weight loss, and long-term therapy resulted in a reduction of IL-6 levels.[52] So, the explanation could be that metformin-associated reduction of weight before significant changes in IL-6 parameter perhaps involves different mechanisms of action.

Overall, the strength of this study is stronger than any single study, as the included primary studies are quite homogeneous.

First, the criteria of diagnosis of PCOS were clearly defined. Besides, subgroup analysis and meta-regression analysis were carried out on the basis of several potential relevant factors. Although significant heterogeneity was detected, the sensitivity analysis did not show that a single study influenced the pooled results and no publication bias was detected. This would undoubtedly enhance the persuasiveness of the study.

Nevertheless, several limitations must be admitted when considering the generalizability of these data. First, the most important limitation is the scarcity of high-quality, multicenter,

Table 7

Subgroup meta-analysis of the included studies for IL-6.

Subgroup analysis	Number of studies	Random-effects SMD (95% CI)	I^2	P for heterogeneity
Age				
<28	4	−0.57 (−1.83 to 0.69)	94.5%	0
≥28	3	−0.39 (−0.79 to 0.02)	0%	.39
Country				
Eastern	1	−0.48 (−1.26 to 0.31)	90.9%	0
Western	6	−0.20 (−0.49 to 0.09)	0%	.436
BMI				
<30	1	−1.99 (−2.33 to −1.65)	90.9%	0
≥30	6	−0.20 (−0.49 to 0.09)	0%	.436
Months				
≤3	2	−1.7 (−2.02 to 1.38)	83.5%	0
>3	5	−0.24 (−0.55 to 0.06)	3.7%	.386
Dose, mg/d				
<2000	5	−0.94 (−1.17 to −0.70)	94.2%	.000
≥2000	2	0.24 (−0.35 to 0.82)	2.8%	.311

BMI = body mass index, IL-6 = interleukin-6, SMD = standard mean differences.

large sample standard RCTs that directly assess the efficacy of metformin treatment in women with PCOS. Second, there was a wide gap of the number of objects in the recruited trails, ranging from 9 to 97, which may weaken the strength of pooled studies. Third, most of the studies included in the meta-analysis contained small numbers of cases, which is probably the reason for significant heterogeneity. Furthermore, obvious heterogeneity across the included studies was not eliminated by the subgroup analyses and the meta-regression analysis also could not determine the source of heterogeneity; it might reflect clinical heterogeneity related to physical activity, PCOS phenotypes, concomitant subclinical inflammatory diseases, etc.

5. Conclusion

This meta-analysis showed a significant decrease of serum CRP levels, especially in obese women, but no significant changes in IL-6 levels after metformin treatment in women with PCOS. In general, the data support that early metformin therapy may ameliorate the state of chronic inflammation in women with PCOS. Further investigation is required to determine whether these findings may prove to be of clinical significance for PCOS patients. Considering the obvious heterogeneity reported in the literature, further well-designed investigations with larger samples are needed to ascertain the long-term effects of metformin on chronic inflammation in PCOS.

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