Meta-Analysis

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Homocysteine, vitamin B12, and folate circulating levels in women with and without polycystic ovary syndrome: A systematic review and meta-analysis

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Abstract

Background: Some studies have reported that homocysteine, vitamin B12, and folic acid levels are associated with polycystic ovary syndrome (PCOS), whereas other studies yielded controversial results.

Objectives: This study aimed to systematize the available evidence of homocysteine, vitamin B12, and folate levels in women with and without PCOS.

Design: Systematic review and meta-analysis

Data Sources and Methods: A systematic search without language restrictions was performed on PubMed, Ovid/Medline, Scopus, Embase, and Web of Science. In addition, the reference lists of the selected studies were reviewed. The Newcastle–Ottawa Scale was employed to evaluate the quality of studies. The means and standard deviations of the outcomes were pooled as standardized mean differences (SMDs) with 95% confidence intervals (CI). Furthermore, the DerSimonian and Laird method was employed for the quantitative synthesis.

Results: A total of 75 studies met the eligibility criteria for at least one outcome. Patients with PCOS had higher circulating homocysteine levels than those without (SMD: 0.82; 95% CI: 0.62–1.02, $n = 70$ studies, $p < 0.001$). This trend remained in the sensitivity and subgroup analyses by world regions of studies, assay methods, and insulin resistance. No significant differences were observed in circulating vitamin B12 (SMD: −0.11; 95% CI: −0.25 to 0.03; $n = 17$ studies, $p = 0.13$) and folate levels (SMD: −0.2; 95% CI: −0.68 to 0.27; $n = 17$ studies, $p = 0.41$) between patients with and without PCOS.

Conclusions: (i) Patients with PCOS exhibited significantly higher homocysteine levels than those without, and (ii) no significant differences were observed in both vitamin B12 and folate levels in women with and without PCOS.

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Introduction

Polycystic ovary syndrome (PCOS) is an endocrine disorder that affects approximately 116 million women of reproductive age (3.4%) worldwide.[1,2] The burden of PCOS ascends to 0.43 million disability adjusted life years, and its incidence exhibits an upward trend.[3] PCOS entails hirsutism, anovulation, and polycystic ovaries.[4,5] About 40% of women with PCOS exhibit insulin resistance that can lead to the development of other long-term health conditions, such as diabetes mellitus, infertility, high blood pressure, and coronary diseases.[6] Although the etiopathogenesis of PCOS remains unclear, various studies in recent years have focused on genetic mechanisms and the role of adipokines.[7,8]

Part of the main approaches to PCOS treatment has been traditionally addressed on the relief of symptoms, lifestyle changes, dietary modification, increase in physical activity, and other environmental changes (e.g., limit alcohol intake).[4,9] Obesity is a cofactor in and increases the negative consequences of PCOS, such as glucose intolerance and dyslipidemia, which leads to adverse cardiovascular complications.[10,11] Dietary modification includes increased consumption of unrefined cereals, beans, fruits, vegetables, lean meat, nuts, seeds and oils, food groups that provide complex carbohydrates, healthy fats, high-quality protein, and micronutrients necessary to improve PCOS condition.[9,12] Among all nutrients, there is evidence that B complex vitamins, specifically vitamin B12 and folate, exert a beneficial effect on fecundability and fertility. Owing to their role in homocysteine metabolism, intervention studies demonstrated that folate and vitamin B12 supplementation can improve the metabolic profiles of women with PCOS.[13]

Vitamin B12 and folate deficiency can be caused by not only an inadequate diet but also other conditions, such as medications or excessive alcohol intake. Furthermore, gastrointestinal conditions, such as malabsorption, can explain the low serum levels of these vitamins.[14] Insulin resistance is a frequent manifestation in women with PCOS and increases the risk of cardiovascular or metabolic diseases.[15,16] In this context, these diseases have also been associated with elevated serum homocysteine levels. Likewise, folate and vitamin B12 are necessary for the metabolization of homocysteine; thus, a deficiency of these nutrients could increase the production of homocysteine, thereby exacerbating metabolic and cardiovascular problems.[17] However, there may be other mechanisms that could increase homocysteine levels, such as the presence of chronic inflammation and liver or genetic alterations.[17–21] Therefore, it is important to synthesize the available evidence regarding the comparison of the serum levels of homocysteine, folate, and vitamin B12 between healthy women and women with PCOS. This systematic review and meta-analysis aimed to evaluate circulating homocysteine, vitamin B12, and folate levels in women with and without PCOS.

Methods

Registration, report guidelines, and systematic searches

This manuscript was drafted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement (see the PRISMA checklist in Supplemental Table S1).[22] A summarized version of the protocol was uploaded to the International Prospective Register of Systematic Review and registered under the code CRD42023432883.

The search strategy was established in line with the Peer Review of Electronic Search Strategies Guidelines.[23] The search formula was based on MeSH, Emtree, and free terms. The entire search strategy is provided as a Supplemental Table S2. For this systematic review, hand-searching, citation matching, and date or language restrictions were not applied. PubMed, Web of Science, OVID Medline, Embase, and Scopus were searched. No search was run in preprints repositories, and the reference lists of the included studies were reviewed to seek for additional potential eligible records. The systematic search was simultaneously run in all databases on May 29, 2024.

Eligibility criteria and data extraction

Studies that evaluated the serum levels of homocysteine, vitamin B12, or folate in women with and without PCOS were searched. The inclusion criteria were studies (i) with cross-sectional, case–control, or cohort designs, (ii) that included adolescent and adult women, and (iii) that reported the serum levels of homocysteine, vitamin B12, or folate in women with and without PCOS (control group). PCOS was defined according to the respective meta-analyzed articles and based on international recommendations as specified.

After retrieving records from all the databases, duplicates were removed using EndNote 20.1 and Rayyan QCRI (Rayyan Systems Inc., Doha, Qatar).[24] These software were used to screen the titles, abstracts, and full texts of the articles. The screening process was independently performed by two authors (JRUB and AAHM). The full text from the remaining articles were independently reviewed by these authors. Any conflict or discrepancy concerning the inclusion of articles at

any stage of the selection process was resolved by consensus. Afterward, four authors (JRUB, EAAB, EAHB, and JCCG) independently extracted the data from each of the included studies in a preset Google Sheets. In the same manner, any conflict was resolved by consensus. The extracted data included the first author, publication date, study title, study design, study location, PCOS criteria, number of women, age, sex, and serum levels of vitamin B12, folate, and homocysteine in women with PCOS, women PCOS with insulin resistance (PCOS-IR), women with PCOS without insulin resistance (PCOS-NIR), and control group.

Quality assessment and publication bias

Two authors critically and independently evaluated the included studies using the Newcastle–Ottawa Scale[25] for the cohort and case–control studies, and an adaptation of the Newcastle–Ottawa Scale for cross-sectional studies. Risk of bias was categorized as having either a low risk of bias (⩾7 stars) or a high risk of bias (⩽6 stars). Publication bias was evaluated using the Egger test, considering $p > 0.1$ as indicative of no publication bias.[26] Publication bias was also evaluated using funnel plots and, if needed, the trim and fill method.[27]

Data synthesis and analyses

The effect size of each study was calculated using the mean and standard deviation (SD) of the markers values for women with PCOS versus those without PCOS (control group) and pooled as standardized mean difference (SMD) with 95% confidence interval (CI). RevMan (Cochrane Collaboration©, United Kingdom) was used for the statistical analysis. Values expressed as medians and their interquartile ranges were transformed into means and their corresponding SDs using Hozo et al.'s method.[28] Random-effects meta-analyses were conducted using the DerSimonian and Laird method.[29] The heterogeneity of the meta-analyses was analyzed using the I^2 statistic ($p \geqslant 60\%$ was considered to indicate high heterogeneity) and Cochran's Q statistic ($p < 0.1$ was considered as a sign of heterogeneity). Subgroup analyses were conducted by world regions, assay methods, study design, and insulin resistance (PCOS-IR versus PCOS-NIR versus control group). Furthermore, sensitivity analyses were conducted using only studies with low risk of bias.

Results

Study selection and study characteristics

The systematic search led to the identification of 1348 articles, of which 808 remained after the removal of duplicates. In the screening of titles and abstracts, 634 studies were excluded. In turn, full-text screening left 75 studies that met all the eligibility criteria.[15,30–103] The flowchart of the selection process is shown in Figure 1.

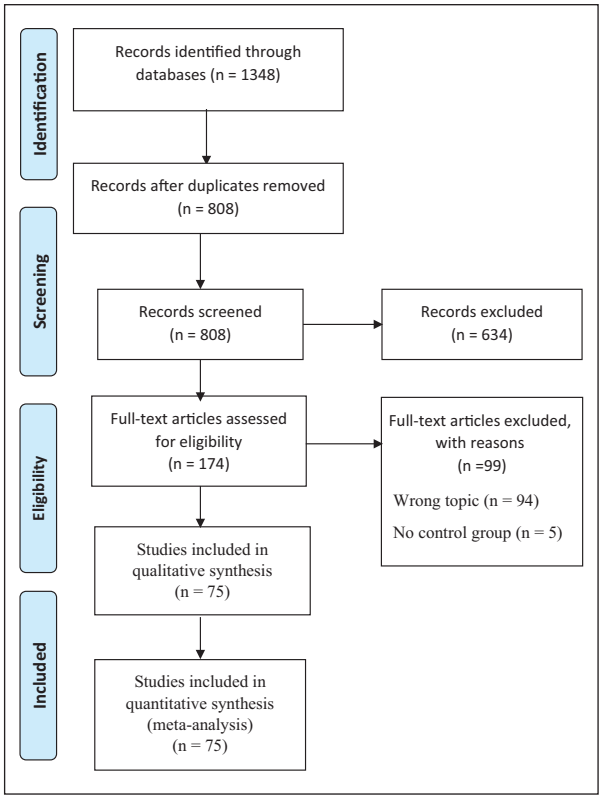


Figure 1. PRISMA flow diagram.
PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analysis.

