The Association Between the Risk of Hypertensive Disorders of Pregnancy and Folic Acid: A Systematic Review and Meta-Analysis

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ABSTRACT -- Purpose: Although folic acid (FA) supplementation has been shown to reduce general cardiovascular risks, its impact on hypertensive disorders of pregnancy (HDP) is unclear. We performed a systematic review and meta-analysis to clarify the association between FA and the risk of HDP (pre-eclampsia (PE) and gestational hypertension (GH)). Methods: PubMed, EmBase, and Cochrane Library were searched up to June 18, 2020, stratified by type of disease, initiation time of FA, form of FA and pre-conception Body Mass Index (BMI). The quality assessment of included studies was evaluated using Newcastle-Ottawa Scale (NOS) for cohort studies and Cochrane Collaboration’s Risk of Bias Assessment Tool for randomized controlled trials (RCTs). Between-study heterogeneity was quantified using Cochran’s Q-statistic and I² statistics. Sensitivity analysis was performed by excluding the studies one by one, and publication bias was analyzed using funnel plots. Results: Twenty studies with 359041 patients were identified for inclusion in the meta-analysis which included 3 RCTs and 17 cohort studies. Pooled estimates showed RR of 0.83 (95%CI 0.74-0.93, P=0.0008) for association between low dose FA (LD-FA) and the risk of PE, but LD-FA was not associated with GH (RR 1.05, 95% CI 0.97-1.13, P=0.20). In addition, the results of subgroup analysis showed that post-conception LD-FA had a 31% decreased risk of PE (RR 0.69, 95% CI 0.59-0.80, P<0.00001), and LD-FA in patients with pre-conception BMI<25 kg/m² had a 32% decreased risk of PE (RR 0.68, 95% CI 0.56-0.81, P<0.0001). Conclusions: LD-FA significantly decreased the risk of PE but not GH, and post-conception LD-FA and pre-conception BMI<25 kg/m² were considered as protective factors to reduce the risk of PE.

INTRODUCTION

Hypertensive disorders of pregnancy (HDP) accounts for 16% of maternal deaths and is also associated with severe morbidity, long term disability and death among both mothers and their babies (1). This group of diseases includes pre-eclampsia (PE) and eclampsia, gestational hypertension (GH) and chronic hypertension. In addition, some evidence showed that women with HDP were at increased risk of developing chronic kidney disease, cardiovascular disease (CVD) and even cardiovascular mortality (2-3). Therefore, effective prevention and treatment of HDP is critical. At present, several clinical practice guidelines have clearly recommended that aspirin could be used for the prevention of HDP in high-risk pregnant women (1-3). A meta-analysis which with focus on the general cardiovascular health indicated a 10% lower risk of stroke and a 4% lower risk of overall CVD with folic acid (FA) supplementation (4). However, studies investigating the use of FA to prevent HDP have yielded inconsistent results. Currently, the relationship between FA and HDP has been widely studied (5-27), and five meta-analyses with inconsistent results have been published (28-32). Two meta-analyses demonstrated that FA was not associated with the risk of GH or PE (28-29). Two demonstrated that multivitamin containing FA (Vit-FA) could significantly lower GH or PE risk (30-31). And one showed no significant difference between FA alone and Vit-FA in reducing PE (32). The latest retrieval deadline for these meta-analyses was Aug. 2017. Since then, six other studies have been published. In addition, FA initiation time, and pre-conception Body Mass Index (BMI) were used as grouping conditions in some cohort studies, so these
factors are an important opportunity for subgroup analysis of meta-analysis.

The purpose of this study was to perform a systematic review and meta-analysis to clarify the association between FA and the risk of HDP.

MATERIALS AND METHODS

Search Strategy
We conducted a comprehensive search in PubMed, EmBase, and Cochrane Library from the date of their inception up to June 18, 2020. The search terms were composed of the following: “folic acid”, “vitamin b9”, “preeclampsia”, “pregnancy induced hypertension”, “gestational hypertension”, “hypertensive disorders of pregnancy”. To identify potential publications, we also examined the references of relevant reviews and meta-analysis. The details of the search strategy were summarized in the Appendix 1.

Study Selection
Inclusion criteria: (1) population-based studies, including cohort studies, case-control studies, and randomized controlled trials (RCTs). (2) the exposure factor is FA or Vit-FA. (3) the outcome is GH or PE. (4) the written language is English. Exclusion criteria: (1) not original studies (e.g., reviews, meta-analysis, commentaries, case reports, editorials and letters). (2) conducted in animals. (3) studies without control group (comparison between different dose or FA alone and Vit-FA) (4) studies lack the necessary data (e.g., non-full text articles, unpublished trials).

Data Extraction
Two investigators (YHY, XMS) assessed the eligibility of retrieved articles and extracted relevant data from each eligible study using a standardized form independently. From included studies, the following information were extracted: study characteristics (first author’s name, year of publication, study design, range of years in the studies, country), participants’ characteristics (population size, gestational age at recruitment, age, pre-conception BMI, FA dose, the initiation time of FA (pre-conception or post-conception), type of disease (GH or PE), the HRs, RRs, ORs with 95% CIs of GH/PE adjusted for confounders.

Outcomes
The primary outcome was the risk of PE. The secondary outcome was the risk of GH.

Quality Assessment
The methodological quality assessment of included studies was independently assessed by two reviewers (YHY, XMS) according to the Newcastle-Ottawa Scale (NOS) and the Cochrane Collaboration’s Risk of Bias Assessment Tool. Two reviewers (XRW, XF) resolved the disagreements, and the final consensus was reached by all four reviewers. For cohort studies, the NOS includes three aspects with a nine-point scale: selection of study population, compatibility of the study groups, and ascertainment of outcomes, and the scores of 0–3, 4–6, and 7–9 were interpreted as low, moderate, or high quality. For RCTs, the Cochrane Collaboration’s Tool includes six aspects with seven items: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other bias.

Statistical analysis
This meta-analysis was conducted by Review Manager for Windows (version 5.3) using the generic inverse variance method. Between-study heterogeneity was quantified using Cochran’s Q-statistic and $I^2$ statistics and a value of $P<0.10$ or an $I^2>50\%$ was considered statistically significant for heterogeneity among studies. The random-effect model was used if it was significant; otherwise, the fixed-effect model was acceptable. Subgroup analyses were performed according to the type of disease (GH or PE), initiation time of FA (pre-conception or post-conception), form of FA (Vit-FA or FA alone) and pre-conception BMI (BMI<25 kg/m$^2$ or BMI $\geq$ 25 kg/m$^2$), respectively. The sensitivity analysis was conducted by excluding the studies one by one to evaluate the stability of results without estimation bias from individual study. The funnel plots were used to evaluate publication bias qualitatively.

RESULTS

Search Results
The search strategy yielded 1294 abstracts. Manual searching identified 4 additional articles. A total of 1269 articles were excluded after de-duplicating and the review of abstracts, leaving 29 studies for full-text review for eligibility. Overall, 20 studies with 359041 patients were identified that were eligible for inclusion in the meta-analysis.$^{5,24}$ The whole literature search process was summarized in the Figure 1.
Study Characteristics
This meta-analysis included 3 RCTs and 17 cohort studies. Characteristics of the 20 included studies in the meta-analysis were presented in Table 1 and Table 2. These studies were published between 2002 and 2020. The study populations ranged from 215 to 193554 participants, with a total number of 359041. There were 12 studies that didn’t clearly describe the initiation time of FA, 4 studies that began taking FA in post-conception period, and 1 study in pre-conception period. FA alone or Vit-FA was 0.2-1mg in most studies, except for 3 RCTs which used high dose (4-5mg) FA. The ascertainment of GH/PE were from medical records and self-report, and there are 14 studies evaluating the risk of PE alone and 6 studies evaluating the risk of both GH and PE. In addition, 5 articles performed a subgroup analysis based on the pre-conception BMI. The ORs, RRs and HRs in 18 studies were adjusted based on the potential confounders.

High Dose (HD)-FA and the Risk of PE
Three studies compared risk of PE with HD-FA. The results showed that HD-FA was not associated with PE (3 studies, RR 1.11, 95% CI 0.75-1.62, P=0.61; Figure 2). No obvious asymmetry was found in the funnel plot (Appendix 4-Figure 1), which indicated that publication bias was unlikely in the analysis. The results of sensitivity analysis showed no substantial modification of the estimates after exclusion of individual study one by one.

Low Dose (LD)-FA and the Risk of HDP(GH/PE)
Eighteen studies compared risk of HDP with LD-FA. Stratification by GH and PE showed that LD-FA significantly decreased the risk of PE (18 studies, RR 0.83, 95%CI 0.74-0.93, P=0.0008, Figure 3), but was not associated with GH (6 studies, RR 1.05, 95% CI 0.97-1.13, P=0.20; Figure 3). No obvious asymmetry was found in the funnel plot (Appendix 4-Figure 2), which indicated that publication bias was unlikely in the analysis. The results of sensitivity analysis showed that the association between LD-FA and increased risk of PE in PE subgroup was reliable.

Pre-conception or Post-conception LD-FA and the Risk of PE
11 studies with 23 results compared the association between peri-conception LD-FA and the risk of PE. According to the initiation time (Pre-conception or Post-conception), a subgroup analysis was...
Figure 2. Forest plot for High Dose Folic Acid and the risk of Hypertensive Disorders of Pregnancy

Figure 3. Forest plot for Low Dose Folic Acid and the risk of Hypertensive Disorders of Pregnancy in subgroup analysis by type of disease (Gestational hypertension or Pre-eclampsia)

performed. The results showed that pre-conception LD-FA was not associated with the PE (9 studies, RR 0.92, 95% CI 0.84-1.02, P=0.12, Figure 4), but post-conception LD-FA had a 31% decreased risk of PE (9 studies, RR 0.69, 95% CI 0.59-0.80, P<0.00001; Figure 4). No obvious asymmetry was found in the funnel plot (Appendix 4-Figure 3), which indicated that publication bias was unlikely in the analysis. The results of sensitivity analysis showed that the association between post-conception LD-FA and the decreased risk of PE was reliable.

LD-FA in patients with pre-conception BMI<25 kg/m² or BMI≥25 kg/m² and the Risk of PE

According to the pre-conception BMI (BMI<25 kg/m² or BMI≥25 kg/m²), a subgroup analysis was performed to compare the association between LD-
Figure 4. Forest plot for Low Dose Folic Acid and the risk of Hypertensive Disorders of Pregnancy in subgroup analysis by initiation time (pre-conception or post-conception)

FA and the risk of PE in 4 studies with 14 results. The results showed that LD-FA in patients with pre-conception BMI<25 kg/m² had a 32% decreased risk of PE (4 studies, RR 0.68, 95%CI 0.56-0.81, P<0.0001, Figure 5), but no difference was found in patients with pre-conception BMI ≥ 25 kg/m² (4 studies, RR 0.87, 95%CI 0.71-1.05, P=0.14; Figure 5). No obvious asymmetry was found in the funnel plot (Appendix 4-Figure 4), which indicated that publication bias was unlikely in the analysis. The results of sensitivity analysis showed that the association between LD-FA and the decreased risk of PE in pre-conception BMI<25 kg/m² subgroup was reliable.

FA alone or Vit-FA and the Risk of PE
According to the form of FA (FA alone or Vit-FA), a subgroup analysis was performed in 8 studies with 13 results. The results showed that Vit-FA significantly decreased the risk of PE (4 studies, RR 0.82, 95%CI 0.68-0.98, P=0.03, Figure 6), but no reduction in PE was found in patients receiving FA alone (7 studies, RR 0.86, 95%CI 0.65-1.13, P=0.27; Figure 6). Asymmetry can be seen in the funnel plot (Appendix 4-Figure 5), especially the Vit-FA subgroup, indicating that there is publication bias in the analysis. In addition, after excluding Bodnar 2006, P-value changed to 0.86 (RR, 95%CI 0.73-1.01, P=0.06), after excluding Catov 2009, P-value changed to 0.83 (RR, 95%CI 0.66-1.04, P=0.10), which indicated that the association between Vit-FA and the decreased risk of PE was not reliable.

DISCUSSION

Our meta-analysis of 20 studies involving 359041 patients found that LD-FA was associated with the decreased risk of PE but not GH. In addition, post-conception LD-FA and pre-conception BMI<25 kg/m² were considered as protective factors to reduce the risk of PE.

Some studies of the association between FA and the risk of GH or PE have shown a potential protective effect (5, 7, 9, 11, 15-17, 19), and Wen...
Figure 5. Forest plot for Low Dose Folic Acid and the risk of Hypertensive Disorders of Pregnancy in subgroup analysis by pre-conception Body Mass Index (BMI<25 kg/m² or BMI≥25 kg/m²)

Figure 6. Forest plot for Low Dose Folic Acid and the risk of Hypertensive Disorders of Pregnancy in subgroup analysis by form of Folic Acid (Multivitamin containing FA or FA alone)

(22) has shown that maternal exposure to FA antagonists appears to increase the risk of placenta-mediated adverse outcomes of pregnancy (33). It has been proved that elevated plasma homocysteine (Hcy) level was associated with increased risk for various forms of CVD, and it was also significantly elevated in women with PE (34). The possible pathogenic mechanisms involve damage of endothelial cell, reduction of vascular flexibility, changes in the hemostatic process and promotion of the development of inflammation. The elevated Hcy may arise from genetic defect of blood pressure, the altered process of DNA and protein synthesis of placental growth and development, and the imbalance between FA and vitamin B₁₂ (24).
Table 1. The characteristics of included studies

| Author, year | Design, years, country | Characteristics | FA initiation time | Dose | Gestational age (weeks) at recruitment | Age(years) | Pre-conception BMI(kg/m2) |
|--------------|------------------------|----------------|-------------------|------|---------------------------------------|------------|---------------------------|
| Li, 2020     | Prospective cohort, 2013-, China | 4853 participants from the Tongji Maternal and Child Health Cohort with information on periconceptional FA supplement use and diagnosis of GH/PE, 1161 and 161 women were diagnosed with GH and PE | pre-conception or post-conception | at least 0.4mg/day | NR | 28.6 (SD 3.4) | 20.6 (SD 2.6) |
| Corsi, 2020  | Randomized Controlled Trial, 2011-2015, Canada | 428 women pregnant with twins who were aged 18 y or older, between 8 and 16 completed weeks' gestation and had at least one high risk factor for PE | post-conception | 4mg/day | 13.8 (SD 1.9, FA) 14.0 (SD 1.8, non-FA) | 31.5 (SD5.2, FA) 31.4 (SD5.0, non-FA) | NR |
| Wen, 2018    | Randomized Controlled Trial, 2011-2015, Canada | 2301 pregnant women with at least one high risk factor for PE (1144 to the FA group and 1157 to the placebo group) | post-conception | 4mg/day | 14 (SD 1.9) | 31 (SD 5.4) | 34 (SD 8.6, FA) 34 (SD 13, non-FA) |
| Ocampo, 2017 | Retrospective cohort, 2004-2014, USA | 3247 participants recruited from the Mother-ToBaby (MTB) network, 2697 were early users and 471 were late users of FA-containing supplements, and 79 were nonusers during their pregnancy | pre-conception or post-conception | NR | NR | 32.3 (SD 4.7, pre-conception-FA) 30.4 (SD 6.0, post-conception-FA) 32.8 (SD 5.6, non-FA) | 24.5 (SD 5.2, pre-conception FA) 25.9 (SD 6.1, post-conception FA) 25.7 (SD 7.3, non-FA) |
| Wen, 2016    | Prospective cohort, 2002-2008, Canada | 7669 pregnant women who were less than 20 weeks gestational age in Ottawa, ON and Kingston, ON, Canada | pre-conception or post-conception | most 1.0 mg or higher | NR | NR | NR |
| Liu, 2015    | Prospective cohort, 2010-2012, China | 10179 women (7864 FA supplement, 2315 non-FA) with live singleton births in Lanzhou, China | pre-conception or post-conception | NR | NR | NR | NR |
| Wang, 2015   | Prospective cohort, 2010-2012, China | 10041 pregnant women with gestational age ≥ 20 weeks and aged 18 years or older, without chronic hypertension, gestational hypertension and mental illness | pre-conception or post-conception | NR | NR | NR | NR |
| Martinussen, 2015 | Prospective cohort,1996-2000, USA | 3647 women (1710 from the AIP study and 1937 from the NIP study) who were followed from the first trimester of pregnancy in New England, USA | pre-conception or post-conception | NR | NR | NR | NR |

Table 1 continues...
| Author, Year | Study Design | Study Population | Sample Size | Maternal Age | Maternal Vitamin D Status | Other Variables |
|-------------|--------------|-----------------|-------------|--------------|--------------------------|----------------|
| Kim, 2014   | Retrospective cohort, 2009-2010, South Korea | 215 participants with singleton pregnancies at the Korea University Anam Hospital | NR          | NR           | NR                       | 31.9 (SD 3.9,FA) 31.3 (SD 4.7,non-FA) |
| Vanderlelie, 2014 | Prospective cohort, 2006-2011, Australia | 2619 mothers with live births in Queensland, Australia | post-conception | most 0.8 mg/day | NR                       | NR |
| Li, 2013    | Retrospective cohort, 1993-1995, China | 193554 women in 2 southern provinces (Jiangsu Province and Zhejiang Province) | pre-conception or post-conception | 0.4mg/day | NR                       | 24.21 (SD 2.47,FA) 25.54 (SD 3.77,non-FA) |
| Timmermans, 2011 | Prospective cohort, 2002-2006, Netherlands | 9778 women at early pregnancy in Rotterdam, the Netherlands | pre-conception or post-conception | 0.4-0.5mg/day | NR                       | 29.8 (IQR 19.7-39.0, post-conception) 31.6 (IQR 22.6-39.5, pre-conception) 27.8 (IQR 17.9-39.2, non-FA) |
| Catov, 2011 | Prospective cohort, 1997-2003, Denmark | 35897 pregnant women in the Danish National Birth Cohort | pre-conception or post-conception | Vit-FA: most FA0.2mg/day | 10.7 (SD 3.5,FA) 10.9 (SD 3.8, Vit-FA) 11.4 (SD 4.3,non-FA) | NR |
| Catov, 2009 | Prospective cohort, 1997-2003, Denmark | 28601 pregnant women in the Danish National Birth Cohort | pre-conception or post-conception | FA: 0.4mg/day Vit-FA: most FA0.2mg/day | 10.8 (SD 3.4,FA) 10.9 (SD 3.5 Vit-FA) 11.2 (SD 3.8,non-FA) | NR |
| Bukowski, 2009 | Prospective cohort, 1999-2002, USA | 34480 women who delivered singleton pregnancies with a gestational age of 10 week 3 d through 13 week 6 d | pre-conception | NR | NR | 27.8 (IQR 23.5–32.5,non-FA) 31.1 (IQR 27.1–34.5,<1 year) 33.1 (IQR29.4–36.3,>1 year) |
| Wen, 2008 | Prospective cohort, 2002-2005, Canada | 2951 pregnant women with prenatal care visit (12-20 weeks’ gestation) at the Ottawa Hospital and Kingston General Hospital | pre-conception or post-conception | most 1.0 mg or higher | NR | NR |
| Oken, 2007 | Prospective cohort, 1999-2002, USA | 1718 participants with live births in Project Viva | NR | normal women: 0.952(SD 0.467)mg PE: 0.854(SD 0.297)mg GH: 1.019(SD 0.486)mg | 10.4 | NR |

Table 1 continues...
Table 2. The clinical outcomes of included studies

| Author, year | Outcome-GH/PE | FA (n) | FA-GH/PE (n) | non-FA (n) | non-FA-GH/PE (n) | FA-GH/PE risk RR/OR/HR-95% CI | Adjusted for |
|--------------|---------------|--------|--------------|------------|-----------------|-----------------------------|--------------|
| Li, 2020     | GH-FA800-pre-conception | 358    | 107          | 646        | 145             | 1.32 (1.06-1.64)            | age, education, work status, monthly income, ethnicity, history of abnormal pregnancy, pre-conception BMI, smoking, drinking, family history of diabetes mellitus, family history of hypertension, other supplement use, gestational diabetes mellitus, and gestational weight gain |
|              | PE-FA800-pre-conception   | 358    | 17           | 646        | 18              | 1.45 (0.74-2.83)            | age, education, work status, monthly income, ethnicity, history of abnormal pregnancy, pre-conception BMI, smoking, drinking, family history of diabetes mellitus, family history of hypertension, other supplement use, gestational diabetes mellitus, and gestational weight gain |
|              | GH-FA400-pre-conception   | 573    | 143          | 646        | 145             | 1.13 (0.92-1.39)            | age, education, work status, monthly income, ethnicity, history of abnormal pregnancy, pre-conception BMI, smoking, drinking, family history of diabetes mellitus, family history of hypertension, other supplement use, gestational diabetes mellitus, and gestational weight gain |
|              | PE-FA400-pre-conception   | 573    | 19           | 646        | 18              | 1.29 (0.66-2.51)            | age, education, work status, monthly income, ethnicity, history of abnormal pregnancy, pre-conception BMI, smoking, drinking, family history of diabetes mellitus, family history of hypertension, other supplement use, gestational diabetes mellitus, and gestational weight gain |
|              | GH-FA800-post-conception  | 1504   | 358          | 646        | 145             | 1.04 (0.87-1.24)            | age, education, work status, monthly income, ethnicity, history of abnormal pregnancy, pre-conception BMI, smoking, drinking, family history of diabetes mellitus, family history of hypertension, other supplement use, gestational diabetes mellitus, and gestational weight gain |
|              | PE-FA800-post-conception  | 1504   | 50           | 646        | 145             | 1.03 (0.59-1.79)            | age, education, work status, monthly income, ethnicity, history of abnormal pregnancy, pre-conception BMI, smoking, drinking, family history of diabetes mellitus, family history of hypertension, other supplement use, gestational diabetes mellitus, and gestational weight gain |
|              | GH-FA400-post-conception  | 1772   | 408          | 646        | 145             | 1.04 (0.88-1.24)            | age, education, work status, monthly income, ethnicity, history of abnormal pregnancy, pre-conception BMI, smoking, drinking, family history of diabetes mellitus, family history of hypertension, other supplement use, gestational diabetes mellitus, and gestational weight gain |
|              | PE-FA400-post-conception  | 1772   | 57           | 646        | 18              | 1.25 (0.72-2.18)            | age, education, work status, monthly income, ethnicity, history of abnormal pregnancy, pre-conception BMI, smoking, drinking, family history of diabetes mellitus, family history of hypertension, other supplement use, gestational diabetes mellitus, and gestational weight gain |
| Corsi, 2020  | PE              | 215    | 37           | 213        | 21              | 1.58 (0.95-2.63)            | age, smoking, parity, country, use of aspirin, and history of preeclampsia |
| Wen, 2018    | PE              | 1174   | 169          | 1157       | 156             | 1.10 (0.90-1.34)            | parity, maternal age, and cigarette smoking |
| Ocampo, 2017 | GH-pre-conception | 2697   | 95           | 79         | 3               | 0.91 (0.27-3.05)            | education, race/ethnicity, parity, pre-conception BMI, diabetes status, asthma status, autoimmune disease status, cigarettes smoked per day, maternal age, gravidity, previous spontaneous abortion or stillbirth, antidepressant use, cohort study and enrollment year |
|              | PE-pre-conception  | 2697   | 94           | 79         | 6               | 0.42 (0.17-1.05)            | education, race/ethnicity, parity, pre-conception BMI, diabetes status, asthma status, autoimmune disease status, cigarettes smoked per day, maternal age, gravidity, previous spontaneous abortion or stillbirth, antidepressant use, cohort study and enrollment year |
|              | GH-post-conception  | 471    | 14           | 79         | 3               | 0.76 (0.21-2.83)            | education, race/ethnicity, parity, pre-conception BMI, diabetes status, asthma status, autoimmune disease status, cigarettes smoked per day, maternal age, gravidity, previous spontaneous abortion or stillbirth, antidepressant use, cohort study and enrollment year |
|              | PE-post-conception  | 471    | 29           | 79         | 6               | 0.55 (0.21-1.46)            | education, race/ethnicity, parity, pre-conception BMI, diabetes status, asthma status, autoimmune disease status, cigarettes smoked per day, maternal age, gravidity, previous spontaneous abortion or stillbirth, antidepressant use, cohort study and enrollment year |

FA, folic acid; Vit-FA, multivitamin containing folic acid; HD-FA, high dose folic acid; LD-FA, low dose folic acid; GH, gestational hypertension; PE, pre-eclampsia; BMI, Body Mass Index; IQR, interquartile range; SD, standard deviation; NR, not reported.
| Wen, 2016 | PE-FA | 625 | 24 | 404 | 17 | 0.76 (0.36-1.62) | maternal age, previous health problem (chronic hypertension), history of preeclampsia, diabetes, smoking, and parity |
| Catov, 2009 | PE-pre-conception | 3034 | 96 | 404 | 17 | 0.75 (0.45-1.25) |
| Catov, 2011 | PE-post-conception | 4018 | 127 | 404 | 17 | 0.75 (0.46-1.23) |
| Liu, 2015 | PE | 7864 | 206 | 2315 | 109 | 0.56 (0.44-0.70) | NR |
| Wang, 2015 | PE | 794 | 238 | 265 | 115 | 0.61 (0.43-0.87) | maternal age, education level, parity, maternal diabetes, pre-conception BMI, weight gain during pregnancy, family monthly income per capita, multiple birth, maternal employment status, parity, maternal smoking in pregnancy and any miscarriages or stillbirths in previous pregnancies |
| Martinussen, 2015 | PE-pre-conception | 1590 | NR | 2057 | NR | 0.80 (0.60-1.20) | study (AIP or NIP), maternal age, maternal ethnicity, maternal education, maternal marital status, parity, maternal smoking in pregnancy and any miscarriages or stillbirths in previous pregnancies |
| | PE-post-conception | 3301 | NR | 346 | NR | 1.10 (0.60-2.10) |
| | BMI<25-PE-pre-conception | NR | NR | NR | NR | 0.60 (0.40-1.00) |
| | BMI<25-PE-post-conception | NR | NR | NR | NR | 1.00 (0.50-2.00) |
| | BMI>25-PE-pre-conception | NR | NR | NR | NR | 1.00 (0.50-2.00) |
| | BMI>25-PE-post-conception | NR | NR | NR | NR | 1.40 (0.50-3.50) |
| Kim, 2014 | PE | 134 | 6 | 81 | 12 | 0.27 (0.09-0.76) | parity, familial monthly income, preeclampsia and prenatal intake of folic acid |
| Vanderlelie, 2014 | PE-FA | 476 | 6 | 1066 | 31 | 0.33 (0.14-0.75) | maternal age, parity, gestational diabetes, indigenous status and maternal smoking |
| | PE- Vit-FA | 719 | 7 | 1066 | 31 | 0.42 (0.13-0.98) |
| | BMI<25-PE-FA | 283 | 4 | 616 | 12 | 0.72 (0.39-1.35) |
| | BMI<25-PE-Vit-FA | 406 | 4 | 616 | 12 | 0.60 (0.39-1.36) |
| | BMI>25-PE-FA | 193 | 5 | 450 | 21 | 0.55 (0.31-0.96) |
| | BMI>25-PE-Vit-FA | 313 | 3 | 450 | 21 | 0.48 (0.27-0.86) |
| Li, 2013 | GH | 32919 | 3226 | 100823 | 9477 | 1.09 (1.04-1.14) | age (continuous), body mass index (continuous), education, occupation, parity, and multiple births |
| | PE | 32919 | 888 | 100823 | 2420 | 1.21 (1.11-1.31) |
| Timmermans, 2011 | GH-pre-conception | 2362 | 165 | 1770 | 48 | 1.10 (0.70-1.70) | gestational age, age, BMI, parity, ethnicity, education, smoking, alcohol consumption, antenatal care |
| | GH-post-conception | 1861 | 87 | 1770 | 48 | 1.40 (0.90-2.10) |
| | PE-pre-conception | 2362 | 45 | 1770 | 39 | 0.80 (0.50-1.40) |
| | PE-post-conception | 1861 | 41 | 1770 | 39 | 0.90 (0.50-1.30) |
| | GH-pre-conception | 2362 | 165 | 1770 | 48 | 1.10 (0.70-1.70) |
| | GH-post-conception | 1861 | 87 | 1770 | 48 | 1.40 (0.90-2.10) |
| | PE-pre-conception | 2362 | 45 | 1770 | 39 | 0.80 (0.50-1.40) |
| | PE-post-conception | 1861 | 41 | 1770 | 39 | 0.90 (0.50-1.30) |
| Catov, 2011 | PE-FA | 2609 | 65 | 11503 | 265 | 1.08 (0.83-1.41) | NR |
| | PE-Vit-FA | 21789 | 479 | 11503 | 265 | 0.95 (0.82-1.11) |
| Catov, 2009 | PE-pre-conception- Vit-FA | 6655 | NR | 7582 | NR | 0.88 (0.70-1.10) | body mass index, smoking, parity, chronic hypertension, gestational age at recruitment, and partial multivitamin use |
| | PE-pre-conception- Vit-FA | 3666 | NR | 7582 | NR | 0.74 (0.56-0.98) |
| | PE-pre-conception-FA | NR | NR | NR | NR | 0.98 (0.50-1.92) |
| | PE-post-conception-FA | NR | NR | NR | NR | 0.95 (0.55-1.66) |
| | BMI<25-PE-pre-conception-Vit-FA | NR | NR | NR | NR | 0.78 (0.57-1.06) |
| | BMI>25-PE-pre-conception-Vit-FA | NR | NR | NR | NR | 0.63 (0.42-0.93) |

*Table 2 continues...*
| Study, Year | BMI<25-PE-pre-conception-Vit-FA | BMI<25-PE-post-conception-Vit-FA | PE-pre-conception<1year | PE-pre-conception>1year | Maternal age, body mass index, race and ethnicity, educational level, marital status, smoking, parity and history of prior preterm birth |
|-------------|---------------------------------|---------------------------------|------------------------|------------------------|------------------------------------------------------------------------------------------------------------------|
| Bukowski, 2009 | 12444 295 15259 381 | 6777 157 15259 381 | 1.03 (0.86-1.23) | 1.05 (0.85-1.31) | maternal age, body mass index, race and ethnicity, educational level, marital status, smoking, parity and history of prior preterm birth |
| Wen, 2008 | 2713 59 238 12 | 421 12 238 12 | 0.37 (0.18-0.75) | 0.46 (0.16-1.31) | maternal age, ethnic background, educational level, parity, previous preeclampsia, chronic hypertension, diabetes, pre-conception body mass index, household income, gestational age at recruitment, and cigarette smoking during pregnancy |
| Oken, 2007 | 1031 24 238 12 | 1682 35 238 12 | 0.46 (0.23-0.91) | 0.41 (0.22-0.78) | maternal age, race (black, Hispanic, white, other), education (college graduate, < College graduate), and parity (0, 1+) |
| Bodnar, 2006 | 860 33 975 43 | 860 21 975 42 | 0.29 (0.12-0.65) | 1.08 (0.52-2.25) | race (Black, other), marital status (married, unmarried), parity (primiparous, multiparous), pre-conceptional physical activity (yes, no), and poverty index ratio (130%, 131–299%, 300%) |
| Charles, 2005 | 448 8 1890 51 | 459 7 1890 51 | 0.46 (0.20-1.05) | 0.59 (0.26-1.32) | smoking at booking, social class, parity, gestation at booking, height of women, weight of women at booking and gestation at delivery |
| Hernández-Díaz, 2002 | 448 8 1890 51 | 459 7 1890 51 | 0.53 (0.36-0.80) | 0.63 (0.28-1.45) | geographic region, family income, and maternal age, pre-conception weight, parity, twin pregnancy, diabetes, smoking, and education |

FA, folic acid; Vit-FA, multivitamin containing folic acid; HD-FA, high dose folic acid; LD-FA, low dose folic acid; GH, gestational hypertension; PE, pre-eclampsia; BMI, Body Mass Index; HR, hazard ratio; OR, odds ratio; RR, relative risk; CI, confidence interval; NR, not reported
may be most effective to prevent PE which was consistent with our finding that post-conception FA could reduce the risk of PE rather than pre-conception FA (22). It should be noted that pre-conception FA is a necessary measure to prevent neural tube defects (NTDs). Therefore, it is a more clinically appropriate choice to extend the use of FA into the later trimesters rather than starting it after pregnancy.

Another finding from our analysis was that FA supplementation could have a significant protective effect among women with pre-conception BMI<25 kg/m² but not statistically significant among overweight women (pre-conception BMI≥25 kg/m²). Firstly, the pathogenic mechanisms of PE may be different among women with BMI<25 kg/m² and BMI≥25 kg/m². The excess risk of PE might be associated with overweight or even obese status. Additionally, most pregnant women take low dose of FA, but both FA alone and multivitamin may not be adequate to overcome the metabolic disturbances of overweight or obese status (7, 11).

Our meta-analysis had several advantages. First, RCTs of HD-FA supplementation were analyzed separately from LD-FA to avoid bias, as the relationship between HD-FA and PE is still controversial. In addition, we did the subgroup analysis and the results demonstrated that post-conception LD-FA and pre-conception BMI<25 kg/m² were protective factors for pregnant women. We also acknowledged some limitations in this study. First, it has been recommended for women planning pregnancy to use FA until the third month of pregnancy to prevent NTDs. However, the discontinued time of FA is unclear for the pregnant women included in this study, particularly pre-conception FA. Therefore, the association of post-conception FA and the decreased risk of PE need to be further confirmed. Secondly, most studies included in this study did not consider the effects of serum FA and serum Hcy. Meanwhile, the genotype of MTHFR was not considered in most studies but it was also an important factor because the studies included came from different countries. And it was difficult to accurately evaluate the dose of dietary FA, which could cause the bias of final results. In addition, although the result indicated that Vit-FA might be more meaningful than FA alone to reduce the risk of PE, it was hard to further compare the effects of FA with Vit-FA due to the few clinical studies. Lastly, only few studies have been conducted in pregnant women with high-risk (20.22-23). Thus, we need more high-quality clinical studies to confirm the findings of this study, such as detailed documentation for FA dose, start and end time, form of FA, high-risk factors, and detection of genotype of MTHFR and serum FA.

CONCLUSIONS
Our meta-analysis found that LD-FA tended to decrease the risk of PE. In addition, post-conception LD-FA and pre-conception BMI<25 kg/m² were considered as protective factors to reduce the risk of PE. Therefore, we recommend that pregnant women use LD-FA throughout the pregnancy period to avoid the occurrence of PE. More importantly, a more convincing result require the inclusion of more rigorous RCTs.

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CONFLICT OF INTEREST
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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SUPLIMETARY DATA

Appendix 1

PUBMED: 251

("vitamin b9"[Text Word] OR "folic acid"[Text Word]) AND ("preeclampsia"[Text Word] OR "pregnancy induced hypertension"[Text Word]) OR "gestational hypertension"[Text Word])

EMBASE: 950

|   |   |   |
|---|---|---|
| 1 | *folic acid/ or vitamin b9.mp. | 23490 |
| 2 | folic acid.mp. | 77966 |
| 3 | *preeclampsia/ or preeclampsia.mp. | 63544 |
| 4 | gestational hypertension.mp. or *maternal hypertension/ | 9880 |
| 5 | pregnancy induced hypertension.mp. | 5374 |
| 6 | hypertensive disorders of pregnancy.mp. | 3420 |
| 7 | 1 or 2 | 78075 |
| 8 | 3 or 4 or 5 or 6 | 70227 |
| 9 | 7 and 8 | 950 |

COCHRANE: 93

#1 (vitamin b9):ti,ab,kw
#2 MeSH descriptor: [Folic Acid] this term only
#3 (folic acid):ti,ab,kw
#4 (#1) OR (#2) OR (#3)
#5 MeSH descriptor: [Pre-Eclampsia] this term only
#6 (preeclampsia):ti,ab,kw
#7 (pre-eclampsia):ti,ab,kw
#8 MeSH descriptor: [Hypertension, Pregnancy-Induced] this term only
#9 (pregnancy induced hypertension):ti,ab,kw
#10 (gestational hypertension):ti,ab,kw
#11 (#5) OR (#6) OR (#7) OR (#8) OR (#9 OR #10)
#12 (#11) AND (#4)
Appendix 2. Table A. Quality of observational studies (indicators from New-Castle-Ottawa scale)

| Study                  | 1a | 2b | 3c | 4d | 5Ae | 5Bf | 6g | 7h | 8i | Total quality scores |
|------------------------|----|----|----|----|-----|-----|----|----|----|---------------------|
| Li, 2020               | Yes| Yes| Yes| Yes| Yes | Yes | Yes| Yes| Yes| 9                   |
| Ocampo, 2017           | Yes| Yes| Yes| Yes| Yes | Yes | Yes| Yes| Yes| 9                   |
| Wen, 2016              | Yes| Yes| Yes| Yes| No  | Yes | Yes| Yes| Yes| 8                   |
| Liu, 2015              | Yes| Yes| Yes| Yes| No  | Yes | Yes| Yes| Yes| 7                   |
| Wang, 2015             | Yes| Yes| Yes| Yes| Yes | Yes | Yes| Yes| Yes| 9                   |
| Martinussen, 2015      | Yes| Yes| Yes| Yes| Yes | Yes | Yes| Yes| Yes| 9                   |
| Kim, 2014              | Yes| Yes| No  | Yes| Yes | Yes | