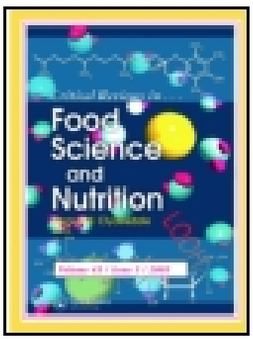

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REVIEW

Beneficial effects of folic acid supplementation on lipid markers in adults: A GRADE-assessed systematic review and dose-response meta-analysis of data from 21,787 participants in 34 randomized controlled trials

Omid Asbaghi[a], Damoon Ashtary-Larky[b], Reza Bagheri[c], Behzad Nazarian[a], Hadi Pourmirzaei Olyaei[d], Mahnaz Rezaei Kelishadi[e], Michael Nordvall[f], Alexei Wong[f], Frédéric Dutheil[g], and Amirmansour Alavi Naeini[e]

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ABSTRACT

Folic acid supplementation has received considerable attention in the literature, yet there is a large discrepancy in its effects on lipid markers in adults. Therefore, this systematic review and meta-analysis of 38 randomized controlled trials (RCTs) evaluated the effects of folic acid supplementation on triglyceride (TG), total cholesterol (TC), low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol concentrations in a cohort of 21,787 participants. A systematic search current as of March 2021 was performed in PubMed/Medline, Scopus, Web of Science, and Embase using relevant keywords to identify eligible studies. A fix or random-effects model was used to estimate the weighted mean difference (WMD) and 95% confidence intervals (CIs). Thirty-four RCTs were included in this meta-analysis. The pooled analysis revealed that serum TG (WMD: −9.78 mg/dL; 95% CI: −15.5 to −4.00; $p = 0.001$, I^2=0.0%, $p = 0.965$) and TC (WMD: −3.96 mg/dL; 95% CI: −6.71 to −1.21; $p = 0.005$, I^2=46.9%, $p = 0.001$) concentrations were significantly reduced following folic acid supplementation compared to placebo. However, folic acid supplementation did not affect serum concentrations of LDL (WMD: −0.97 mg/dL; 95% CI: −6.82 to 4.89; $p = 0.746$, I^2=60.6%, $p < 0.001$) or HDL cholesterol (WMD: 0.44 mg/dL; 95% CI: −0.53 to 1.41; $p = 0.378$, I^2= 0.0%, $p = 0.831$). A significant dose-response relationship was observed between the dose of folic acid supplementation and serum concentrations of HDL cholesterols ($r = 2.22$, $p = 0.047$). Folic acid supplementation reduced serum concentrations of TG and TC without affecting LDL or HDL cholesterols. Future large RCTs on various populations are needed to show further beneficial effects of folic acid supplementation on lipid profile.

KEYWORDS

Dyslipidemia; folate; folic acid; lipid profile; meta-analysis

Introduction

Metabolic imbalances such as dyslipidemia, which is described as increased concentrations of total cholesterol (TC), low-density lipoprotein (LDL), triglycerides (TG), and/or reduced high-density lipoprotein (HDL), are definitively involved in cardiovascular disease (CVD) progression (Smedts et al. 2012). Dyslipidemia is a multifactorial disorder of lipoprotein metabolism that results from interactions between genetic and environmental factors that promote atherosclerosis and the progression of cardiovascular disorders in conditions such as type 2 diabetes mellitus (T2DM) (Banach et al. 2014; Bibbins-Domingo et al. 2016; Rizzo et al. 2013). Lipid profile dysregulation is considered one of the leading public health concerns worldwide as associated rates of CVD have rapidly escalated in developed countries. Alarmingly, it is predicted that the CVD mortality rate will approach if not exceed 22.2 million individuals worldwide by 2030 (World Health Organization 2014). All considered, dyslipidemia has been directly linked to arterial damage and atherosclerosis (Mierzecki et al. 2013; Tarchalski, Guzik, and Wysocki 2003), diminished quality of life (QOL) due to poor lipid control, increased morbidity and mortality, and higher healthcare-associated financial burdens. Therefore, it is essential to elucidate on methods to reduce CVD risk factors and improve QOL through regulation of lipid profile (Pourmasoumi et al. 2018). One such method of approach has focused on the effects of select vitamins on lipid profile (Daviddi et al. 2019; Wang et al. 2016), particularly the role folate and/or folic acid have on favorably manipulating cholesterol, triglycerides, and other lipid markers. Previously, the beneficial effects of some vitamins and minerals on metabolic factors have been demonstrated (Asbaghi, Fatemeh et al. 2020; Asbaghi, Moradi et al. 2021;

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Asbaghi, Naeini et al. 2021; Asbaghi, Sadeghian, Fouladvand et al. 2020; Asbaghi, Sadeghian, Nazarian et al. 2020).

Folate belongs to the vitamin B family and is naturally present in various foods, including vegetables, fruits, nuts, beans, and eggs (Winkels et al. 2007). Folic acid is the oxidized form of folate and is scarcely found in nature but rather is added to enriched or fortified foods. A wide range of bioavailability for folate and folic acid has been reported (McNulty and Pentieva 2004) with an estimated 50% of naturally occurring folate and 85% of folic acid in fortified food being digested, absorbed, and metabolized (Ebara 2017). Further, folic acid is a water-soluble cofactor of numerous enzymes, which may be produced by plants and bacteria but not in humans and other animals, and is a known essential nutrient. Folate deficiency accompanies many diseases and disorders such as fetal neural tube defects, cognitive dysfunction, cancer, and CVD (Donnelly 2001; Lee et al. 2011; Moens et al. 2008; Scaglione and Panzavolta 2014), and thus folic acid food fortification and/or supplementation each have received attention to address these and other neurological and psychiatric health concerns (Bazzano 2009). The effectiveness of folic acid supplementation in lowering the stroke-related risk of hypercholesterolemia (Qin et al. 2016) as well as plasma homocysteine concentrations has come under scrutiny as a viable approach to lower CVD incidence. Additional and complementary research has indicated that folic acid supplementation may also diminish CVD risk factors by reducing LDL-C concentrations (Owoyele et al. 2006) and, as a form of primary prevention in low-dose folic acid supplementation, has been suggested as a means to improve lipid markers (Mierzecki et al. 2013) such as LDL-C concentrations and LDL-C/HDL-C and TC/HDL-C ratios (Vijayakumar et al. 2017).

While numerous investigations have shown favorable effects of folic acid supplementation on lipid profile in various disease states, its overall effect in the adult population varies from study to study (Bahmani, et al. 2018; Liem et al. 2003; Mierzecki et al. 2015; Qin et al. 2016). Moreover, some previous studies failed to show any beneficial effects of folic acid supplementation on lipid profile (Aarsand and Carlsen 1998; Asemi et al. 2016; Doshi et al. 2001). Therefore, we have conducted this systematic review and meta-analysis to formulate a clear role of folic acid supplementation on lipid markers in adults.

Materials and methods

This study was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) protocol for conducting and disseminating systematic reviews and meta-analyses (Moher et al. 2009).

Search strategy

To find interrelated studies on folic acid supplementation in adults, we performed a comprehensive literature search in online databases including PubMed/Medline, Scopus, Web of Science, and Embase for the time period up to March 2021. The following MeSH and non-MeSH terms were used in our search strategy: (("folate"[Title/Abstract] OR "folic acid"[Title/Abstract] OR "Vitamin M"[Title/Abstract] OR "Vitamin B9"[Title/Abstract] OR "Folacin"[Title/Abstract] OR "Folvite"[Title/Abstract] OR "Pteroylglutamic Acid"[Title/Abstract] OR "folates"[Title/Abstract] OR "tetrahydrofolates"[Title/Abstract] OR "Formyltetrahydrofolates"[Title/Abstract]) AND (Triglyceride[Title/Abstract] OR Triacylglycerol[Title/Abstract] OR cholesterol[Title/Abstract] OR Lipoprotein[Title/Abstract] OR "very low density lipoprotein"[Title/Abstract] OR VLDL[Title/Abstract] OR "low density lipoprotein"[Title/Abstract] OR LDL[Title/Abstract] OR LDL-C[Title/Abstract] OR "high density lipoprotein"[Title/Abstract] OR HDL[Title/Abstract] OR HDL-C[Title/Abstract] OR "lipid profile"[Title/Abstract])) AND (Intervention[Title/Abstract] OR "Intervention Study"[Title/Abstract] OR "Intervention Studies"[Title/Abstract] OR "controlled trial"[Title/Abstract] OR randomized[Title/Abstract] OR randomized[Title/Abstract] OR random[Title/Abstract] OR randomly[Title/Abstract] OR placebo[Title/Abstract] OR "clinical trial"[Title/Abstract] OR Trial[Title/Abstract] OR "randomized controlled trial"[Title/Abstract] OR "randomized clinical trial"[Title/Abstract] OR RCT[Title/Abstract] OR blinded[Title/Abstract] OR "double blind"[Title/Abstract] OR "double blinded"[Title/Abstract] OR trial[Title/Abstract] OR "clinical trial"[Title/Abstract] OR trials[Title/Abstract] OR "Pragmatic Clinical Trial"[Title/Abstract] OR "Cross-Over Studies"[Title/Abstract] OR "Cross-Over"[Title/Abstract] OR "Cross-Over Study"[Title/Abstract] OR parallel[Title/Abstract] OR "parallel study"[Title/Abstract] OR "parallel trial"[Title/Abstract]).

Search parameters were not restricted to publication date or original printed language. References from all relevant peer-reviewed investigations were consulted and cross-referenced against database searches to avoid omitting publications. All citations were subsequently included in the Endnote screening software and consequently, duplicate citations were removed from consideration in this study.

Inclusion criteria

In the present study, consideration was given to studies meeting all of the following criteria: (1) RCTs, (2) studies on the adult population regardless of sex ($\geq$18 years), (3) use of a folic acid supplementation intervention/regimen, (4) a folic acid supplementation intervention utilizing a pre and post-study design, (5) a placebo or control group study design with serum lipid markers as an outcome for both groups, and (6) in the event of multiple cohort data publications from a single larger dataset, the more comprehensive article, whenever possible, was utilized in the present study. Studies containing more than one intervention group meeting the above criteria were considered independent datasets to determine overall effect size.

Exclusion criteria

Characteristics of the literature not meeting the inclusion criteria for this study as determined through the acquisition of full-text studies included: (1) cross-sectional and case-control design studies, (2) non RCT review studies, (3) ecological studies, (4) control group manipulation of any sort, and (5) studies lacking a placebo or control group, of non-randomized research design, and/or performed on participants not meeting the minimum age criteria (<18 years).

Data extraction

Two independent investigators (OA and BN) completed data extraction from each qualified study not excluded and meeting the inclusion criteria. Extracted data contained the name of the primary investigator, year of publication, study location of origin, study design, participant group size (placebo/control and intervention), participant demographics [(mean ± SD age and body mass index (BMI), and sex)], folic acid supplementation dose, duration of intervention, mean ± SD lipid marker alterations for both intervention and control groups, and any confounding variables utilized or accounted for in the RCT. Dataset values were converted to the most common units of expression, whenever possible, for purposes of data analysis.

Quality assessment

Study quality was measured by two independent reviewers (OA and BN) using the Cochrane Collaboration modified risk of bias tool, which determines study bias in seven domains, including random sequence generation, allocation concealment, reporting bias, performance bias, detection bias, attrition bias, and other potential sources of bias (Higgins et al. 2011). Consequently, terms including "Low", "High", or "Unclear" were used to classify each domain of study bias. Dissimilarities between independent reviewers on the level of study bias in each domain were evaluated and resolved by the corresponding author.

Statistical analysis

Weighted mean differences (WMD) and SDs of lipid profile (TC, TG, LDL, and HDL cholesterol concentrations) from studies both intervention and control groups were extracted and used to generate overall effect sizes as determined by the random-effects model approach of DerSimonian and Laird (DerSimonian and Laird 1986). Additionally, when mean changes were not reported following folic acid supplementation (e.g., only mean lipid value at baseline and again at post-intervention were noted in the study), the following formula was used to derive such changes: mean change = final post-intervention lipid marker value − baseline value for the same; and subsequently, changes in SDs were calculated by the following formula (Borenstein et al. 2011):

$$\text{SD change} = \sqrt{[(\text{SD baseline})\hat{2} + (\text{SD final})\hat{2} - (2R \times \text{SD baseline} \times \text{SD}).}$$

Outcome variables (TG, TC, LDL, and HDL cholesterol concentrations) reported in mmol/L were converted to mg/dL through readily available and generic conversion tables. Moreover, reported standard errors (SEs), 95% CIs, and interquartile ranges (IQRs) were converted to SDs using the method of Hozo et al. (Hozo, Djulbegovic, and Hozo 2005). Subsequently, a random-effects model, which incorporates between-study variations, was utilized to determine overall lipid marker effect size. Heterogeneity between studies was performed using Cochran's Q test and analyzed by an I-square (I^2) statistic (Higgins et al. 2003) where I^2 >40% or $p < 0.01$ was considered as having high between-study heterogeneity (Higgins and Thompson 2002). To detect other potential sources of heterogeneity, subgroup analyses/comparisons were performed according to pre-established criteria and included study duration (<12 and ≥12 weeks), folic acid supplementation dosage (<5 and ≥5 mg/d), baseline serum concentrations of TG (<150 and ≥150 mg/dL), TC (<200 and ≥200 mg/dL), LDL (<100 and ≥100 mg/dL), and HDL cholesterols (<50 and ≥50 mg/dL), health status (healthy and unhealthy/diseased state), reported sex (both, male and female) and study quality (high/moderate). Meta-regression was used to differentiate confounding variables and linear relations among the effect and sample sizes, duration of intervention, and intervention dosage (Mitchell 2012). Sensitivity analysis was undertaken to determine the individual study effect on the overall estimation of effect (Tobias 1999). The possibility of publication bias was further verified through Begg's test and funnel plots (Egger et al. 1997). Statistical analysis was carried out using STATA, version 11.2 (Stata Corp, College Station, TX). For all analyses, p-values <0.05 were considered statistically significant.

Certainty assessment

The overall certainty of evidence across studies was evaluated according to the guidelines of the GRADE (Grading of Recommendations Assessment, Development, and Evaluation) working group (gradeworkinggroup.org). According to the corresponding evaluation criteria, the quality of evidence was subsequently classified into four categories, including high, moderate, low, and very low (Guyatt et al. 2008).

Results

Study selection

The initial databases search yielded 2793 studies, 839 of which were removed due to duplication. Another 1904 studies were excluded for the following reasoning: unrelated title and abstract not warranting full-text review (n = 1566), animal studies (n = 170), and review studies (n = 168). Consequently, 50 relevant studies remained for full-text review and meta-analysis consideration. Among these, 16 studies were excluded because of a lack of necessary data reporting or other required information as outlined above in the inclusion/exclusion criteria. Finally, 34 studies achieving all necessary criteria were included for meta-analysis in the present study (Figure 1).

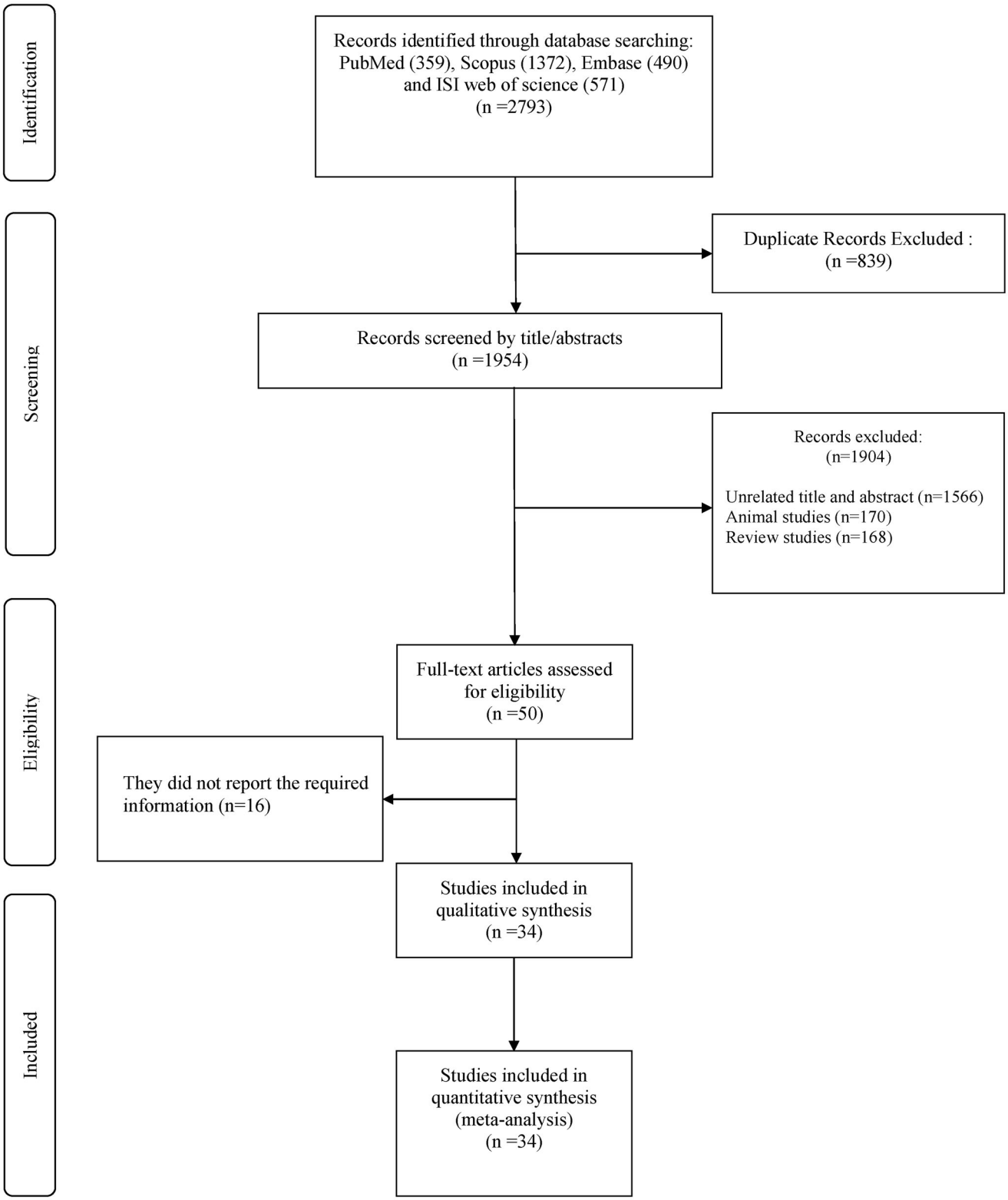


Figure 1. Flowchart of study selection for inclusion studies.

Study characteristics

Study characteristics of the 34 studies (Aarsand and Carlsen 1998; Aghamohammadi Khiavi, Pourghassem, and Aliasgharzadeh 2011; Araki et al. 2006; Asemi, Karamali, and Esmaillzadeh 2014; Asemi et al. 2016; Bahmani, et al. 2018; Cagnacci, Cannoletta, and Volpe 2009; Doshi et al. 2001; Doshi et al. 2002; Gargari, Aghamohammadi, and Aliasgharzadeh 2011; Grigoletti et al. 2013; Hashemi et al. 2016; Kilicdag et al. 2005; Lim, Choi, and Choue 2008; Mangoni et al. 2003; Mangoni et al. 2005; McGregor, Shand, and Lynn 2000; Melenovsky et al. 2003; Moat et al. 2006; Moens et al. 2007; Palomba et al. 2010; Qin et al. 2016; Sheu et al. 2005; Shidfar et al. 2009; Solini, Santini, and Ferrannini 2006; Talari et al. 2016; Title et al. 2000; Title

et al. 2006; Toprak et al. 2005; Verhaar et al. 1999; Villa et al. 2005; Weijun et al. 2008; Wilmink et al. 2000; Woo et al. 1999) included 38 intervention arms and are shown in Table 1. In total, 21,787 participants were included (case = 10,889 and control = 10,898) in these publications dated from between 1998 and 2018. Twenty-seven studies were designed as parallel studies with the remaining seven as crossover design. Study durations ranged from between two and 234 weeks with sample sizes ranging from eight to 11,862 participants. Participants' demographic characteristics included age ranging from 21 to 65 years and baseline BMI varying between 21.4 and 30.7 $kg.m^{-2}$. While the majority of investigations (21) enrolled both sexes, ten and three studies exclusively utilized female and male participants, respectively. Quality assessment characteristics of studies are provided in Table 2.

Meta-analysis

The effects of folic acid supplementation on TG

Outcomes analysis of the 29 studies that measured TG concentrations following folic acid supplementation (Aarsand and Carlsen 1998; Aghamohammadi Khiavi, Pourghassem, and Aliasgharzadeh 2011; Asemi, Karamali, and Esmaillzadeh 2014; Asemi et al. 2016; Bahmani, et al. 2018; Cagnacci, Cannoletta, and Volpe 2009; Doshi et al. 2001; Doshi et al. 2002; Gargari, Aghamohammadi, and Aliasgharzadeh 2011; Grigoletti et al. 2013; Hashemi et al. 2016; Kilicdag et al. 2005; Lim, Choi, and Choue 2008; Mangoni et al. 2003; McGregor, Shand, and Lynn 2000; Melenovsky et al. 2003; Moat et al. 2006; Moens et al. 2007; Palomba et al. 2010; Sheu et al. 2005; Shidfar et al. 2009; Solini, Santini, and Ferrannini 2006; Talari et al. 2016; Title et al. 2000; Title et al. 2006; Verhaar et al. 1999; Villa et al. 2005; Weijun et al. 2008; Wilmink et al. 2000) containing 32 arms in total (751 cases and 755 controls) indicated an overall effect of a significant reduction in TG concentrations [(WMD: −9.78 mg/dL; 95% CI: −15.5 to −4.00; $p = 0.001$, $I^2 = 0.0\%$, $p = 0.965$);(Figure 2A)]. In addition, subgroup analyses demonstrated that folic acid supplementation reduced TG concentrations in female and unhealthy participants to within normal ranges (<150 mg/dL). In addition, high quality studies, long-term intervention (≥12 weeks) and low dose supplementation (<5 g/d) significantly reduced TG concentrations (Table 3).

The effects of folic acid supplementation on TC

Based on the results of 31 studies (Aarsand and Carlsen 1998; Aghamohammadi Khiavi, Pourghassem, and Aliasgharzadeh 2011; Araki et al. 2006; Asemi, Karamali, and Esmaillzadeh 2014; Asemi et al. 2016; Bahmani, et al. 2018; Cagnacci, Cannoletta, and Volpe 2009; Doshi et al. 2001; Doshi et al. 2002; Gargari, Aghamohammadi, and Aliasgharzadeh 2011; Grigoletti et al. 2013; Kilicdag et al. 2005; Lim, Choi, and Choue 2008; Mangoni et al. 2003; Mangoni et al. 2005; McGregor, Shand, and Lynn 2000; Melenovsky et al. 2003; Moat et al. 2006; Palomba et al. 2010; Qin et al. 2016; Sheu et al. 2005; Shidfar et al. 2009; Solini, Santini, and Ferrannini 2006; Talari et al. 2016; Title et al. 2000; Title et al. 2006; Verhaar et al. 1999; Villa et al. 2005; Weijun et al. 2008; Wilmink et al. 2000; Woo et al. 1999) containing 35 total effects (10,806 cases and 10,816 controls), there was a significant decrease in TC concentrations following folic acid supplementation [(WMD: −3.96 mg/dL; 95% CI: −6.71 to −1.21; $p = 0.005$, $I^2 = 46.9\%$, $p = 0.001$); (Figure 2B)] regardless of study duration. Subgroup analyses showed that baseline TC concentrations, intervention dose, and sex are the sources of heterogeneity. In addition, meta-analysis results revealed that TC concentrations were significantly decreased following high-quality studies as well as high dose interventions (≥5 mg/d) in unhealthy and female participants (Table 3).

The effects of folic acid supplementation on LDL cholesterol

Overall result from 24 studies (Aghamohammadi Khiavi, Pourghassem, and Aliasgharzadeh 2011; Araki et al. 2006; Asemi, Karamali, and Esmaillzadeh 2014; Asemi et al. 2016; Bahmani, et al. 2018; Doshi et al. 2001; Doshi et al. 2002; Gargari, Aghamohammadi, and Aliasgharzadeh 2011; Hashemi et al. 2016; Kilicdag et al. 2005; Lim, Choi, and Choue 2008; Mangoni et al. 2005; McGregor, Shand, and Lynn 2000; Moat et al. 2006; Moens et al. 2007; Palomba et al. 2010; Sheu et al. 2005; Shidfar et al. 2009; Talari et al. 2016; Title et al. 2000; Title et al. 2006; Toprak et al. 2005; Villa et al. 2005; Weijun et al. 2008) containing 27 total effect sizes (648 cases and 650 controls) did not reveal significant alterations in LDL cholesterol concentrations [(WMD: −0.97 mg/dL; 95% CI: −6.82 to 4.89; $p = 0.746$, $I^2 = 60.6\%$, $p < 0.001$); (Figure 2C)]. Subgroup analyses demonstrated that baseline serum LDL cholesterol concentrations, study duration, intervention dose, health status, and sex are the sources of heterogeneity. Following low dose folic acid supplementation (<5 mg/d), LDL cholesterol concentrations were significantly elevated, although when given in higher doses (≥5 mg/d), such significant elevation was eliminated (Table 3).

The effects of folic acid supplementation on HDL cholesterol

Pooled effect sizes from 31 studies (Aarsand and Carlsen 1998; Aghamohammadi Khiavi, Pourghassem, and Aliasgharzadeh 2011; Araki et al. 2006; Asemi, Karamali, and Esmaillzadeh 2014; Asemi et al. 2016; Bahmani, et al. 2018; Cagnacci, Cannoletta, and Volpe 2009; Doshi et al. 2001; Doshi et al. 2002; Gargari, Aghamohammadi, and Aliasgharzadeh 2011; Grigoletti et al. 2013; Hashemi et al. 2016; Kilicdag et al. 2005; Lim, Choi, and Choue 2008; Mangoni et al. 2005; McGregor, Shand, and Lynn 2000; Melenovsky et al. 2003; Moat et al. 2006; Moens et al. 2007; Palomba et al. 2010; Sheu et al. 2005; Shidfar et al. 2009; Solini, Santini, and Ferrannini 2006; Talari et al. 2016; Title

Table 1. Characteristic of included studies in meta-analysis.

Studies	Country	Study Design	Participants	Sample Size and Sex	Sample size IG	Sample size CG	Trial Duration (Weeks)	Age (Mean ± SD) IG	Age (Mean ± SD) CG	BMI (Mean ± SD) IG	BMI (Mean ± SD) CG	Intervention Folic acid dose (mg/d)	Intervention Control group
Aarsand and Carlsen 1998	Norway	RA/DB/PC	Type 2 Diabetes	28: 21 M, 7 F	14	14	12	56.7 ± 10.47	61.6 ± 9.35	29.2 ± 5.23	28.3 ± 4.11	0.25	Placebo
Verhaar et al. 1999	Netherland	RA/DB/PC	Hypercholesterolemia	20: 14 M, 6 F	20	20	4	35 ± 13.41	35 ± 13.41	23.4 ± 3.13	23.4 ± 3.13	5	Placebo
Woo et al. 1999	China	RA/DB/PC	Hyperhomocystinemia	17: 15 M, 2 F	17	17	8	54 ± 10	54 ± 10	NR	NR	10	Placebo
Title et al. 2000	Canada	RA/DB/PC	Coronary Artery Disease	50: 40 M, 10 F	25	25	17	57.2 ± 9.8	60.6 ± 8.6	NR	NR	5	Placebo
McGregor, Shand, and Lynn 2000 (A)	New Zealand	RA/DB/PC	Continuous ambulatory peritoneal dialysis	8	8	8	12	54.4 ± 9.5	54.4 ± 9.5	NR	NR	5	Placebo
McGregor, Shand, and Lynn 2000 (B)	New Zealand	RA/DB/PC	hemodialysis	13	13	13	12	53.8 ± 14.7	53.8 ± 14.7	NR	NR	5	Placebo
Wilmink et al. 2000	Netherlands	RA/DB/PC	Endothelial Dysfunction	40	20	20	2	23 ± 3.4	23 ± 3.4	21.9 ± 2.7	22.8 ± 2.6	10	Placebo
Doshi et al. 2001	United kingdom	RA/PC	Coronary Artery Disease	50: 44 M, 6 F	50	50	6	57 ± 8	57 ± 8	28.5 ± 4.4	28.5 ± 4.4	5	Placebo
Doshi et al. 2002	United kingdom	RA/PC	Coronary Artery Disease	33: 30 M 3 F	16	17	6	55 ± 7	56 ± 7	NR	NR	5	Placebo
Melenovsky et al. 2003	Prague	RA	hyperlipidemia	37: 22 M, 15 F	18	19	9	50.3 ± 10	50.1 ± 9.6	27.7 ± 2.9	29.6 ± 4.8	10	No intervention
Mangoni et al. 2003	United Kingdom	RA/PC	healthy smokers	24: 9 M, 15 F	12	12	4	39.7 ± 11.77	36 ± 12.47	25.7 ± 2.77	24.9 ± 3.11	5	Placebo
Kilicdag et al. 2005	Turkey	RA	polycystic ovarian syndrome patients	31: 31 F	17	14	12	24.94 ± 6.67	24.14 ± 6.92	28.58 ± 5.43	26.02 ± 5.98	0.348	No intervention
Villa et al. 2005	Italy	RA/PC	Postmenopausal	20: 20 F	10	10	8	55.4 ± 6.95	53.1 ± 3.79	29.7 ± 4.74	26.91 ± 5.88	7.5	Placebo
Mangoni et al. 2005	Australia	RA/DB/PC	Type 2 Diabetes	26: 14 M,12F	13	13	4	55.3 ± 4.32	57.6 ± 4.68	30.5 ± 3.96	32.3 ± 4.68	5	Placebo
Sheu et al. 2005	Taiwan	RA/DB/PC	obese women	74: 74 F	36	38	12	43 ± 12	40 ± 12.32	29.6 ± 3.6	29.3 ± 4.93	5	Placebo
Toprak et al. 2005	Turkey	RA/PC	postmenopausal	40: 40 F	20	20	12	51 ± 7	52 ± 5	29.1 ± 3.5	27.6 ± 3.5	5	Placebo
Moat et al. 2006 (A)	USA	RA/DB/PC	coronary artery disease	59: 52 M, 7 F	30	29	6	61 ± 7	61 ± 7	28.5 ± 4.4	29.6 ± 4.1	0.4	Placebo
Moat et al. 2006 (B)	USA	RA/DB/PC	coronary artery disease	54: 46 M, 8 F	25	29	6	60 ± 7	61 ± 7	29.9 ± 4.4	29.6 ± 4.1	5	Placebo
Title et al. 2006	Canada	RA/DB/PC	type 2 diabetes	19: 9 M,10F	19	19	2	54.5 ± 5.9	54.5 ± 5.9	NR	NR	10	Placebo
Solini, Santini, and Ferrannini 2006	Italy	RA/PC	overweight subjects	60: 19 M, 41 F	30	30	12	50 ± 7	49 ± 8	27.5 ± 0.6	27.4 ± 0.6	2.5	Placebo
Araki et al. 2006	Japan	RA/DB/PC	young Japanese male subjects.	17: 17 M	9	8	2	22.3 ± 3	21.9 ± 1.2	21.4 ± 4.2	21.4 ± 4.2	0.8	Placebo
Moens et al. 2007	Belgium	RA/DB/PC	Acute myocardial infarction	40: 35 M, 5 F	20	20	6	57 ± 11	56 ± 14	NR	NR	10	Placebo
Lim, Choi, and Choue 2008	South Korea	RA	hyperlipidemic patients	40: 23 M, 17 F	20	20	12	46.7 ± 6.5	43.4 ± 10.7	25.4 ± 1.9	24.8 ± 2.3	5	No intervention
Weijun et al. 2008	China	RA	Type 2 Diabetes	60: 27 M, 33 F	30	30	2	59.4 ± 10.4	57.9 ± 10.4	25.1 ± 3.2	25 ± 2.8	5	No intervention
Shidfar et al. 2009	Iran	RA/DB/PC	Hypercholesterolemic Adults	40: 16 M, 24 F	20	20	8	44 ± 7.06	45 ± 7.78	27.06 ± 2.64	26.05 ± 2.17	5	Placebo
Cagnacci, Cannoletta, and Volpe 2009	Italy	RA/DB/PC	Postmenopausal	30: 30 F	15	15	3	55.8 ± 4.26	54.5 ± 4.64	26.3 ± 5.03	27.5 ± 5.03	15	Placebo
Palomba et al. 2010	Italy	RA/DB/PC	polycystic ovary syndrome	47: 47 F	23	24	25	26.9 ± 3.1	26.4 ± 2.8	27.9 ± 2.6	28.1 ± 3.1	0.4	Placebo
Aghamohammadi Khiavi, Pourghassem, and Aliasgharzadeh 2011	Iran	RA/DB/PC	Type 2 Diabetes Mellitus	68: 68 M	34	34	8	58.7 ± 7.2	55.6 ± 9.3	27.4 ± 3.2	27.8 ± 4	5	Placebo
Gargari, Aghamohammadi, and Aliasgharzadeh 2011	Iran	RA/DB/PC	overweight and obese men with type 2 diabetes	48: 48 M	24	24	8	59.4 ± 7.6	57 ± 10.1	28.8 ± 2.7	28.5 ± 3.3	5	Placebo
Grigoletti et al. 2013	Brazil	RA/DB/PC	HIV-infected individuals	30: 14 M, 16 F	15	15	4	45 ± 7.74	45 ± 7.74	23.9 ± 4.96	23.9 ± 3.11	5	Placebo
Asemi, Karamali, and Esmaillzadeh 2014 (A)	Iran	RA/DB/PC	overweight women with polycystic ovary syndrome	54: 54 F	27	27	8	24.3 ± 5.0	24.7 ± 5.0	27.2 ± 5.0	27.9 ± 4.7	1	Placebo
Asemi, Karamali, and Esmaillzadeh 2014 (B)	Iran	RA/DB/PC	overweight women with polycystic ovary syndrome	54: 54 F	27	27	8	25.1 ± 4.5	24.7 ± 5.0	29.3 ± 4.6	27.9 ± 4.7	5	Placebo
Talari et al. 2016	Iran	RA/DB/PC	Metabolic Syndrome	60: 26 M, 34 F	30	30	12	62.1 ± 9.6	65.4 ± 11.5	29.8 ± 3.8	29.8 ± 4.4	5	Placebo
Asemi et al. 2016	Iran	RA/DB/PC	Cervical intraepithelial neoplasia grade 1	58: 58 F	29	29	25	36.8 ± 8.8	39.1 ± 9.1	28.2 ± 3.5	29.8 ± 6.4	5	Placebo
Hashemi et al. 2016	Iran	RA/TB/PC	Pre-eclamptic patients	85: 85 F	43	42	8	30.82 ± 4.08	31.2 ± 4.3	25.19 ± 2.53	24.63 ± 2.64	5	Placebo
Qin et al. 2016 (A)	China	RA/DB	Hypertensive Patients with Total Cholesterol < 200 mg/dL	8304: 3879 M, 4425 F	4117	4187	234	60.2 ± 7.7	60.1 ± 7.7	24.3 ± 3.7	24.3 ± 3.7	0.8	No intervention
Qin et al. 2016 (B)	China	RA/DB	Hypertensive Patients with Total Cholesterol ≥ 200 mg/dL	11862: 4360 M, 7502 F	5963	5899	234	59.9 ± 7.4	59.9 ± 7.4	25.4 ± 3.6	25.3 ± 3.6	0.8	No intervention
Bahmani, et al. 2018	Iran	RA/DB/PC	Endometrial Hyperplasia	60: 60 F	30	30	12	44.4 ± 6.5	44.7 ± 3.1	30.7 ± 4.6	30.5 ± 3.8	5	Placebo

Abbreviations. IG, intervention group; CG, control group; DB, double-blinded; SB, single-blinded; PC, placebo-controlled; CO, controlled; RA, randomized; NR, not reported; F, Female; M, Male; NR, not reported.

Table 2. Quality assessment.

Studies	Random sequence generation	Allocation concealment	Selective reporting	Other sources of bias	Blinding (participants and personnel)	Blinding (outcome assessment)	Incomplete outcome data	General quality
Aarsand and Carlsen 1998	L	H	H	H	L	H	H	Moderate
Verhaar et al. 1999	L	H	H	H	L	H	L	High
Woo et al. 1999	L	H	H	H	L	H	L	High
Title et al. 2000	L	H	L	H	L	H	L	High
McGregor, Shand, and Lynn 2000	L	H	L	H	L	H	L	High
Wilmink et al. 2000	L	H	H	H	L	H	L	High
Doshi et al. 2001	L	H	L	H	H	H	L	High
Doshi et al. 2002	L	H	L	H	H	H	L	High
Melenovsky et al. 2003	L	H	H	H	H	H	L	Moderate
Mangoni et al. 2003	L	H	H	H	H	H	L	Moderate
Kilicdag et al. 2005	L	H	L	H	H	H	L	High
Villa et al. 2005	L	H	L	H	H	H	L	High
Mangoni et al. 2005	L	H	L	H	L	H	H	High
Sheu et al. 2005	L	H	L	H	L	H	L	High
Toprak et al. 2005	L	H	H	H	H	H	L	Moderate
Moat et al. 2006	L	H	L	H	L	H	L	High
Title et al. 2006	L	H	L	H	L	H	L	High
Solini, Santini, and Ferrannini 2006	L	H	H	H	H	H	L	Moderate
Araki et al. 2006	L	H	L	H	L	H	L	High
Moens et al. 2007	L	H	H	H	L	H	L	High
Lim, Choi, and Choue 2008	L	H	L	H	H	H	L	High
Weijun et al. 2008	L	H	H	H	H	H	L	Moderate
Shidfar et al. 2009	L	H	L	H	L	H	L	High
Cagnacci, Cannoletta, and Volpe 2009	L	H	H	H	L	H	L	High
Palomba et al. 2010	L	H	L	H	L	H	L	High
Aghamohammadi Khiavi, Pourghassem, and Aliasgharzadeh 2011	L	H	L	H	L	H	L	High
Gargari, Aghamohammadi, and Aliasgharzadeh 2011	L	H	L	H	L	H	L	High
Grigoletti et al. 2013	L	H	H	H	L	H	L	High
Asemi, Karamali, and Esmaillzadeh 2014	L	U	L	L	L	L	L	High
Talari et al. 2016	L	H	L	H	L	H	L	High
Asemi et al. 2016	L	H	L	H	L	H	L	High
Hashemi et al. 2016	L	L	H	H	L	L	L	High
Qin et al. 2016	L	H	H	H	L	H	L	High
Bahmani, et al. 2018	L	H	L	H	L	H	L	High

Abbreviations. L, low; H, high; U, unclear.

et al. 2000; Title et al. 2006; Toprak et al. 2005; Verhaar et al. 1999; Villa et al. 2005; Weijun et al. 2008; Wilmink et al. 2000) containing 34 arms (780 cases and 783 controls) did not reveal a significant change in HDL cholesterol concentrations (WMD: 0.44 mg/dL; 95% CI: −0.53 to 1.41; $p = 0.378$, $I^2 = 0.0\%$, $p = 0.831$) following folic acid supplementation. Subgroup analyses demonstrated similar results (Figure 2D and Table 3).

Publication bias

According to Begg's regression test, there was no evidence of publication bias for studies examining the effect of folic acid supplementation on TG ($p = 685$), TC ($p = 1.000$), and LDL ($p = 0.123$). However, there was significant publication bias for HDL cholesterol ($p = 0.041$), which was visually confirmed after funnel plot analysis (Figure 3A–D).

Meta-regression analysis

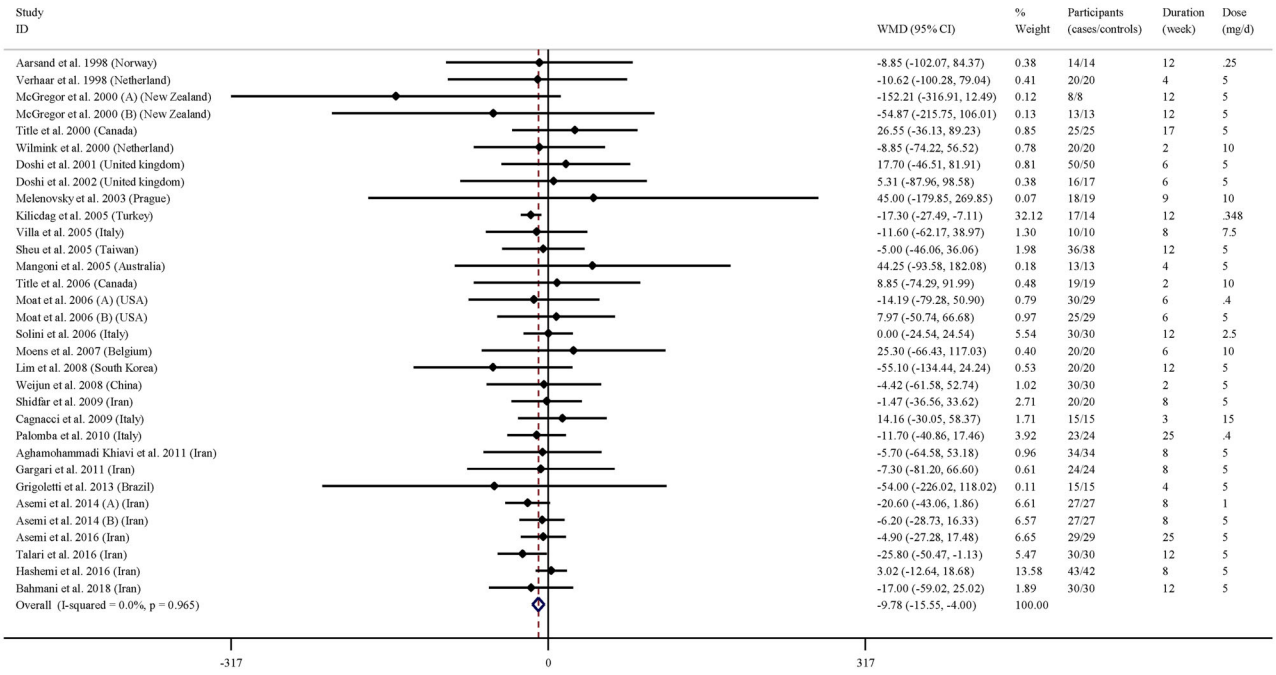
We performed a meta-regression analysis to investigate the potential association between a decrease in TG, TC, LDL and HDL concentrations with dose and duration of folic acid supplementation (mg/d). Meta-regression analysis showed no significant linear relationships between folic acid supplementation dose and changes in TG ($p = 0.620$), TC ($p = 0.738$), and LDL ($p = 0.143$) and HDL ($p = 0.652$) cholesterol (Figure 4A–D) concentrations. In addition, meta-regression for intervention duration did not show associations between decreased TG ($p = 0.785$), TC ($p = 0.704$), and LDL ($p = 0.469$) and HDL ($p = 0.659$) concentrations and duration of folic acid supplementation (Figure 5A–D).

Non-linear dose and duration responses

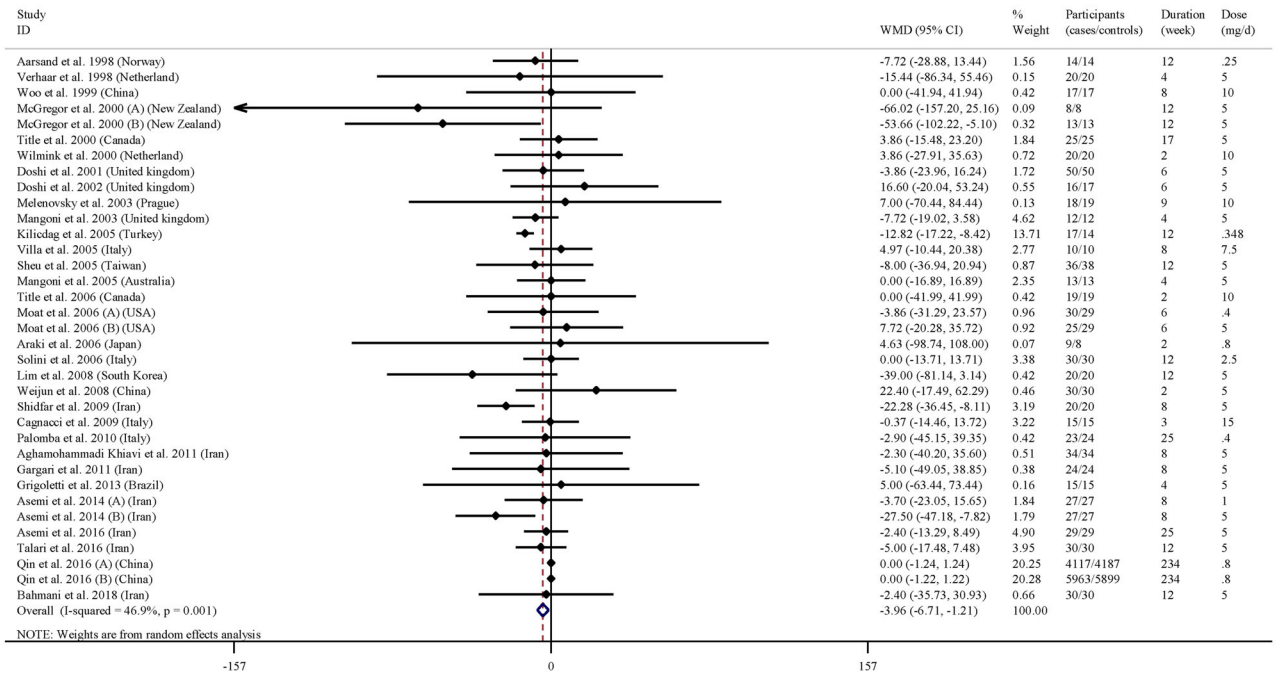
Dose-response analysis showed that folic acid supplementation significantly altered HDL based on dose ($r = 2.22$, p-nonlinearity = 0.047) in a non-linear fashion. Contrastingly, there were no significant associations demonstrated for all other lipid marker outcomes for non-linear dose-response (Figure 6A–D) and duration (Figure 7A–D).

Sensitivity analysis

Upon removing individual study effects for sensitivity analysis, the overall lipid marker category results did not significantly change for TG, LDL, and HDL cholesterol concentrations with one exception. However, after removing the study by Kilicdag et al. (Kilicdag et al. 2005) the overall result of TC was significantly changed (WMD: −0.37 mg/dL;



A) TG



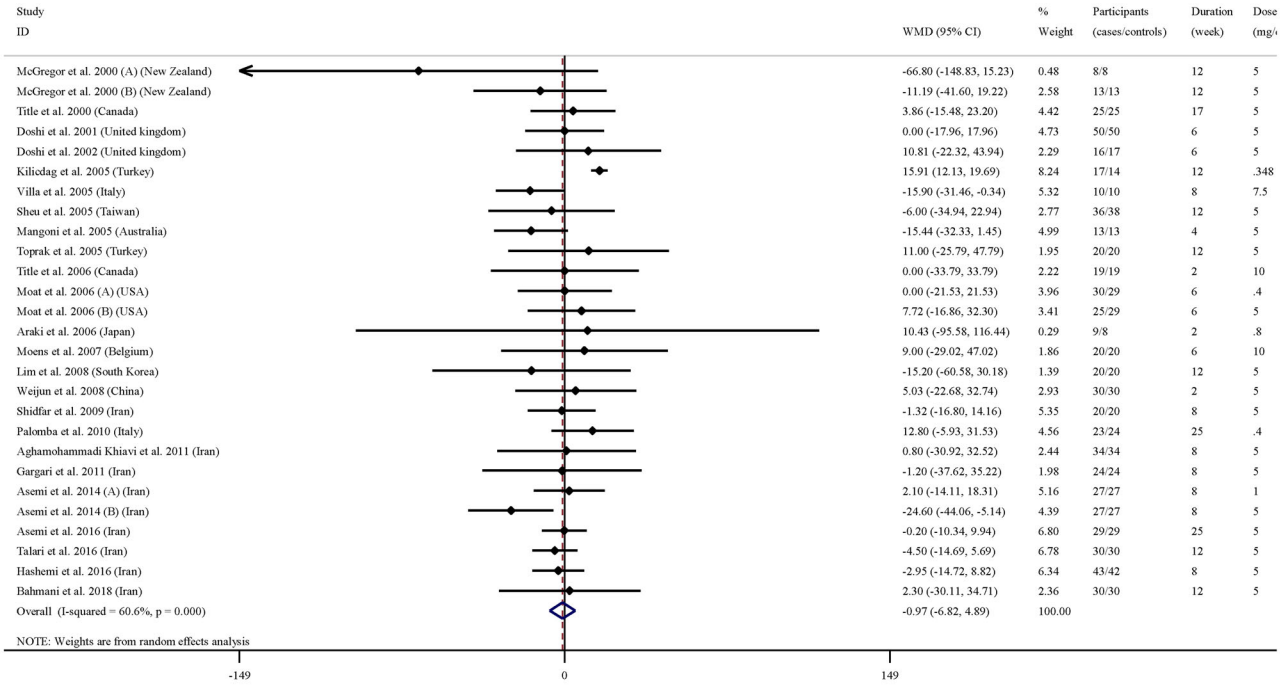
B) TC

Figure 2. Forest plot detailing weighted mean difference and 95% confidence intervals (CIs) for the effect of folic acid supplementation on; A) TG; B) TC; C) LDL cholesterol; and D) HDL cholesterol.

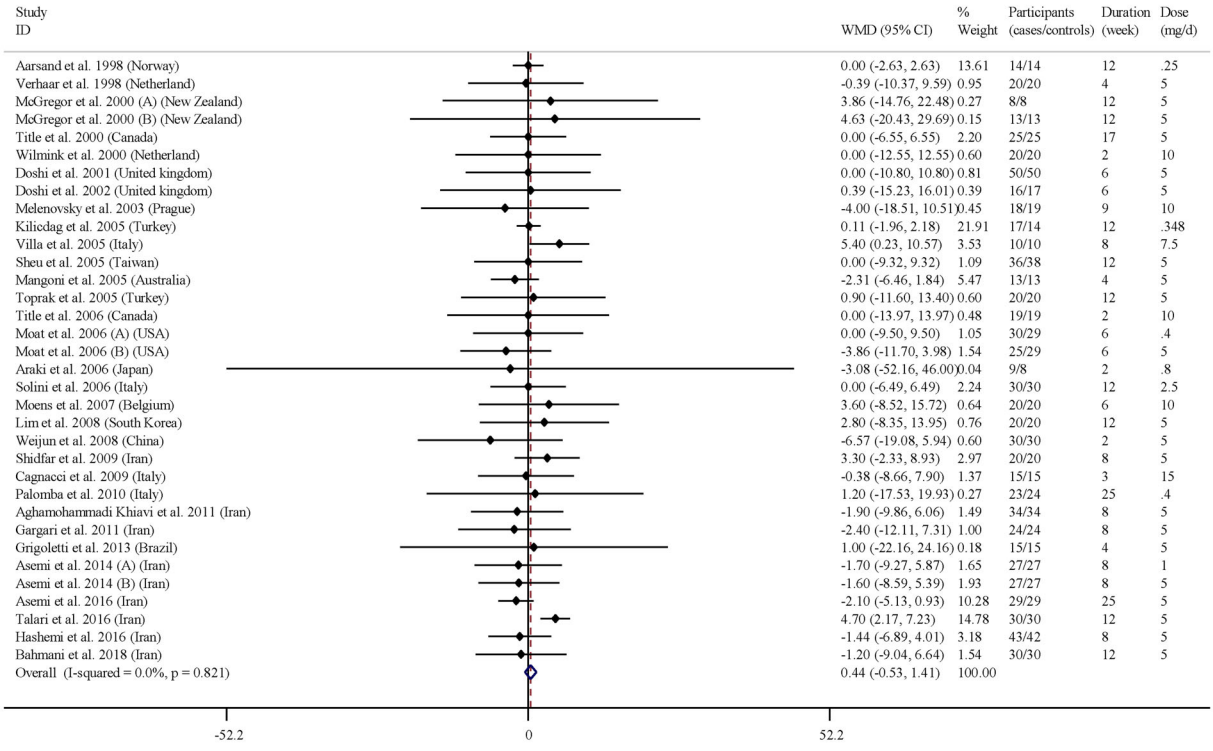
95% CI: −1.42, 0.67). Additionally, we conducted sensitivity analysis based on study quality. In this regard, we demonstrated that serum concentrations of TG (WMD: −0.70 mg/dL; 95% CI: −22.5, 21.10) and TC (WMD: −3.86 mg/dL; 95% CI: −11.72, 4.00) were changed after removing studies with high quality.

Grading of evidence

The GRADE protocol was used to evaluate the certainty of the evidence (Table 4) and determined the effect evaluates of TG to be of high quality. The evidence relating to TC and HDL concentrations was downgraded to moderate



C) LDL cholesterol



D) HDL cholesterol

Figure 2. Continued.

Table 3. Overall Effect and subgroup analyses of folic acid supplementation on lipid markers.

	NO	Sample size (cases/controls)	WMD (95%CI)	P within group	heterogeneity			
					P heterogeneity	I2	P between sub-groups	Tau2
Subgroup analyses of acid folic supplementation on triglycerides.								
Overall effect	32	751/755	−9.78 (-15.55, −4.00)	**0.001**	0.965	0.0%		
Baseline TG (mg/dL)								
<150	16	375/373	−11.24 (-17.49, −4.98)	**<0.001**	0.876	0.0%	0.235	0.0
≥150	16	376/382	−1.41 (-16.37, 13.55)	0.853	0.927	0.0%		0.0
Trial duration (weeks)								
<12	20	438/444	−3.08 (-12.16, 5.99)	0.506	0.998	0.0%	0.061	0.0
≥12	12	313/311	−14.32 (-21.79, −6.84)	**<0.001**	0.623	0.0%		0.0
Intervention dose (mg/d)								
<5	6	146/145	−15.24 (-23.45, −7.02)	**<0.001**	0.858	0.0%	0.067	0.0
≥5	26	605/610	−4.45 (-12.56, 3.65)	0.282	0.976	0.0%		0.0
Health status								
Health	3	69/68	1.05 (-18.69, 20.80)	0.916	0.746	0.0%	0.261	0.0
Unhealthy	29	682/687	−10.78 (-16.82, −4.75)	**<0.001**	0.958	0.0%		0.0
Sex								
Both sexes	19	436/441	−7.46 (-19.73, 4.81)	0.236	0.922	0.0%	0.902	0.0
Female	10	256/313	−10.51 (-17.12, −3.91)	**0.002**	0.627	0.0%		0.0
Male	2	58/58	−6.32 (-52.36, 39.72)	0.788	0.974	0.0%		0.0
Study quality								
High	28	659/662	−10.46 (-16.44, −4.47)	0.001	0.920	0.0%	0.398	0.0%
Moderate	4	92/93	−0.70 (-22.5, 21.10)	0.950	0.976	0.0%		0.0%
Subgroup analyses of acid folic supplementation on total cholesterols.								
Overall effect	35	10806/10816	−3.96 (-6.71, −1.21)	**0.005**	0.001	46.9%		9.31
Baseline TC (mg/dL)								
<200	18	4577/4650	−3.17 (-8.11, 1.77)	0.208	0.009	49.8%	0.021	27.80
≥200	16	6202/6139	−4.71 (-10.38, 0.95)	0.103	0.099	32.8%		29.46
Trial duration (weeks)								
<12	21	451/455	−5.14 (-10.06, −0.22)	**0.040**	0.587	0.0%	0.072	0.0
≥12	14	10355/10361	−3.64 (-6.94, −0.34)	**0.030**	<0.001	69.6%		9.93
Intervention dose (mg/d)								
<5	9	10230/10232	−3.11 (-6.43, 0.21)	0.066	<0.001	75.0%	0.028	8.29
≥5	26	576/584	−5.23 (-9.75, −0.71)	**0.023**	0.351	7.8%		10.31
Health status								
Health	5	75/76	−1.82 (-8.48, 4.84)	0.592	0.751	0.0%	0.738	0.0
Unhealthy	30	10730/10741	−4.46 (-7.55, −1.38)	**0.005**	<0.001	53.2%		10.64
Sex								
Both sexes	23	10525/10536	−0.61 (-2.23, 1.00)	0.459	0.288	12.7%	<0.001	1.14
Female	9	214/214	−6.91 (-13.32, −0.51)	**0.034**	0.125	36.7%		29.38
Male	3	67/66	−2.91 (-30.56, 24.74)	0.836	0.985	0.0%		0.0
Study quality								
High	30	10702/10711	−4.06 (-7.03, −1.09)	0.007	0.001	52.2%	0.427	0.0
Moderate	5	104/105	−3.86 (-11.72, 4.00)	0.336	0.623	0.0%		10.13
Subgroup analyses of acid folic supplementation on LDL cholesterol.								
Overall effect	27	648/650	−0.97 (-6.82, 4.89)	0.746	<0.001	60.6%		104.62
Baseline LDL cholesterol (mg/dL)								
<100	10	266/261	3.37 (-4.61, 11.36)	0.408	0.001	68.1%	<0.001	84.68
≥100	17	382/389	−5.64 (-11.39, 0.10)	0.054	0.525	0.0%		0.0
Trial duration (weeks)								
<12	16	397/399	−4.40 (-9.53, 0.72)	0.093	0.676	0.0%	<0.001	0.0
≥12	11	251/251	2.45 (-6.49, 11.40)	0.591	0.001	65.5%		102.70
Intervention dose (mg/d)								
<5	5	106/102	12.90 (7.04, 18.77)	**<0.001**	0.337	12.1%	<0.001	8.69

Table 3. Continued.

≥5	22	542/548	−4.05 (-8.18, 0.07)	0.055	0.770	0.0%		0.0
Health status								
Health	3	39/38	−11.41 (-25.62, 2.78)	0.115	0.385	0.0%	0.011	0.0
Unhealthy	24	609/612	−0.34 (-6.30, 5.60)	0.909	<0.001	60.0%		96.56
Sex								
Both sexes	14	319/323	−2.67 (-8.23, 2.88)	0.345	0.851	0.0%		0.0
Female	10	262/261	−0.37 (-10.24, 9.50)	0.941	<0.001	79.1%	0.001	164.56
Male	3	67/66	0.44 (-22.88, 23.77)	0.970	0.979	0.0%		0.0
Study quality								
High	25	577/579	−1.44 (-7.57, 4.69)	0.645	<0.001	63.5%	0.965	112.64
Moderate	2	50/50	7.19 (-14.94, 29.32)	0.524	0.799	0.0%		0.0
Subgroup analyses of acid folic supplementation on HDL cholestrol.								
Overall effect	34	780/783	0.44 (-0.53, 1.41)	0.378	0.831	0.0%		
Baseline HDL cholestrol (mg/dL)								
<50	23	550/549	0.71 (-0.38, 1.80)	0.203	0.786	0.0%	0.289	0.0
≥50	11	230/234	−0.56 (-2.65, 1.52)	0.597	0.647	0.0%		0.0
Trial duration (weeks)								
<12	21	485/488	−0.31 (-2.07, 1.45)	0.728	0.961	0.0%	0.318	0.0
≥12	13	295/295	0.76 (-0.40, 1.92)	0.199	0.290	15.3%		0.96
Intervention dose (mg/d)								
<5	7	150/146	−0.00 (-1.52, 1.51)	0.995	1.000	0.0%	0.460	0.0
≥5	27	630/637	0.74 (-0.52, 2.00)	0.250	0.534	0.0%		0.0
Health status								
Health	5	696/700	2.43 (-1.04, 5.91)	0.170	0.671	0.0%	0.249	0.0
Unhealthy	29	84/83	0.26 (-0.74, 1.27)	0.604	0.791	0.0%		0.0
Sex								
Both sexes	20	436/441	1.21 (-0.15, 2.59)	0.082	0.661	0.0%	0.242	0.0
Female	11	277/276	−0.25 (-1.66, 1.15)	0.724	0.751	0.0%		0.0
Male	3	67/66	−2.11 (-8.22, 3.99)	0.497	0.996	0.0%		0.0
Study quality								
High	29	668/670	0.59 (-0.47, 1.66)	0.277	0.695	0.0%	0.495	0.0
Moderate	5	98/99	−0.29 (-2.61, 2.02)	0.802	0.860	0.0%		0.0

Abbreviations. NO, number of observations; CI, confidence interval; WMD, weighted mean differences; mg/d, miligrams per day.

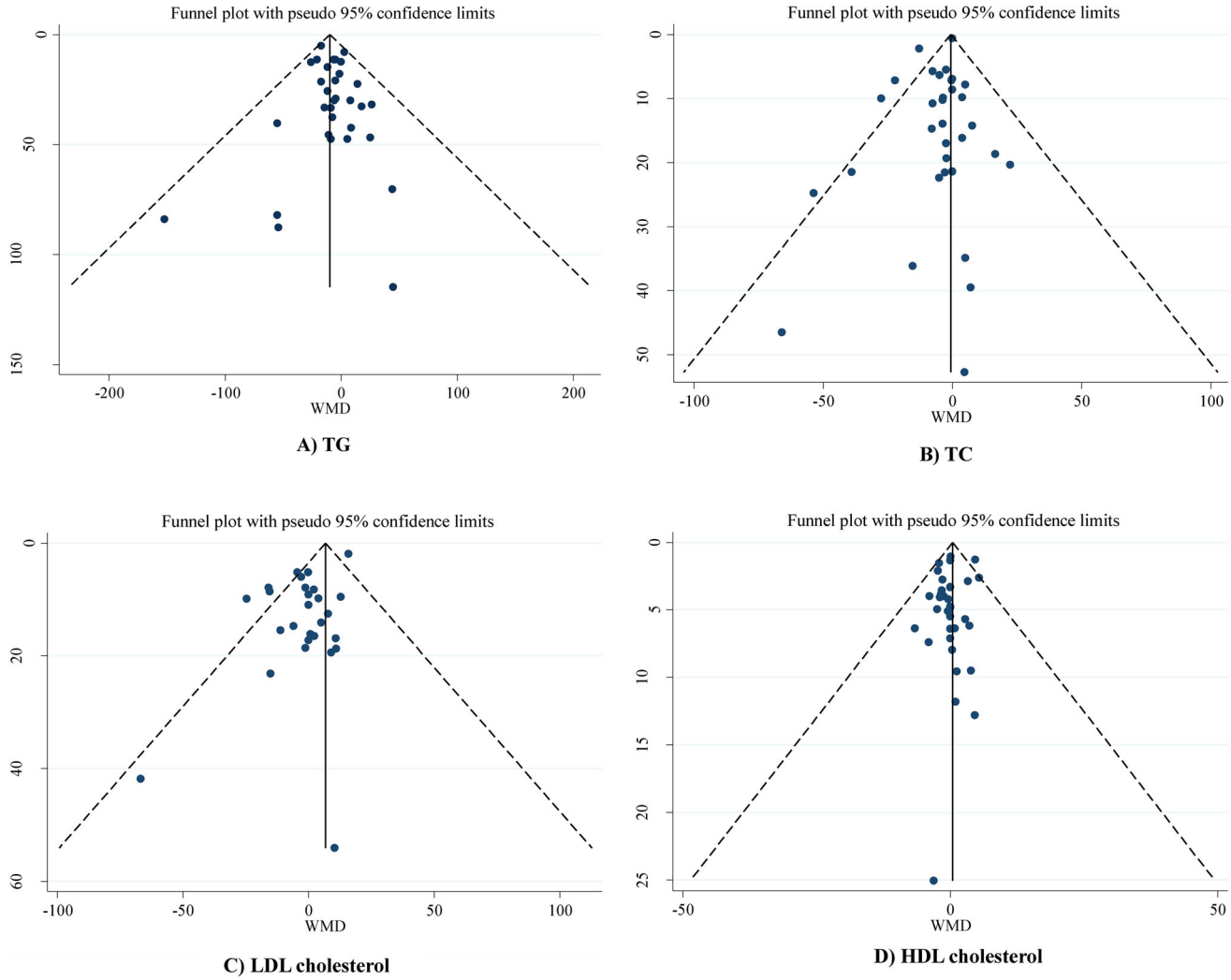


Figure 3. Funnel plot for the effect of folic acid supplementation on; A) TG; B) TC; C) LDL cholesterol; and D) HDL cholesterol.

quality. According to the GRADE protocol, evidence regarding LDL concentrations was identified as low quality due to serious inconsistency, indirectness, and imprecision. The overall quality of the body of evidence presented in this systematic review and meta-analysis was regarded as moderate.

Discussion

This meta-analysis evaluated the effects of folic acid supplementation on lipid markers in the adult population. Accordingly, folic acid supplementation was associated with a significant improvement in TG concentrations but without any significant alterations in LDL and HDL concentrations when compared to controls. Moreover, our analysis illustrated that folic acid supplementation invokes a small but significant reduction in TC concentrations. Subgroup analyses portrayed a novel finding of enhanced folic acid supplementation improvements on lipid markers (TG and TC) in women and patients with metabolic diseases when compared to men and healthy populations. Meta-regression analysis revealed no significant association between dose and duration of folic acid intervention with changes in lipid markers.

The importance of these findings is corroborated by several investigations including animal models reported that folic acid deficiency exacerbates lipid metabolism disorders where supplementation may have a protective effect against the same (Mohammadian et al. 2015; Mutavdzin et al. 2019; Ojeda et al. 2008). Additionally, while there are inconsistencies in reported data, select epidemiological studies have suggested a relationship between low folate concentrations and higher free fatty acids, thus increasing the risk of dyslipidemia (Daviddi et al. 2019; Li et al. 2015; Mahalle et al. 2014). For instance, Shen et al. set to determine the association between serum folate concentrations and measures of cardiovascular health in a cohort of young Chinese women (Shen et al. 2016). They reported that serum folate concentrations were inversely associated with TC concentrations and women with folate deficiency had marginally ($p = 0.05$) higher TG concentrations. Further, a recently published cohort study discovered that participants with higher serum folate had higher serum HDL cholesterol and lower serum triglyceride concentrations (Long et al. 2020). However, during 13 years of follow-up in the AMORIS study, researchers did not observe any relationship between folate and TG and TC concentrations (Essén et al. 2019).

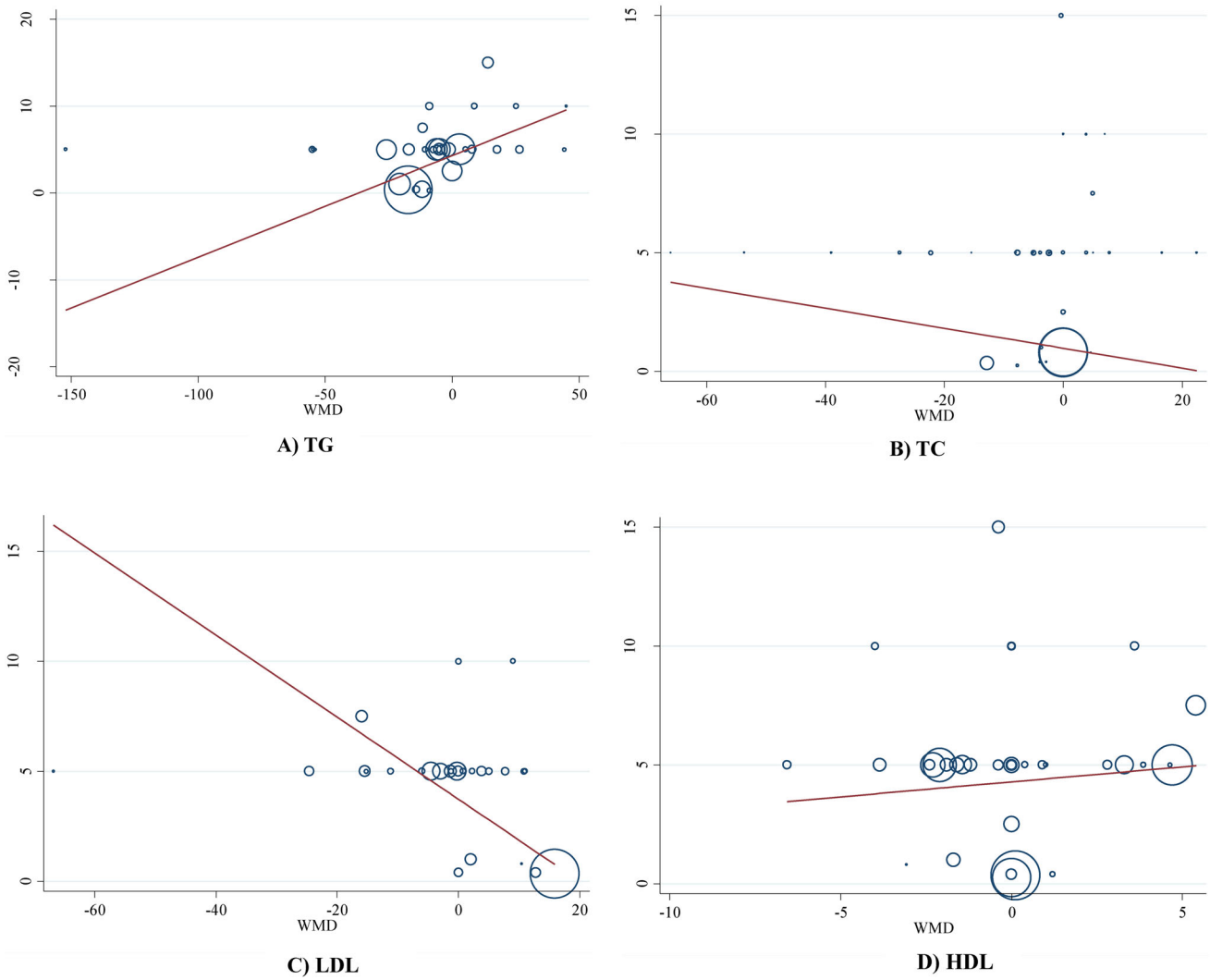


Figure 4. Linear dose-response relations between dose of folic acid supplementation and absolute mean differences in A) TG; B) TC; C) LDL cholesterol; and D) HDL cholesterol.

Our findings suggest that folic acid supplementation may be more effective in the women population, and while sex-specific outcomes of folic acid supplementation remain unclear, differences may be accounted for by lower folate concentration and dietary folate intake in women than men as reported in observational studies (Gargari, Aghamohammadi, and Aliasgharzadeh 2011; Robinson, et al. 1998). Moreover, it has been suggested that the dietary folic acid intake requirements are greater in men than women to achieve the same serum folate concentrations, mainly due to a higher lean mass to overall body mass (Winkels et al. 2008). In addition, several studies reported that men had a much higher prevalence of cigarette smoking and alcohol consumption compared to women and, in general, smokers had lower plasma and red blood cell folate concentrations compared with their nonsmoking counterparts (Bauer, Göhlmann, and Sinning 2007; Cafolla et al. 2000; Wilsnack et al. 2009). On the other hand, some previous studies reported the positive relationship between serum folate and estrogen concentrations and the potential effects of folic acid supplementation in increasing estrogen concentrations among women (Ericson et al. 2010; Sütterlin et al. 2003). Estrogen might improve lipid profile by some possible mechanisms including its anti-inflammatory and antioxidant properties (Geer and Shen 2009). Moreover, estrogen also contributed to the regulation of lipoprotein lipase, which is involved in lipoprotein metabolism (Berg, et al. 2004; Randolph et al. 2011) and can improve lipid profile by decreasing TG and increasing HDL concentrations (Pirim et al. 2014; Rip et al. 2006). Further investigations are indeed needed to evaluate the gender-specific effects of folic acid supplementation on lipid markers.

The possible mechanisms underlying the positive effects of folic acid supplementation on TG and TC concentrations in the general population and particularly in patients with metabolic diseases remain unclear. However, we propose four possible theories warranting further investigation for the relationship between folic acid supplementation and lipid profile variables. Firstly, it seems that individuals with lower folate concentrations at baseline particularly show improvements in lipid markers following folic acid supplementation. It has been mentioned that patients with the metabolic disease are more likely to experience lower serum folate concentrations (Kaplan et al. 1999; Malaguarnera et al.

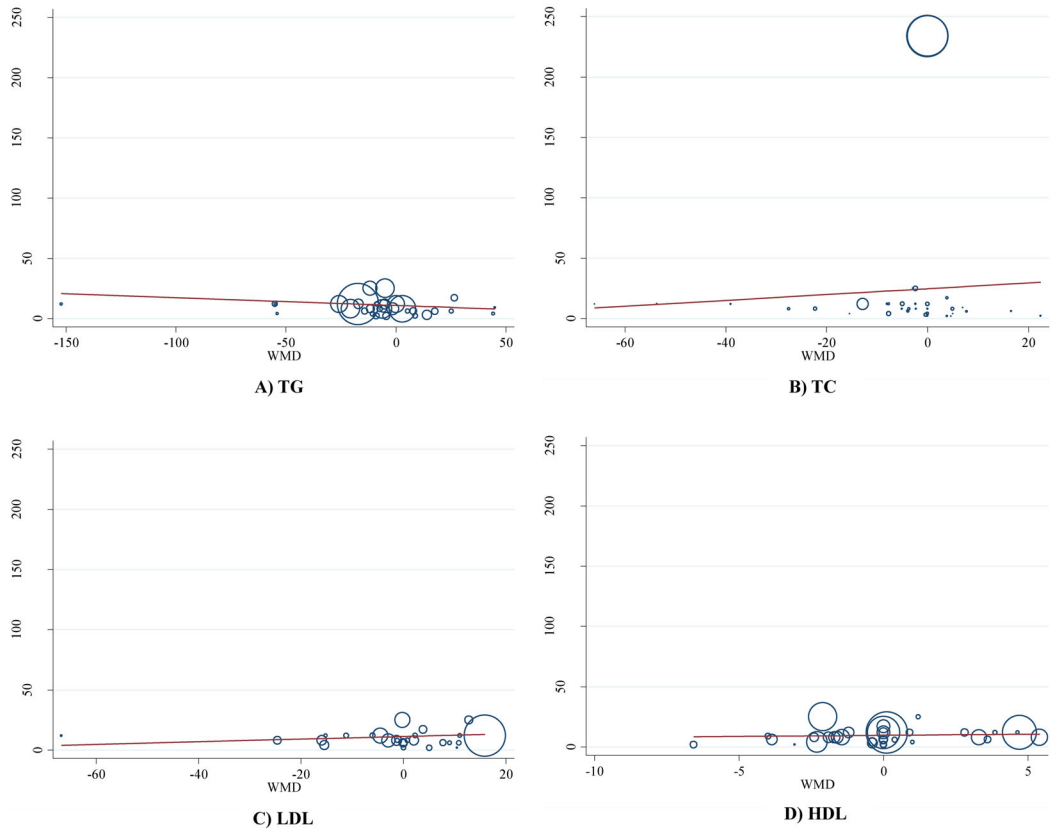


Figure 5. Linear dose-response relations between duration of folic acid supplementation and absolute mean differences in A) TG; B) TC; C) LDL cholesterol; and D) HDL cholesterol.

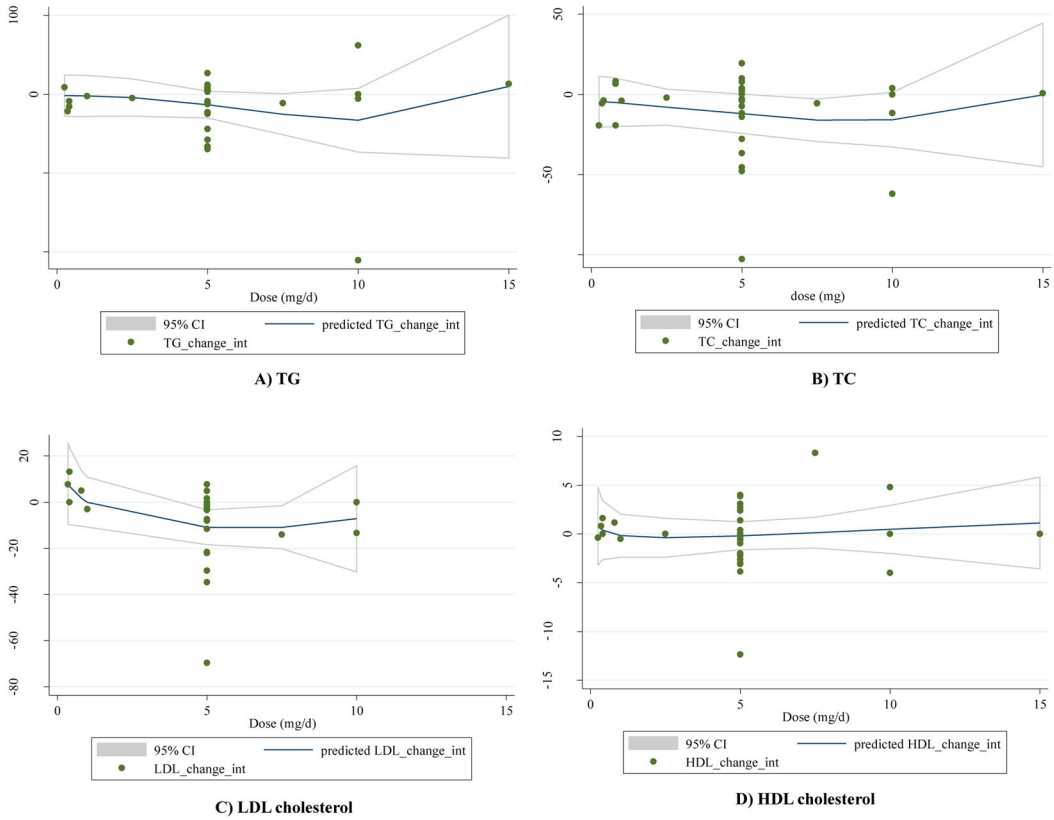


Figure 6. Non-linear dose-response relations between dose of folic acid supplementation and absolute mean differences in A) TG; B) TC; C) LDL cholesterol; and D) HDL cholesterol.

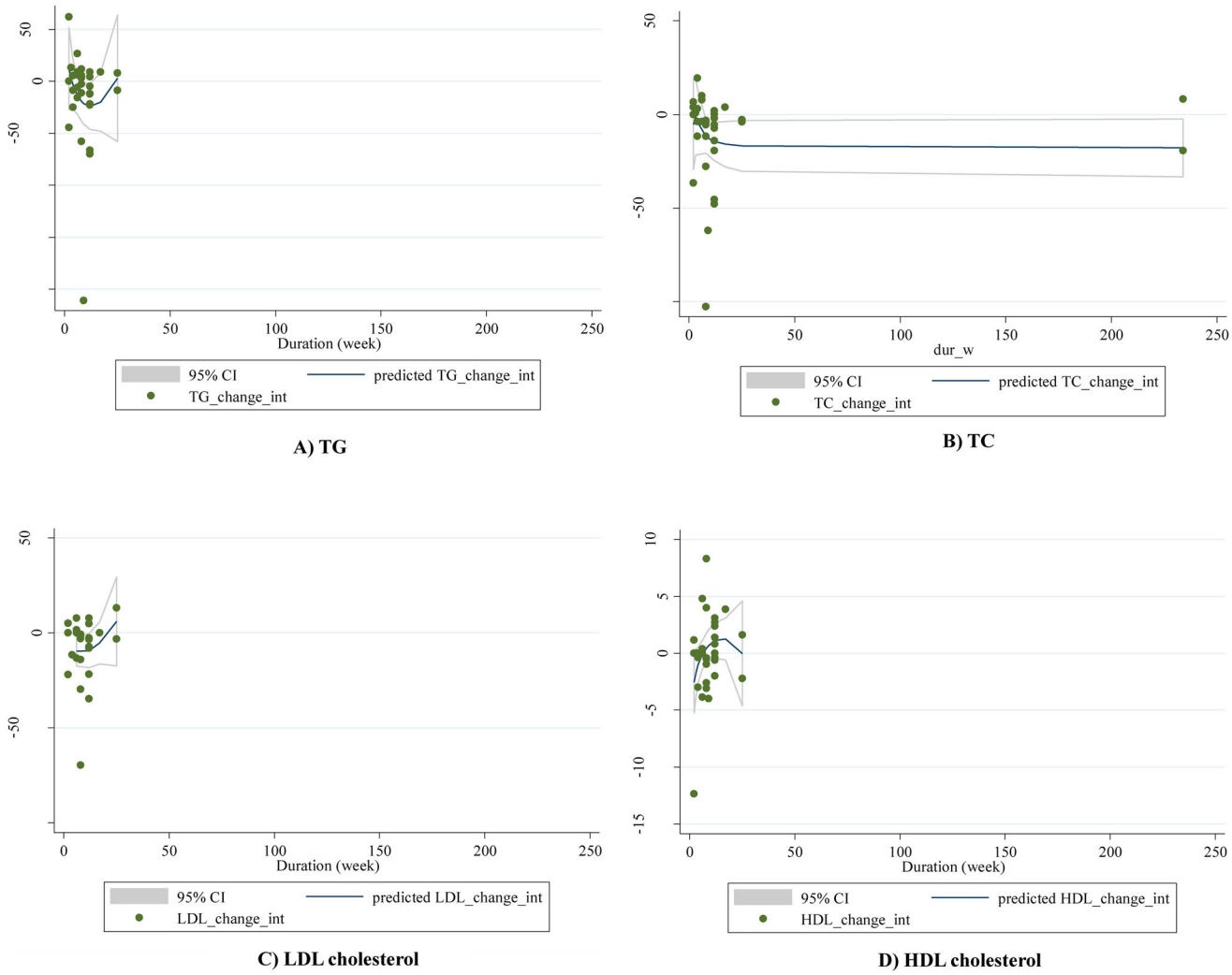


Figure 7. Non-linear dose-response relations between duration of folic acid supplementation and absolute mean differences in A) TG; B) TC; C) LDL cholesterol; and D) HDL cholesterol.

2015; Wang et al. 2020). Others have hypothesized that reestablishing normal folic acid concentrations in those suffering from folate deficiency and/or insufficiency may experience greater beneficial effects, including glycemic profile, compared to individuals with normal serum folate concentrations. Secondly, folate deficiency-induced hyperhomocysteinaemia may promote dyslipidemia (McNulty et al. 2008). Several studies have indicated that folate concentrations are inversely associated with blood concentrations of homocysteine and that serum homocysteine concentrations are strongly correlated with the prevalence of dyslipidemia (Momin, et al. 2017; Yadav, Bhagwat, and Rathod 2006). Moreover, it appears that earlier stages of folate deficiency are expressed by rapidly rising homocysteine concentrations, which appear at a higher range of folate deficiency (serum folate of 10-14 nmol/L) (Rogers et al. 2018). Thirdly, inflammation plays an important role in the development of dyslipidemia (Esteve, Ricart, and Fernández-Real 2005; Tietge 2014). Activation of inflammatory pathways can impair the reverse cholesterol transport to HDL, resulting in hypercholesterolemia, stimulation, synthesis, and accumulation of VLDL (very low-density lipoprotein), and hypertriglyceridemia (Esteve, Ricart, and Fernández-Real 2005). Further, both in vitro and in vivo studies have shown that folic acid supplementation may inhibit proinflammatory processes by inhibiting Nuclear factor kappa B (NF-kB) activation (Bagherieh et al. 2021; Kumar et al. 2015). Therefore, the potential anti-inflammatory effects of folic acid supplementation may prove to be another possible lipid profile improving mechanism. Finally, evidence indicates that oxidative stress is linked with lipid peroxidation and the development of dyslipidemia (Rizzo et al. 2009; Tangvarasittichai 2015). With this in mind, folic acid supplementation has been suggested to impart antioxidant properties (Joshi et al. 2001) by suppressing lipid peroxidation and subsequently initiating the indirect metabolism and decline of lipids (Abd Allah and Badary 2017; Joshi et al. 2001). Further mechanistic investigations are needed to evaluate such possible mechanisms of the effects of folic acid supplementation on lipid markers.

This meta-analysis comes with both strengths and limitations. The primary strengths of this study are the relatively large number of studies meeting rather specific inclusion criteria and subsequent high sample size overall. Another

Table 4. GRADE profile of acid folic supplementation for lipid profile scores in adults.

Quality assessment						Summary of findings		
Outcomes	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication Bias	Case/Control Sample Size	WMD (95%CI)	Quality of evidence
TG	No serious limitations	No serious limitations	Serious Limitations[c]	No Serious Limitations	No serious limitations	751/755	−9.77 (-15.54, −4.00)	⊕⊕⊕⊕ High
TC	No serious limitations	Serious Limitations[a]	Serious Limitations[c]	No Serious Limitations	No serious limitations	10806/10816	−3.95 (-6.70, −1.20)	⊕⊕⊕◯ Moderate
LDL-cholestrol	No serious limitations	Serious Limitations[b]	Serious Limitations[c]	Serious Limitations[d]	No serious limitations	648/650	−0.96 (-6.82, 4.88)	⊕⊕◯◯ Low
HDL-cholestrol	No serious limitations	No serious limitations	Serious Limitations[c]	Serious Limitations[d]	No serious limitations	780/783	0.43 (-0.53, 1.40)	⊕⊕⊕◯ Moderate

aThe test for heterogeneity is significant, and the I^2 is moderate, 46.9%
bThe test for heterogeneity is significant, and the I^2 is moderate, 60.6%
cstudies conducted subcect with varous conditons.
dvalues are distributed within opposite direction across studies.

advantage of this meta-analysis relates to the inclusion of several long-term studies, which certainly has the advantage of documenting long-term effects of folic acid supplementation on lipid markers and allowing comparisons to shorter duration designs (e.g., total cholesterols was shown to decrease to a greater extent in studies of longer duration whereas LDL was significantly reduced only in shorter duration studies). These results are intriguing and warrant further investigation. It should be mentioned that there existed some publication bias and heterogeneity in the analysis as limitations to this study.

In conclusion, folic acid supplementation was associated with significant improvements in TC and TG concentrations but without significant alterations in LDL and HDL concentrations when compared to a control group. Moreover, as a novel finding, women and patients with metabolic diseases demonstrated significant lipid improving effects following folic acid supplementation when compared to their men and healthy population counterparts.

Declaration of interest

The authors declare no conflict of interest.

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