The systematic review led to the identification of 42 case–control, 25 cross-sectional, and 8 cohort studies. A total of 9962 women were included, of whom 4772 were diagnosed with PCOS. The criteria for diagnosing PCOS were according to the Rotterdam Criteria (72 studies),[104] the United States National Institutes of Health (2 studies),[105] and the Homburg criterion (1 study).[106] The methods employed to measure the serum levels of homocysteine, vitamin B12, and folate were chemiluminescence immunoassay, electrochemiluminescence immunoassay, enzyme-linked immunosorbent assay (ELISA), fluorescence polarization immunoassay, high-performance liquid chromatography, enzyme immunoassay, radioimmunoassay, nephelometry immunoassay, spectrophotometry, and electrophoresis (Table 1).

Quality assessment and publication bias

In the quality assessment, 70 studies were found to have a low risk of bias and 5 had a high risk of bias (Supplemental Table S3). Publication bias was observed when the difference of homocysteine and folate serum levels was evaluated in women with and without PCOS (Egger's test, $p < 0.1$), which were corrected using the trim and fill method for homocysteine (SMD: 0.45; 95% CI: 0.22–0.67; Supplemental Figure S1) and folate (SMD: 0.373; 95% CI: −0.118 to 0.864; Supplemental Figure S2).

Table 1. Characteristics of the included studies in women with and without polycystic ovary syndrome, reporting blood homocysteine, vitamin B12, and/or folate levels.

Author	Year	Country	Median/mean/range age (IQR/SD)	Participants (PCOS/control)	PCOS definition criteria	Marker analyzed	Marker mean (SD) in women with PCOS	Marker mean (SD) in control group	Assay method
Agacayak et al.[30]	2015	Turkey	PCOS(+): 26.2 (4) PCOS(−): 27.6 (4.3)	60 (30/30)	Rotterdam	Vitamin B12	256.3 (203.3)	265 (99)	ECLIA
Al-Gareeb et al.[31]	2016	Iraq	18–42	207 (101/106)	Rotterdam	Homocysteine	11.5 (5.41)	8.10 (1.89)	HPLC
Arkhypkina et al.[32]	2016	Ukraine	18–24	90 (70/20)	Rotterdam	Folate	23.54 (0.52)	28.9 (0.7)	Spectrophotometry
						Homocysteine	10.9 (0.3)	8.2 (0.2)	CLIA
Asanidze et al.[33]	2022	Georgia	PCOS(+): 26.8 (3.4) PCOS(−): 25.2 (3.9)	160 (90/70)	Rotterdam	Homocysteine	12.24 (2.5)	7.2 (2.4)	EIA
Atamer et al.[34]	2008	Turkey	PCOS(+): 27.3 (8.3) PCOS(−): 27.9 (8.6)	65 (35/30)	Rotterdam	Homocysteine	11.52 (3.11)	6.32 (2.33)	HPLC
Atanasova et al.[35]	2017	Macedonia	PCOS(+): 23.9 (3.9) PCOS(−): 24.6 (4.8)	116 (73/43)	Rotterdam	Homocysteine	11.98 (2.88)	8.5 (3)	EIA
Badawy et al.[36]	2006	Egypt	PCOS(+): 23.9 (5.62) PCOS(−): 26.7 (4.55)	125 (90/35)	Rotterdam	Homocysteine	14.32 (5.67)	4.67 (3.11)	CLIA
Bahat et al.[37]	2021	Turkey	PCOS(+): 24.84 (5.40) PCOS(−): 25.19 (4.53)	200 (100/100)	Rotterdam	Homocysteine	10.04 (2.16)	8.17 (2)	CLIA
Battaglia et al.[38]	2008	Italy	PCOS(+): 25.2 (4) PCOS(−): 26.5 (4.4)	60 (28/15)	Rotterdam	Homocysteine	11.5 (0.5)	9.8 (1.5)	FPI
Bayrak et al.[39]	2012	Turkey	PCOS(+): 21.8 (6.7) PCOS(−): 22.5 (2.6)	102 (77/25)	Rotterdam	Homocysteine	10.7 (11.1)	9.3 (1.8)	FPI
Bayraktar et al.[40]	2004	Turkey	PCOS(+): 21.4 (3.16) PCOS(−): 21.6 (3.18)	75 (50/25)	National Institutes of Health	Homocysteine	17.7 (3.6)	8.9 (1.9)	HPLC
						Folate	9.3 (3.1)	9.3 (2.7)	CLIA
						Vitamin B12	251.9 (122.3)	156.2 (129.8)	CLIA
Bhushan et al.[41]	2022	India	PCOS(+): 23.94 (4.45) PCOS(−): 23.83 (4.64)	70 (35/35)	Rotterdam	Homocysteine	17.93 (9.86)	10.32 (5.95)	CLIA
Caglar et al.[42]	2011	Turkey	PCOS(+): 22.6 (4) PCOS(−): 23.4 (4)	82 (61/21)	Rotterdam	Homocysteine	10.3 (9.62)	7.5 (3.25)	NR
Calzada et al.[43]	2018	Spain	PCOS(+): 28 (5) PCOS(−): 30 (6)	294 (125/169)	Rotterdam	Homocysteine	10.1 (2.9)	9.3 (2)	Nephelometry
Cao et al.[44]	2022	China	PCOS(+): 29.39 (3.16) PCOS(−): 30.6 (2.84)	96 (66/30)	Rotterdam	Homocysteine	12.17 (3.64)	10.35 (1.92)	NR
						Folate	13.74 (8.81)	15.28 (7.51)	NR
						Vitamin B12	384.24 (154.59)	461.34 (193.83)	NR
Celik et al.[45]	2013	Turkey	PCOS(+): 24.8 (3.80) PCOS(−): 25.02 (3.75)	86 (44/42)	Rotterdam	Homocysteine	18.49 (6.20)	16.84 (5.06)	EIA
Centinkalp et al.[46]	2009	Turkey	PCOS(+): 24.58 (4.61) PCOS(−): 25.48 (3.38)	220 (129/91)	Rotterdam	Homocysteine	2.63 (1.6)	1.33 (0.22)	NR
Chakraborty et al.[15]	2013	India	PCOS(+): 28.95 (4.28) PCOS(−): 29.85 (3.69)	243 (126/117)	Rotterdam	Homocysteine	13.14 (0.61)	8.39 (2.22)	CLIA
Chang et al.[47]	2019	China	PCOS(+): 27.9 (3.3) PCOS(−): 0	1,000 (159/628)	Rotterdam	Homocysteine	7.11 (2.68)	6.73 (2.77)	ELISA
Cleto et al.[48]	2010	Brazil	PCOS(+): 26.2 (6.0) PCOS(−): 27.7 (6.1)	110 (56/54)	Rotterdam	Homocysteine	5.9 (2)	5.1 (1.3)	ECLIA
Czerwonogrodzka-Senczyna et al.[49]	2018	Poland	23–46	175 (105/70)	Rotterdam	Homocysteine	10.23 (3.21)	9.93 (2.94)	FPI

(Continued)

Table 1. (Continued)

Author	Year	Country	Median/mean/range age (IQR/SD)	Participants (PCOS/control)	PCOS definition criteria	Marker analyzed	Marker mean (SD) in women with PCOS	Marker mean (SD) in control group	Assay method
De la Calle et al.[50]	2007	Spain	PCOS(+): 28.9 (5.8) PCOS(−): 28.5 (6.6)	78 (39/39)	Rotterdam	Homocysteine	9 (2.1)	6.4 (1.8)	HPLC
						Folate	7.6 (3.7)	10.2 (3.6)	EIA
						Vitamin B12	339 (119)	386 (133)	EIA
Deveer et al.[51]	2012	Turkey	PCOS(+): 18.16 (3.07) PCOS(−) : 18.20 (2.34)	50 (25/25)	Rotterdam	Homocysteine	11.20 (6)	11.50 (5.23)	CLIA
Devi et al.[52]	2013	India	PCOS(+): 22.75 (5.60) PCOS(−): 24.25 (5.06)	81 (41/40)	Rotterdam	Homocysteine	18.23 (7.07)	12.1 (3.32)	FPI
Dhanalakshmi et al.[53]	2011	India	30–40	100 (50/50)	Rotterdam	Homocysteine	5.42 (0.33)	9.81 (0.24)	NR
						Folate	10.02 (0.68)	7.5 (1.06)	NR
Diwaker et al.[54]	2018	India	>18	90 (50/40)	Rotterdam	Homocysteine	11.8 (5.5)	7.8 (2.29)	FPI
El Gharib et al.[55]	2016	Egypt	PCOS(+): 28.83 (3.47) PCOS(−): 27.65 (3.67)	100 (60/40)	Rotterdam	Homocysteine	26.66 (18.7)	12.97 (5.17)	NR
Eskandari et al.[56]	2016	Iran	PCOS(+): 30.21 (5.41) PCOS(−): 27.67 (5.03)	70 (35/35)	Rotterdam	Homocysteine	13.27 (7.02)	9.29 (2.68)	EIA
Esmaeilzadeh et al.[57]	2017	Iran	PCOS(+): 23.65 (5.08) PCOS(−): 23.73 (3.85)	80 (60/20)	Rotterdam	Homocysteine	11.68 (2.92)	9.31 (2.45)	EIA
						Folate	16.81 (6.52)	15.09 (5.6)	ECLIA
						Vitamin B12	392.82 (137.31)	415.13 (145.38)	ECLIA
Feng et al.[58]	2021	China	PCOS(+): 27.02 (4.76) PCOS(−): 27.44 (4.21)	450 (150/300)	Rotterdam	Homocysteine	10.07 (2.06)	8.13 (1.21)	EIA
						Folate	10.49 (3.83)	11.99 (2.58)	NR
Gözüküçük et al.[59]	2021	Turkey	PCOS(+): 23.9 (3.6) PCOS(−): 24 (2.9)	86 (45/41)	Rotterdam	Homocysteine	10.05 (9.77)	9.87 (9.7)	FPI
Gungor et al.[60]	2021	Turkey	PCOS(+): 24.96 (5.27) PCOS(−): 25.48 (5.36)	200 (109/91)	Rotterdam	Vitamin B12	324 (189.3)	364.71 (127.9)	NR
Gupta et al.[61]	2013	India	PCOS(+): 28.27 (3.36) PCOS(−): 27.4 (2.73)	80 (40/40)	Rotterdam	Homocysteine	6.64 (0.94)	11.04 (4.52)	EIA
Guzelmeric et al.[62]	2007	Turkey	PCOS(+): 23.5 (4.7) PCOS(−): 25.9 (5.6)	70 (44/26)	Rotterdam	Homocysteine	13.30 (4.81)	9.02 (3.36)	FPI
Hamadneh et al.[63]	2021	Jordan	PCOS(+): 23.58 (0.55) PCOS(−): 34.45 (1.07)	150 (77/73)	Rotterdam	Homocysteine	21.3 (1.88)	16.26 (0.97)	HPLC
Harmanci et al.[64]	2013	Turkey	PCOS(+): 22.2 (3.5) PCOS(−): 22.2 (3.5)	46 (23/23)	Rotterdam	Homocysteine	13.1 (5.2)	13.4 (4.9)	HPLC
Hemati et al.[65]	2011	Iran	PCOS(+): 31.09 (8.9) PCOS(−): 29.1 (3.2)	114 (64/50)	Rotterdam	Homocysteine	10.9 (7.2)	6.8 (1.95)	ELISA
Heutling et al.[66]	2008	Germany	PCOS(+): 27.8 (4.7) PCOS(−): 27.8 (5.6)	122 (83/39)	Rotterdam	Homocysteine	9.3 (3.2)	8.5 (2.1)	HPLC
Isbilen et al.[67]	2022	Turkey	PCOS(+): 23 (19–27) PCOS(−): 31 (24–39)	140 (101/39)	Rotterdam	Vitamin B12	223.75 (110.3)	219.75 (67.47)	CLIA
Karadeniz et al.[68]	2010	Turkey	PCOS(+): 24.27 (5.44) PCOS(−): 26.41 (5.65)	156 (86/70)	Rotterdam	Homocysteine	12.315 (3.9)	10.078 (2.247)	FPI
Karaer et al.[69]	2010	Turkey	PCOS(+): 32.6 (6.6) PCOS(−): 35.6 (5.2)	62 (31/31)	Rotterdam	Homocysteine	10.5 (4.7)	8.03 (1.7)	Competitive immunoassay
Kaya et al.[70]	2009	Turkey	PCOS(+): 27.2 (4.1) PCOS(−): 26.7 (3.7)	122 (61/61)	Rotterdam	Homocysteine	13.9 (7.3)	9.4 (2.1)	HPLC
						Folate	9.2 (2.1)	9.7 (1.3)	ECLIA
						Vitamin B12	268.2 (46)	308.9 (68.4)	ECLIA

(Continued)

Table 1. (Continued)

Author	Year	Country	Median/mean/range age (IQR/SD)	Participants (PCOS/control)	PCOS definition criteria	Marker analyzed	Marker mean (SD) in women with PCOS	Marker mean (SD) in control group	Assay method
Kilic-Okman et al.[71]	2004	Turkey	PCOS(+): 23.90 (5.86) PCOS(−): 25.24 (4.28)	54 (29/25)	Rotterdam	Homocysteine	11.11 (5.6)	4.26 (3.1)	CLIA
Krishna et al.[72]	2009	India	26–46	112 (56/56)	Rotterdam	Homocysteine	20.21 (1.99)	18.96 (1.93)	ELISA
Kulhan et al.[73]	2017	Turkey	PCOS(+): 24.06 (6.12) PCOS(−): 25.34 (5.82)	132 (65/67)	Rotterdam	Folate	19.01 (5.32)	10.79 (5.75)	CLIA
						Vitamin B12	314.61 (92.95)	300.23 (87.06)	CLIA
Li et al.[74]	2022	China	PCOS(+): 26.875 (2.658) PCOS(−): 27.262 (3.030)	871 (208/663)	Rotterdam	Homocysteine	15.214 (9.993)	12.563 (7.205)	EIA
Loverro et al.[75]	2002	Italy	PCOS(+): 23.8 (4.06) PCOS(−): 22.2 (3.1)	55 (35/20)	Rotterdam	Homocysteine	10.4 (4.4)	7.2 (1.5)	HPLC
Maleedhu et al.[76]	2014	India	20–35	207 (142/65)	Rotterdam	Homocysteine	10.13 (2.8)	7.13 (2.32)	EIA
Miranda-Furtado et al.[77]	2015	Brazil	PCOS(+): 28.13 (5.45) PCOS(−): 29.62 (5.28)	97 (45/52)	Rotterdam	Homocysteine	7.77 (1.78)	8.27 (6.22)	CLIA
Moran et al.[78]	2008	Australia	PCOS(+): 32.6 (6.6) PCOS(−): 35.6 (5.2)	30 (14/16)	Rotterdam	Folate	18.5 (6.5)	18.7 (11.3)	NR
						Vitamin B12	314.5 (142.2)	277.6 (123.9)	NR
Moti et al.[79]	2015	Iran	PCOS(+): 26.80 (0.67) PCOS(−): 25.10 (0.89)	60 (30/30)	Rotterdam	Homocysteine	12.80 (1.7)	9.30 (1.7)	HPLC
Nafiye et al.[80]	2009	Turkey	PCOS(+): 29.61 (5.12) PCOS(−): 29.71 (4.41)	74 (36/38)	Rotterdam	Homocysteine	7.94 (1.91)	6.77 (2.28)	CLIA
Nassir et al.[81]	2020	Sudan	18–46	300 (200/100)	Rotterdam	Homocysteine	14.9 (2.1)	12.1 (2.5)	CLIA
Nazar et al.[82]	2017	Iraq	20–35	120 (60/60)	Rotterdam	Homocysteine	7.39 (2.4)	14.41 (13.73)	ELISA
Oktem et al.[83]	2009	Turkey	PCOS(+): 25.6 (3.5) PCOS(−): 25.6 (3.8)	62 (31/31)	Rotterdam	Homocysteine	11.3 (4.8)	9.0 (3.2)	FPI
Onwumere et al.[84]	2022	Nigeria	PCOS(+): 32 (27.9–35) PCOS(−): 33.5 (31–35)	60 (30/30)	Rotterdam	Homocysteine	23.74 (18.13)	10 (1.14)	EIA
Orio et al.[85]	2003	Italy	PCOS(+): 22.5 (4.3) PCOS(−): 21.9 (3.2)	140 (70/70)	Rotterdam	Homocysteine	11.3 (3.7)	12.2 (4.5)	HPLC
						Folate	3.78 (1.05)	3.74 (1.01)	Electrophoresis
						Vitamin B12	377.4 (101.5)	384.5 (128.7)	Electrophoresis
Palep-Singh et al.[86]	2008	England	PCOS(+): 31 (29–33) PCOS(−): 35 (32–37)	41 (25/16)	Rotterdam	Homocysteine	9.82 (2.79)	8.03 (2.29)	FPI
						Folate	7.5 (5.7)	10.05 (7.55)	CLIA
						Vitamin B12	394.5 (131.8)	411.5 (112.5)	CLIA
Peña-Paredes et al.[87]	2008	Venezuela	PCOS(+): 24 (3.5) PCOS(−): 23.3 (3)	40 (20/20)	Rotterdam	Homocysteine	11.4 (1.8)	6.7 (1.6)	HPLC
Rekha et al.[88]	2013	India	PCOS(+): 26.10 (4.08) PCOS(−): 27.01 (4.28)	90 (50/40)	Rotterdam	Homocysteine	11.88 (5.55)	7.80 (2.29)	FPI
Sadaria et al.[89]	2021	India	18–35	100 (50/50)	Rotterdam	Homocysteine	13.9 (3.29)	9.91 (2.88)	EIA
Sahin et al.[90]	2007	Turkey	PCOS(+): 21.6 (3.7) PCOS(−): 22.9 (1.8)	40 (20/20)	Rotterdam	Homocysteine	12.8 (5.2)	9.9 (2.7)	FPI
Salehpour et al.[91]	2011	Iran	PCOS(+): 29 (5.9) PCOS(−): 27.3 (5.3)	168 (85/83)	Rotterdam	Homocysteine	16.25 (11.94)	11.58 (3.82)	RIA
Schachter et al.[92]	2015	Israel	28.8 (0.4)	51 (28/23)	Rotterdam	Homocysteine	10.5 (0.9)	10.3 (0.9)	FPI
Soares et al.[93]	2009	Brazil	PCOS(+): 24.5 (3.8) PCOS(−): 24.5 (5.1)	90 (40/50)	Rotterdam	Homocysteine	6.67 (1.78)	6.47 (1.45)	CLIA

(Continued)

Table 1. (Continued)

Author	Year	Country	Median/mean/range age (IQR/SD)	Participants (PCOS/control)	PCOS definition criteria	Marker analyzed	Marker mean (SD) in women with PCOS	Marker mean (SD) in control group	Assay method
Sohrabvand et al.[94]	2009	Iran	PCOS(+): 24.27 (3.75) PCOS(−): 25.62 (4.31)	156 (52/104)	Rotterdam	Homocysteine	12.21 (4.55)	13.68 (4.307)	EIA
						Folate	7.48 (4.37)	5.43 (3.15)	RIA
						Vitamin B12	323.29 (150.44)	360.29 (248.99)	RIA
Suleiman et al.[95]	2018	Iraq	PCOS(+): 26.88 (6.76) PCOS(−): 27.58 (6.45)	90 (50/50)	Rotterdam	Homocysteine	17.00 (4)	9.00 (2.15)	EIA
Tarkun et al.[96]	2005	Turkey	PCOS(+): 24.86 (4.7) PCOS(−): 25.4 (4.07)	75 (40/35)	Rotterdam	Homocysteine	11.5 (2.71)	9.4 (1.8)	CLIA
						Folate	8.9 (3.45)	9.6 (2.7)	CLIA
						Vitamin B12	352.14 (184.11)	373.2 (117.6)	CLIA
Temel et al.[97]	2010	Turkey	PCOS(+): 23.13 (4.28) PCOS(−): 23.30 (2.76)	60 (30/30)	Rotterdam	Homocysteine	16.25 (6.76)	8.91 (6.47)	EIA
Topcu et al.[98]	2005	Turkey	PCOS(+): 27.1 (4.5) PCOS(−): 28.8 (4.4)	54 (28/26)	Rotterdam	Homocysteine	9.3 (3.1)	6.4 (1.2)	NR
Vrbikova et al.[99]	2003	Czech Republic	PCOS(+): 25.8 (7) PCOS(−): 33 (5)	51 (40/11)	National Institutes of Health	Homocysteine	10.1 (2.5)	8.78 (2.75)	HPLC
Wijeyaratne et al.[100]	2004	Sri Lanka	PCOS(+): 27.3 (1.7) PCOS(−): 33 (4.7)	119 (74/45)	Rotterdam	Homocysteine	10.2 (1.9)	9 (3.8)	FPI
Yarali et al.[101]	2001	Turkey	PCOS(+): 27.9 (6.1) PCOS(−): 31.4 (6.5)	60 (30/30)	Rotterdam	Homocysteine	11.2 (4.1)	9 (2.4)	FPI
						Folate	9.3 (4.8)	9.3 (3)	CLIA
						Vitamin B12	250.7 (131.9)	270.1 (114)	CLIA
Yilmaz et al.[102]	2005	Turkey	PCOS(+): 23.2 (4.92) PCOS(−): 23.98 (6.08)	135 (85/50)	Homburg	Homocysteine	14.78 (7.67)	10.45 (5.67)	HPLC
						Folate	12.96 (3.51)	12.78 (3.76)	CLIA
						Vitamin B12	268.49 (56.78)	276.71 (35.89)	CLIA
Yilmaz et al.[103]	2005	Turkey	PCOS(+): 24.12 (4.89) PCOS(−): 24.35 (5.02)	85 (50/35)	Rotterdam	Homocysteine	13.76 (6.28)	9.32 (4.52)	HPLC
						Folate	11.52 (3.14)	11.83 (3.39)	CLIA
						Vitamin B12	312.87 (72.61)	306.12 (68.35)	CLIA

IQR: interquartile range; SD: standard deviation; CLIA: chemiluminescence immunoassay; ECLIA: electrochemiluminescence immunoassay; ELISA: enzyme-linked immunosorbent assay; EIA: enzyme immunoassay; FPI: fluorescence polarization immunoassay; HPLC: high-performance liquid chromatography; NR: not reported; RIA: radio immunoassay; PCOS: polycystic ovary syndrome.

Homocysteine, vitamin B12, and folate meta-analyses

A total of 70 studies ($n=9{,}400$ participants) evaluated the homocysteine levels in women with and without PCOS. Women with PCOS had higher homocysteine levels than controls (SMD: 0.82; 95% CI: 0.62–1.02; $p<0.001$; $I^2=94\%$; Figure 2(a)). A total of 17 studies ($n=1{,}705$ participants) evaluated vitamin B12 levels in women with and without PCOS. No significant difference was observed in vitamin B12 levels between women with PCOS and the controls (SMD: −0.11; 95% CI: −0.25 to 0.03; $p=0.13$; $I^2=50\%$; Figure 2(b)). A total of 17 studies ($n=1{,}945$ participants) evaluated folate levels in women with and without PCOS. No significant difference was observed in the folate levels between women with PCOS and the controls (SMD: −0.2; 95% CI: −0.68 to 0.27; $p=0.41$; $I^2=96\%$; Figure 2(c)).

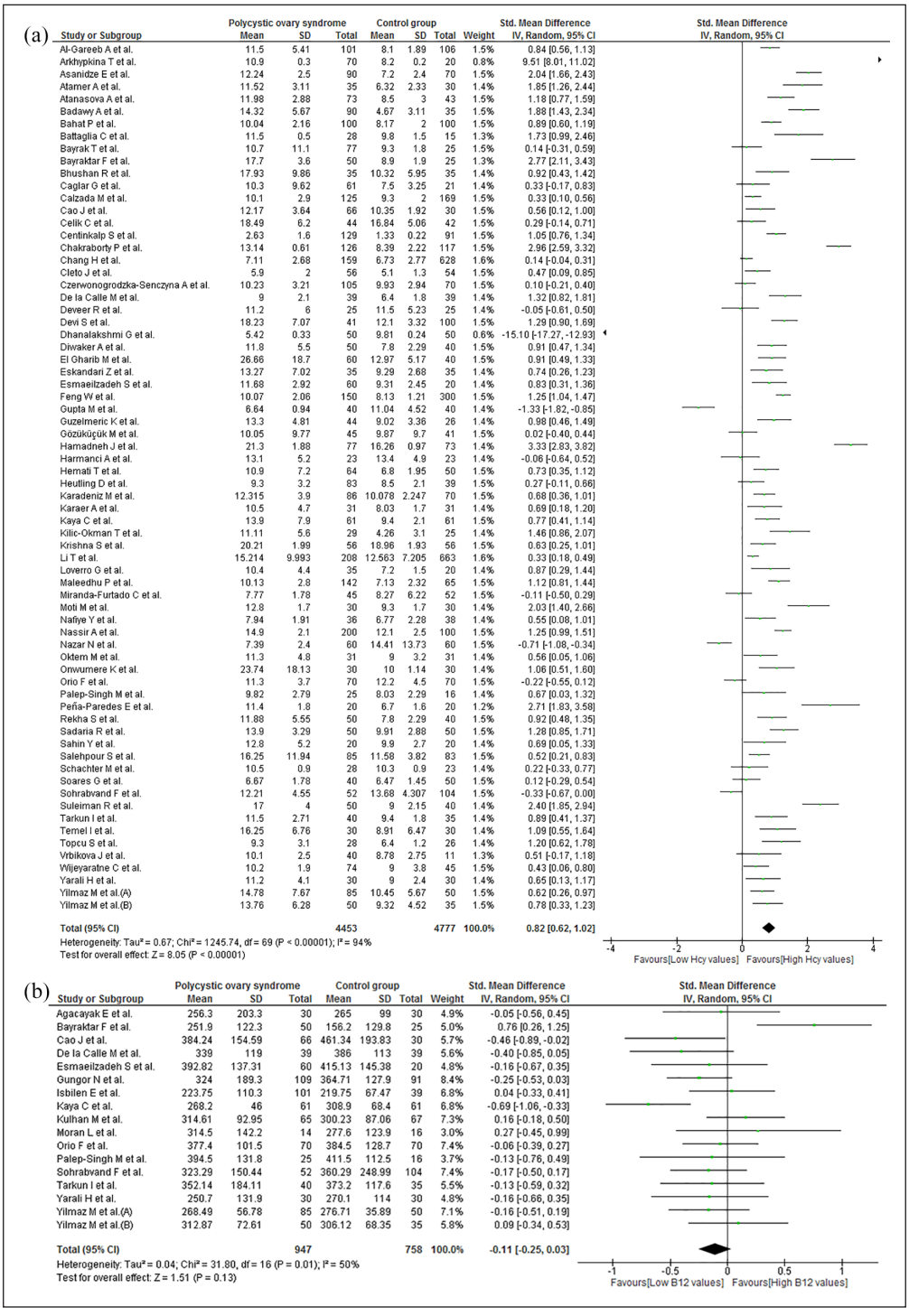


Figure 2. (Continued)

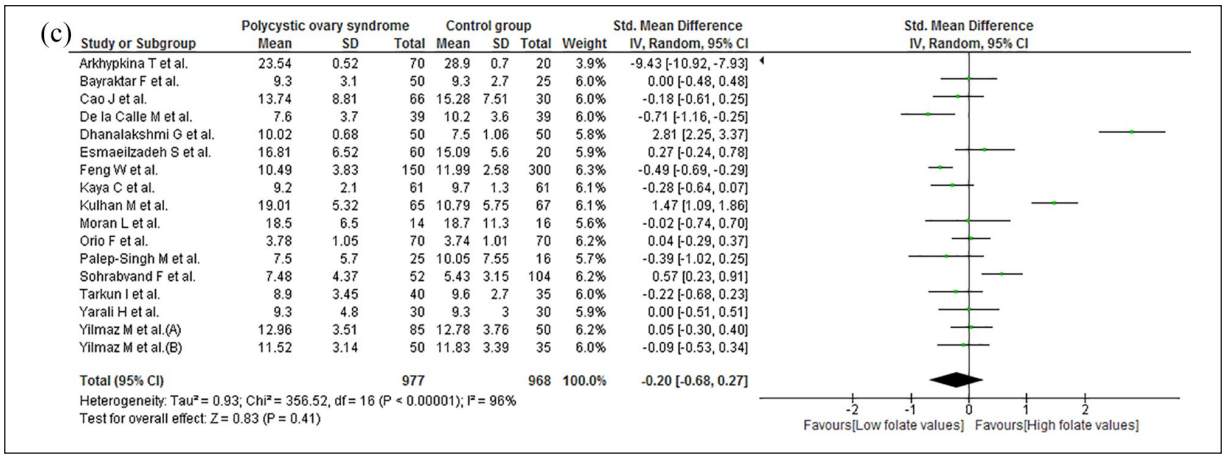


Figure 2. Forest plots of studies comparing (a) homocysteine, (b) vitamin B12, and (c) folate in women with and without polycystic ovary syndrome.

Subgroup and sensitivity analyses

In the subgroup analyses by world regions (Figure 3(a)), assay methods (Figure 3(b)), study designs (Figure 3(c)), and insulin resistance (Figure 3(d) and (e)), the differences in homocysteine levels remained with high heterogeneity, except in studies conducted in America continent and in those that evaluated homocysteine levels using the ELISA method. In the sensitivity analysis that included only studies with low risk of bias, women with PCOS were consistently found to have higher homocysteine levels than those without PCOS (SMD: 0.8; 95% CI: 0.59–1.02; $p < 0.01$) without a decrease in heterogeneity ($I^2 = 95\%$; Supplemental Figure S3).

In the vitamin B12 subgroup analyses by world regions (Figure 4(a)), assay methods (Figure 4(b)), study design (Figure 4(c)), and insulin resistance (Figure 4(d) and (e)) did not exhibit significant differences, except in studies conducted in Asia. In the sensitivity analysis that included only studies with low risk of bias, no significant difference was observed between women with and without PCOS (SMD: −0.08; 95% CI: −0.25 to 0.08; $p = 0.33$; Supplemental Figure S4).

In any of the folate subgroup analyses by world regions (Figure 5(a)), assay methods (Figure 5(b)), study designs (Figure 5(c)), or insulin resistance (Figure 5(d) and (e)) did not significant differences. In the sensitivity analysis, there was no significant difference between women with PCOS and women without PCOS when studies with only low risk of bias were included (SMD: −0.11; 95% CI: −0.71 to 0.49; $p = 0.71$; Supplemental Figure S5).

Discussion

In this study, patients with PCOS exhibited significantly higher homocysteine levels than those without PCOS. This association remained significant in (i) the subgroup analyses by world regions and assay methods and (ii) the sensitivity analyses. No significant differences were observed in vitamin B12 and folate levels between women with and without PCOS. Furthermore, there were no differences in the subgroup nor sensitivity analyses for these two outcomes.

Main homocysteine, vitamin B12, and folate metabolism

Homocysteine is a sulfur-containing amino acid does not present in the dietary protein that is an intermediate of methionine metabolism. It plays a role in the remethylation of methionine[91] and transsulfuration of cysteine,[107] requiring vitamins B6 and B12 as well as folate as cofactors for the synthesis.[107] Homocysteine is a biomarker of general health rather than a causal factor, and its serum level is increased in cardiovascular diseases, bone metabolic disorders, renal dysfunction, and neurodegenerative diseases. It is recycled to methionine or converted to cysteine. All tissues can include a methyl group donated by folate. The causes of prime homocysteine imbalance and hyperhomocysteinemia include genetic factors and, less frequently, nutritional and hormonal factors.[92,108]

Insulin levels may influence homocysteine levels by acting on its glomerular filtration or inhibiting the hepatic cystathionine beta-synthase.[88,92] Patients with PCOS-IR and those with PCOS-non-IR exhibit increased homocysteine levels, and a correlation exists between homocysteine and insulin levels independent of body mass index (BMI).[92] In addition, circulating homocysteine is inversely correlated with sex hormone-binding globulin.[109] Follicular homocysteine is a marker of oocyte quality in patients with PCOS, and hyperhomocysteinemia is associated with infertility.[13] Thus, homocysteinemia has been postulated as a

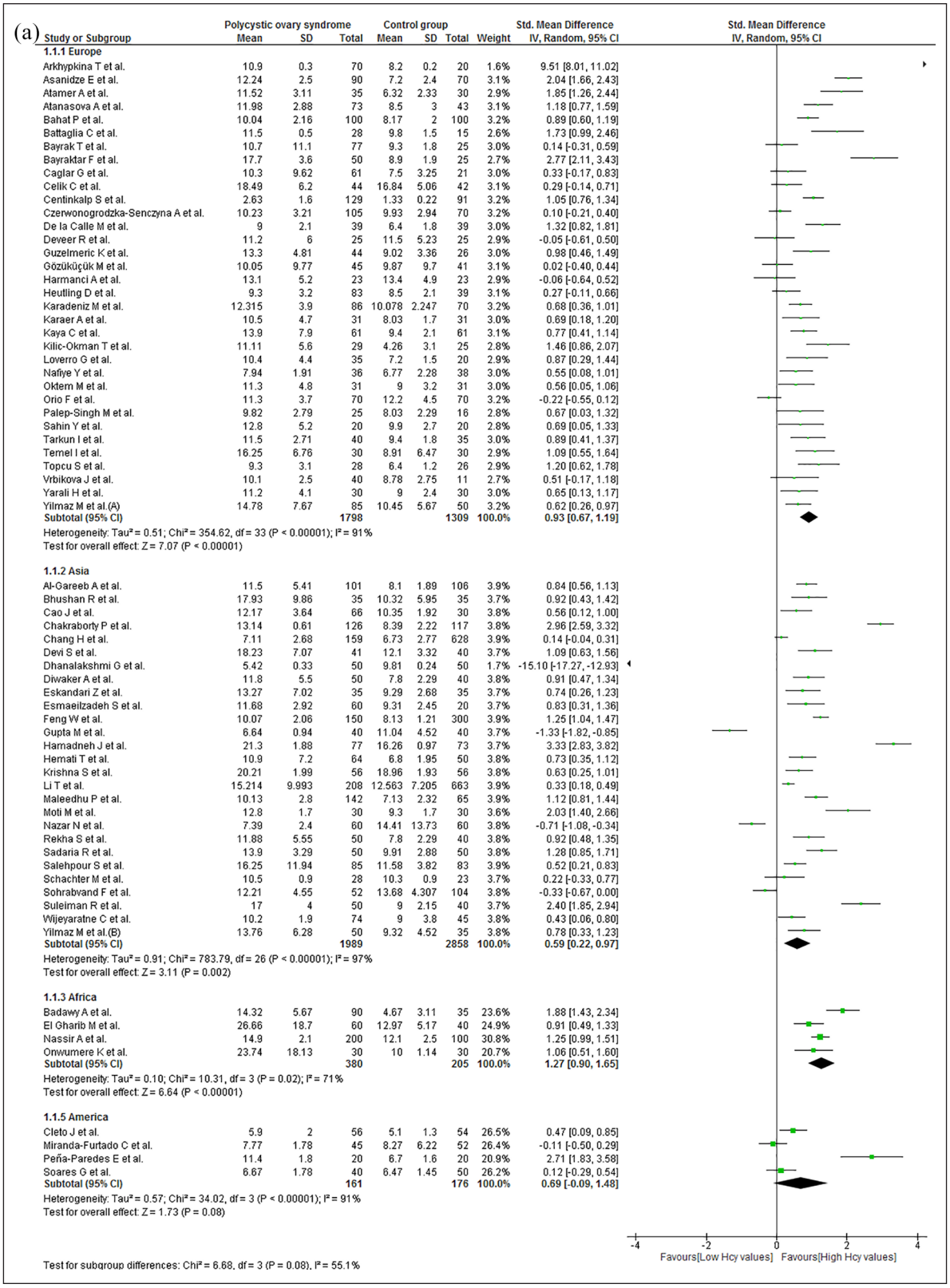
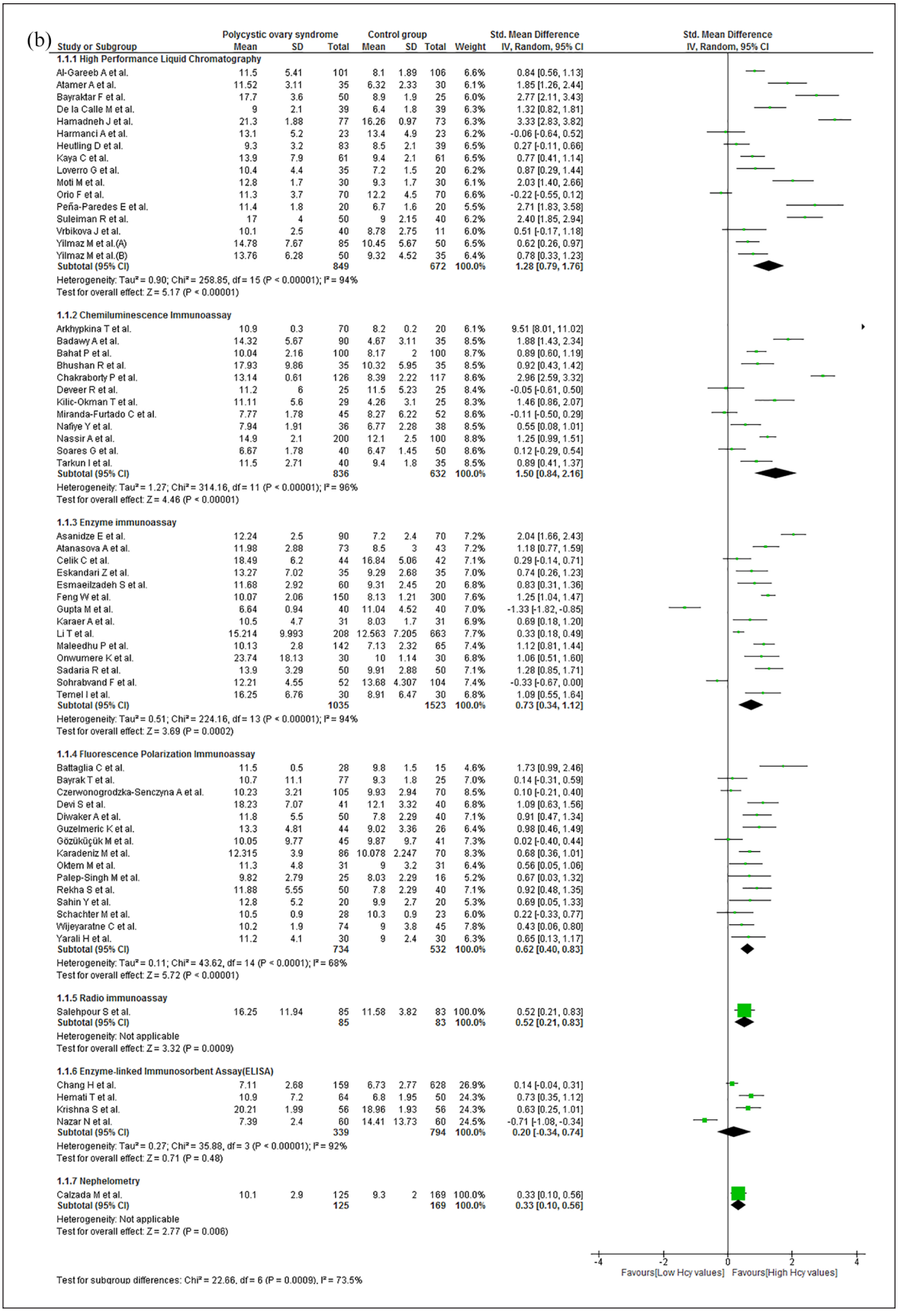


Figure 3. (Continued)



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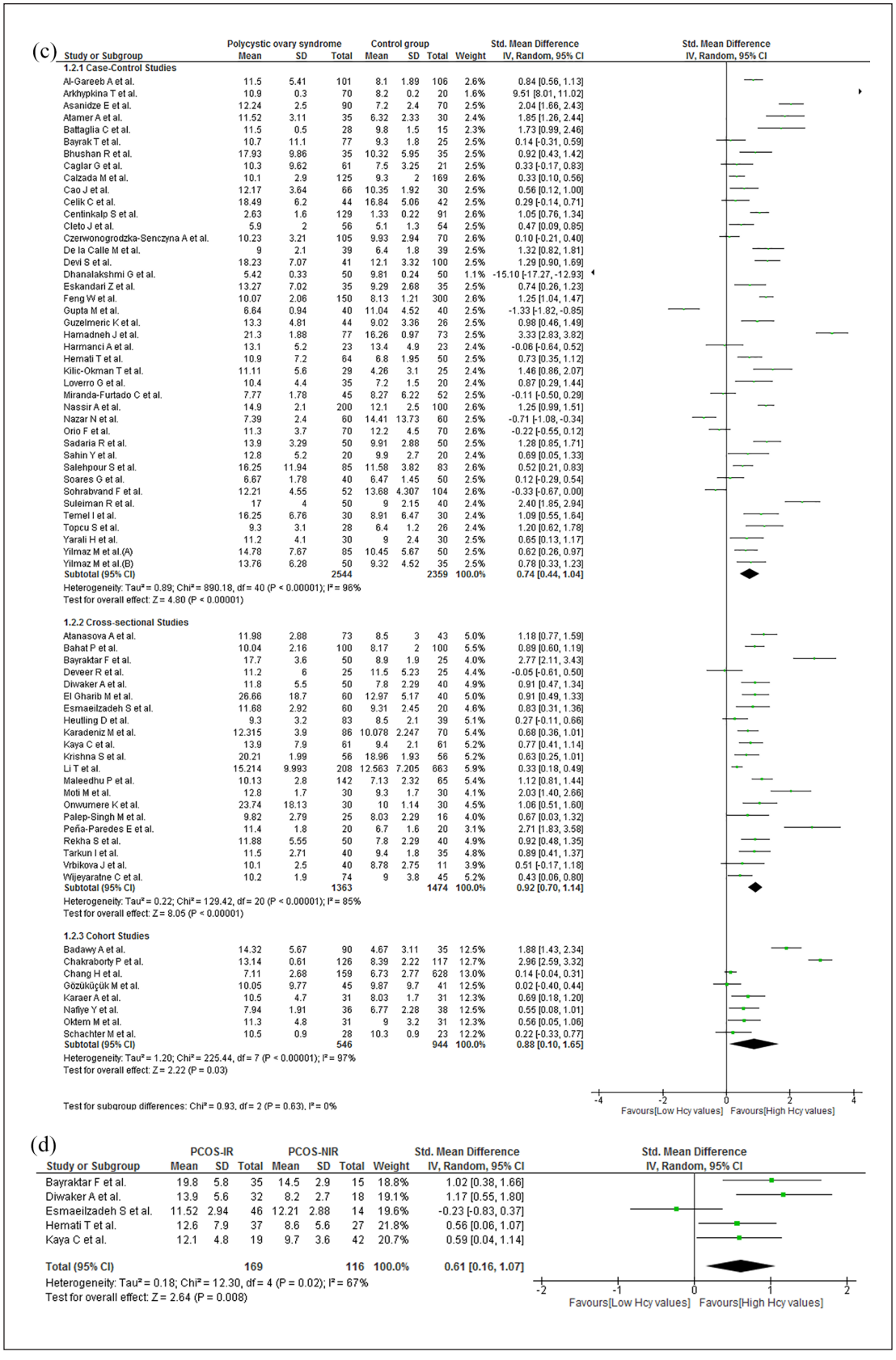

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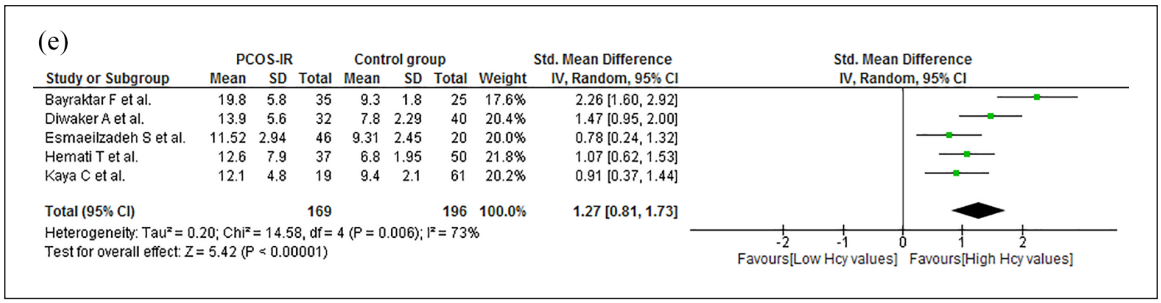


Figure 3. Subgroup analyses comparing homocysteine in women with and without PCOS: (a) by world regions, (b) by assay methods, (c) by study designs (d) by IR in women with PCOS (PCOS-IR versus PCOS-non IR), and (e) by IR in women with PCOS versus women without PCOS.
PCOS: polycystic ovary syndrome; IR: insulin resistance.

tool for oocyte-embryo selection[110] and hyperhomocysteinemia as a risk index of obstetric complications.[111,112]

Homocysteine, vitamin B12, and folate in patients with PCOS

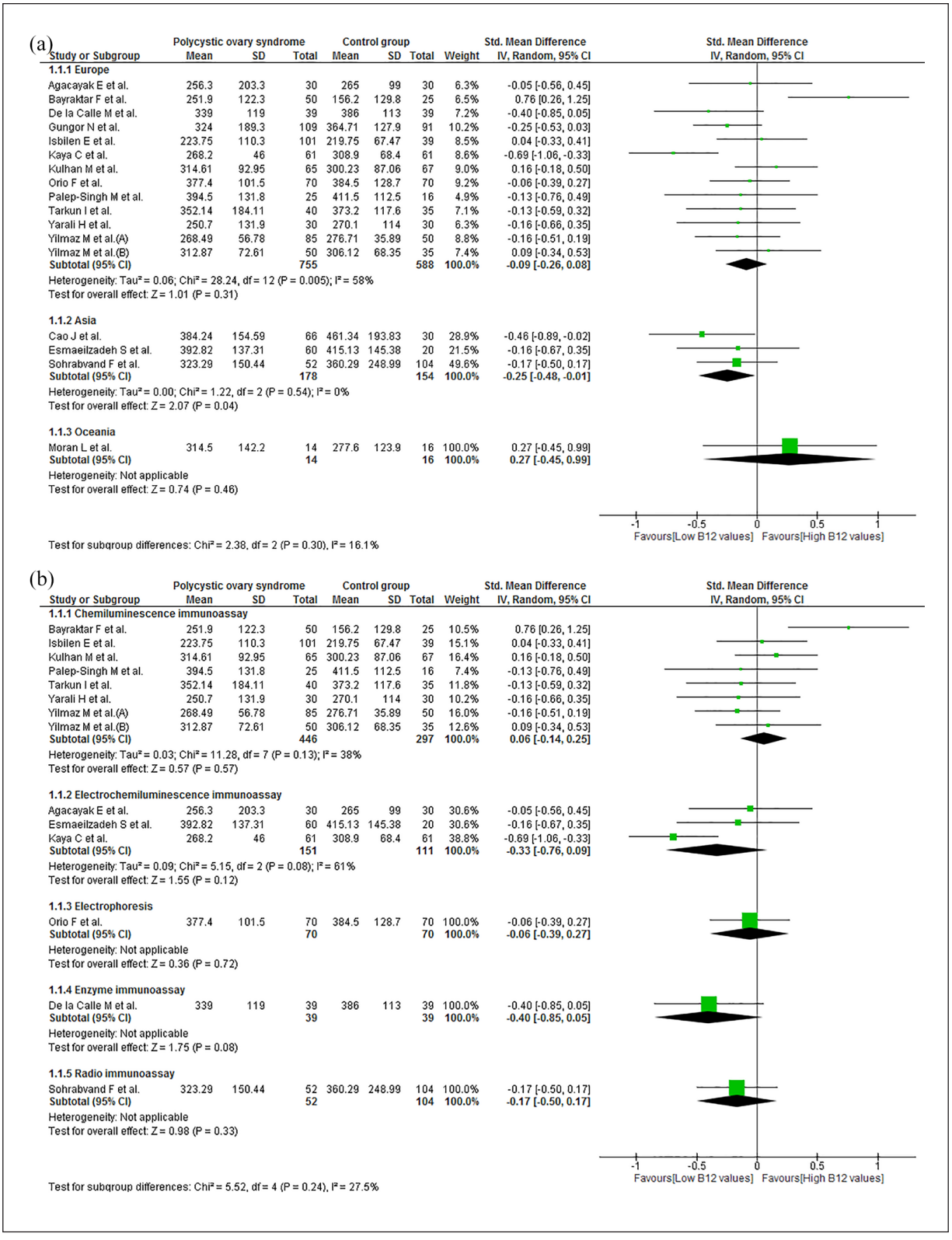
Hyperhomocysteinemia is a long-term health risk for patients with PCOS, including cardiovascular diseases, chronic kidney disease, hypothyroidism, cognitive decline, and osteoporosis.[113,114] A previous systematic review and meta-analysis reported significantly higher homocysteine levels in patients with PCOS than in those without,[115] with the levels being significantly lower in those with PCOS-non-IR than in those with PCOS-IR.[115] Differences in homocysteine levels were also found when the participants were matched for age and BMI.[116] Our systematic review and meta-analysis confirmed similar findings based on an updated comprehensive systematic search across multiple databases.

There are specific underlying mechanisms related to the PCOS pathogenesis and its treatment that account for the high homocysteine levels observed in women with the syndrome. Some determinants of elevated homocysteine levels inherent to PCOS have been analyzed, including the correlations between homocysteine and insulin resistance or hyperandrogenism,[117] although it remains a debated matter. Methylenetetrahydrofolate reductase (MTHFR) polymorphism was previously considered to be responsible for the high homocysteine levels among women with PCOS.[85,118] Nevertheless, a recent meta-analysis did not find evidence supporting the association between MTHFR C677T polymorphism and PCOS.[119] Meanwhile, despite the decrease in insulin resistance, the use of insulin sensitizers such as metformin or rosiglitazone in patients with PCOS to improve ovulation induction and metabolic parameters has been associated with an elevation in serum homocysteine levels in women with type 2 diabetes mellitus.[120] Similarly, regardless of smoking and obesity, the plasma homocysteine levels were increased in patients with PCOS treated with metformin.[121–124] Interestingly, PCOS patients with obesity have higher homocysteine levels than those who have no obesity.[125]

Folate circulating levels are frequently reduced in patients with PCOS, and folate alone or in combination with B vitamins improve fasting insulin and other outcomes.[9,13] The administration of B-group vitamins and folic acid reduces circulating homocysteine in patients with PCOS patients, suggesting that such an administration counteracts the homocysteine-increasing effect of metformin therapy.[122] No significant differences were observed in vitamin B12 and folate between women with and without PCOS.

The pathophysiology of PCOS includes insulin resistance, inflammation, and hormonal imbalances, would drive these links.[17,70,119] However, PCOS affects heterogeneous populations; therefore, further research is warranted to fully elucidate the specific mechanisms underlying B12 and folate level variations in women with PCOS. To the best of our knowledge, this is the first systematic review that assessed serum vitamin B12 and folate concentrations in women with and without PCOS.

It is premature to suggest treating homocysteine to tackle PCOS with our findings. However, considering the association of hyperhomocysteinemia with the increased risks of cardiovascular, cerebrovascular, and thromboembolic diseases, consider it in treatment.[114] Due to the limitations of our study, which are described in the following section, it is impossible to establish temporality or causality. Thus, our findings are merely constrained to associations. Kondapaneni et al.,[16] in their previous nonsystematic literature review, evaluated the importance of homocysteine levels in the overall management of PCOS. They highlighted the need to conduct larger-scale studies with standardized inclusion criteria. In addition, they recommended follow-up on homocysteine levels in women with PCOS at a high risk for complications in their management course.[16]



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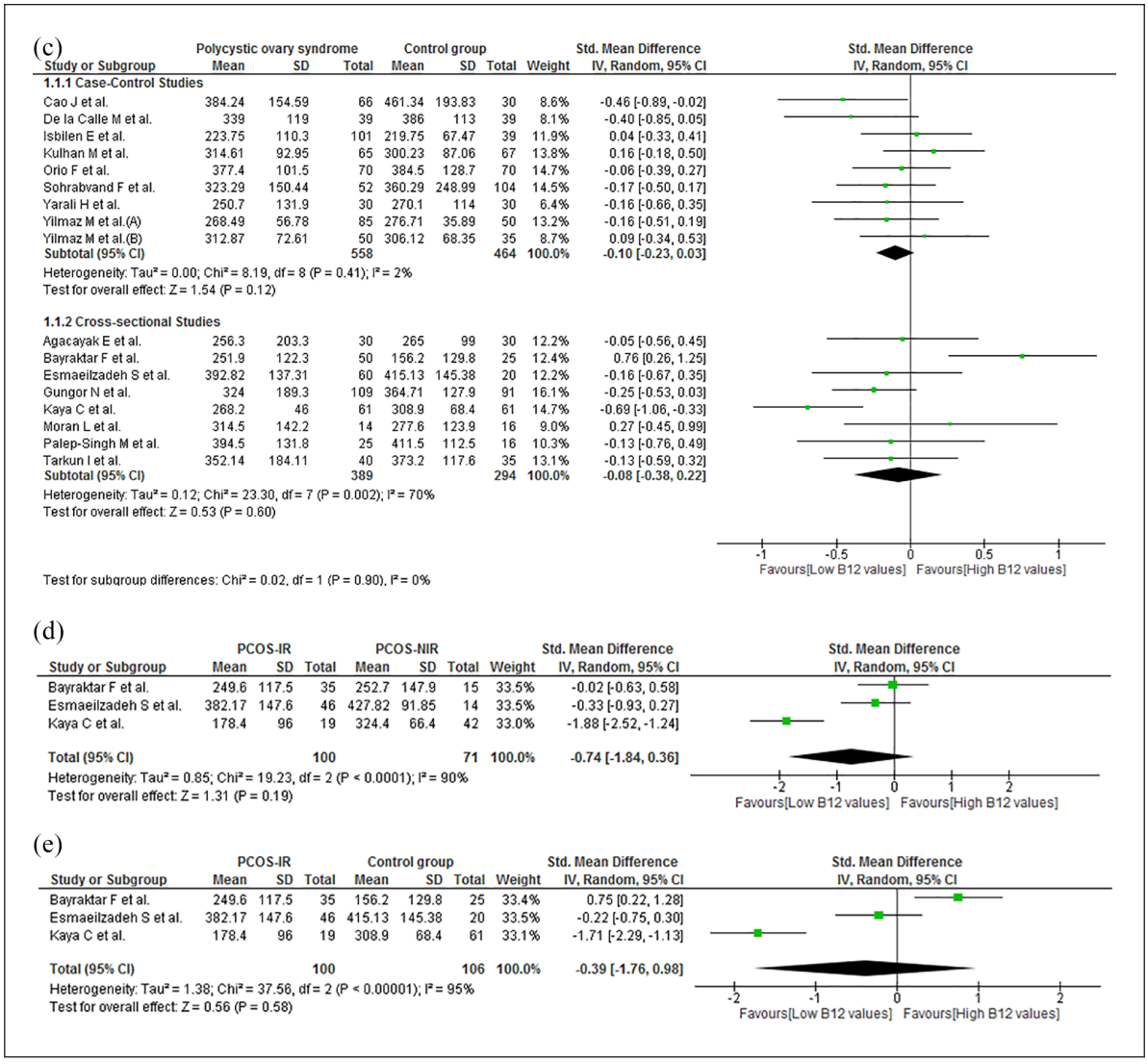
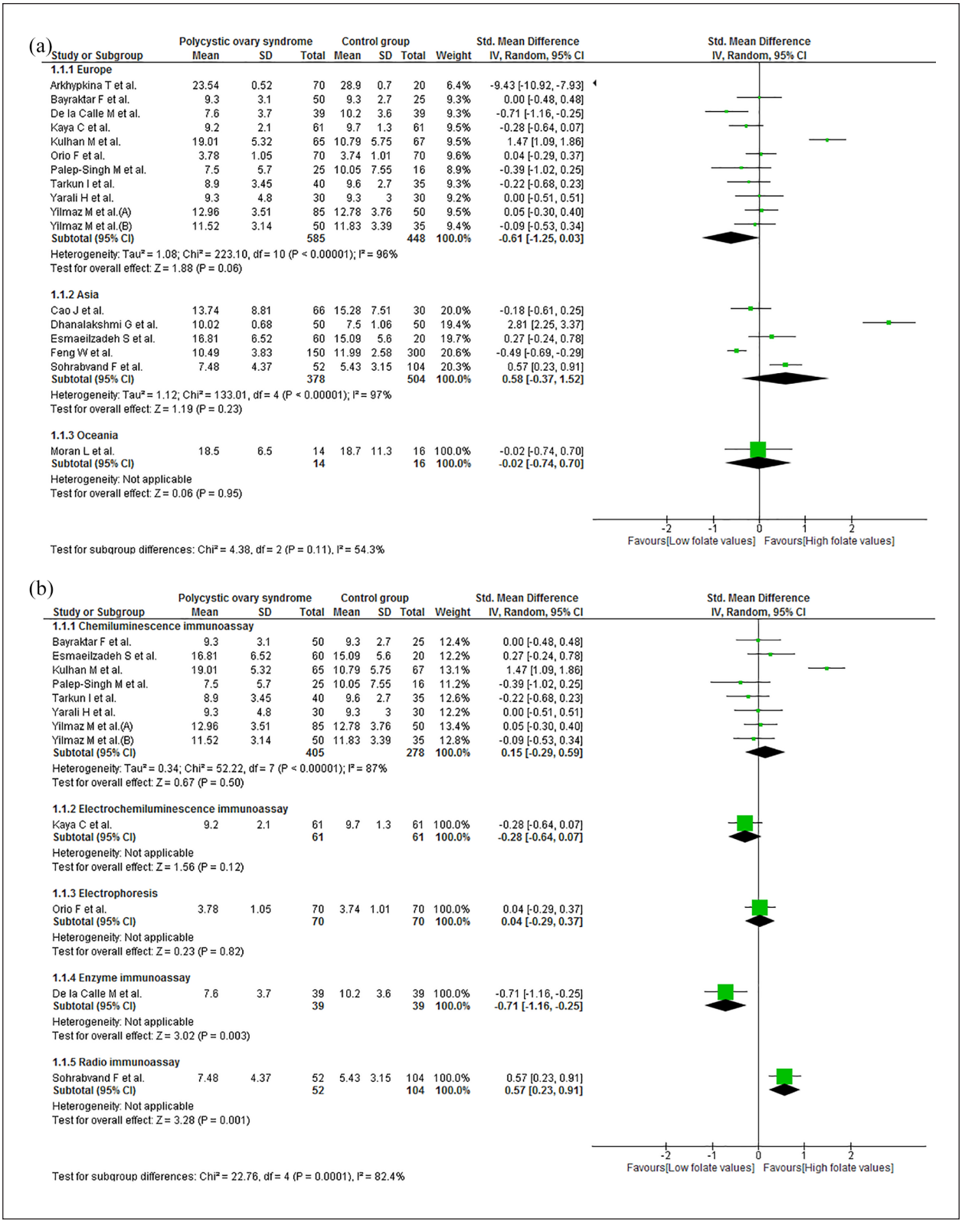


Figure 4. Subgroup analyses comparing vitamin B12 in women with and without PCOS: (a) by world regions, (b) by assay methods, (c) by study designs, (d) by IR in women with PCOS (PCOS-IR versus PCOS-non IR), and (e) by IR in women with PCOS versus those without the syndrome.
PCOS: polycystic ovary syndrome; IR: insulin resistance.

Metformin is widely used as an insulin sensitizer in women with PCOS. Nonetheless, a systematic review revealed that it increased the homocysteine levels and decreased the folic acid levels of nonpregnant women with PCOS.[126] Regarding vitamin B12, another systematic review revealed lower serum levels of this vitamin in patients with PCOS receiving metformin than in the newly diagnosed ones.[127] Similarly, another systematic review showed that folic acid supplementation improved the BMI of women with PCOS and those with high homocysteine levels.[128] Accordingly, the supplementation of folic acid and B vitamins would be important for these patients.[126] A growing evidence has been found regarding the benefits of such a supplementation on the reproductive health outcomes of women with PCOS.[13]

Limitations and strengths

Our findings should be interpreted considering some limitations. First, there is evidence of substantial statistical heterogeneity attributable to the clinical and methodological variations among the analyzed studies. This would be important for future studies to analyze more clinical and analytical covariates to conduct subanalysis by BMI, physical activity, glycosylated hemoglobin levels, dietary regimen, type of population evaluated, center settings, and



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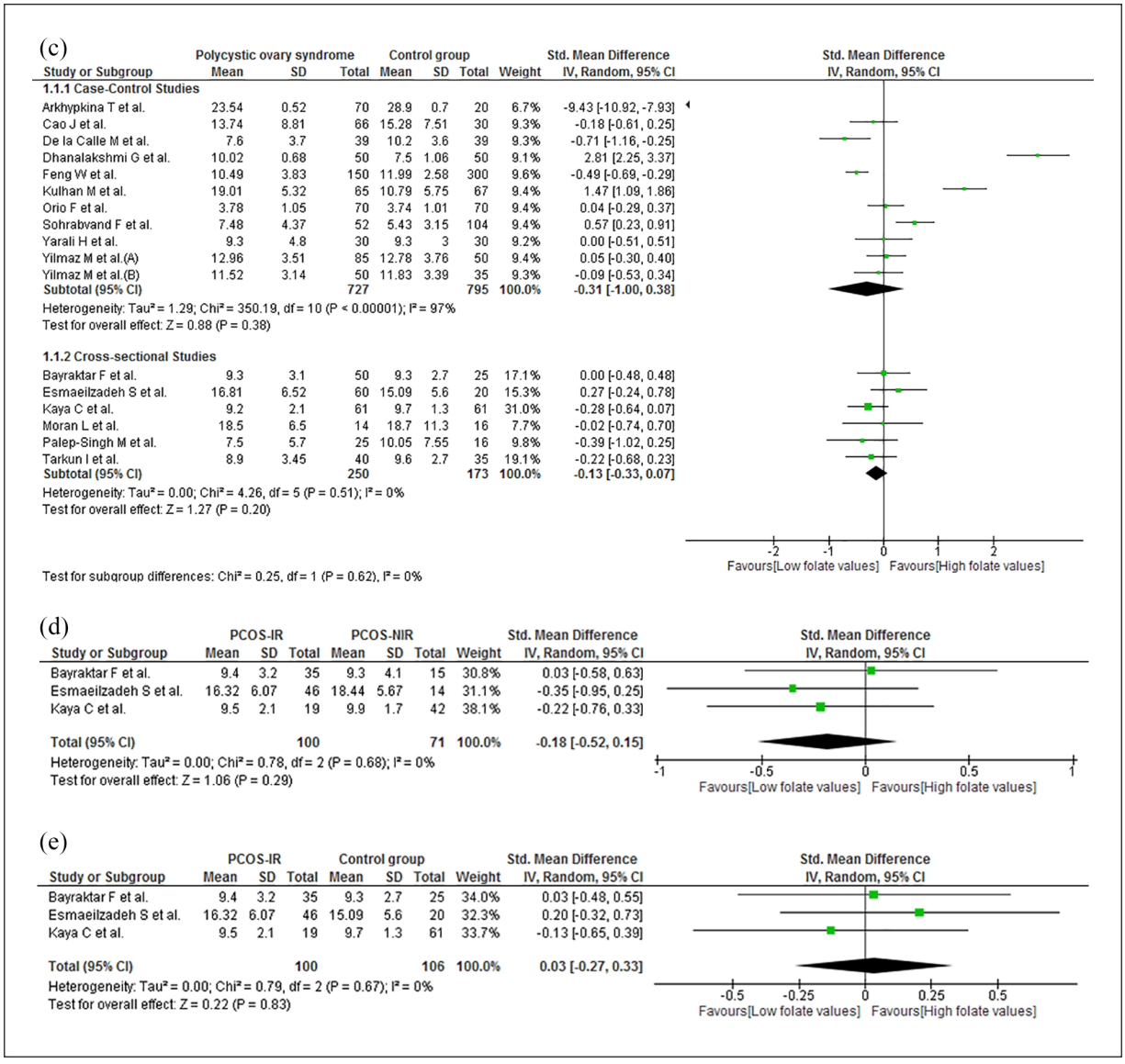

Figure 5. Subgroup analyses comparing folate in women with and without PCOS: (a) by world regions, (b) by assay methods, (c) by study designs (d) by IR in women with PCOS (PCOS-IR versus PCOS non-IR), and (e) by IR in women with PCOS versus women without PCOS.
PCOS: polycystic ovary syndrome; IR: insulin resistance.

others so as to explore the cause of heterogeneity. However, there was no significant reduction in heterogeneity in the sensitivity analysis despite the inclusion of only studies with low risk of bias. Second, most of the effect measures reported in the included studies are subjected to potential confounding. However, in the majority of the reported studies, the effect consistently exhibited the same trend. Third, most of the included studies were from Asia, which raises the need to conduct studies in other populations to evaluate potential variations caused by country-specific factors. Fourth, there is a lack of established threshold values for determining the sensitivity and specificity of homocysteine, vitamin B12, and folate deficiency in women with PCOS. Therefore, future research should aim to address this gap in different contexts and populations. Fifth, the inclusion of a substantial number of cross-sectional studies in our analysis limits the establishment of causality and restricts our findings to associations rather than causal relationships. Nevertheless, our study also has several strengths. Sensitivity analysis and subgroup analyses were conducted according to diagnostic criteria and world regions. In addition, to the best of our knowledge, this systematic review represents the first attempt to assess the serum levels of vitamin B12 and folate in women with and without PCOS.

Conclusions

Patients with PCOS exhibited significantly higher homocysteine levels than those without PCOS. No significant differences were observed in vitamin B12 and folate levels between women with and without PCOS.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Author contribution(s)

Juan R Ulloque-Badaracco: Conceptualization; Methodology; Formal analysis; Writing – original draft; Investigation; Data curation.
Ali Al-kassab-Córdova: Investigation; Writing – original draft; Data curation; Formal analysis.
Enrique A Hernández-Bustamante: Data curation; Investigation; Writing – original draft.
Esteban A Alarcón-Braga: Investigation; Writing – original draft; Data curation.
Juan C Cabrera-Guzmán: Data curation; Investigation; Writing – original draft.
Andres A Horruitiner-Mendoza: Investigation; Data curation; Writing – original draft.
Pamela Robles-Valcárcel: Investigation; Supervision; Writing – original draft.
Vicente A Benites-Zapata: Investigation; Writing – review & editing; Supervision; Formal analysis; Writing – original draft; Conceptualization; Methodology.
Faustino R Pérez-López: Writing – review & editing; Writing –original draft; Investigation; Formal analysis.

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Competing interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Availability of data and materials

All data generated or analyzed during the current study are included in this published article and its Supplemental Material.

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Supplemental material

Supplemental material for this article is available online.

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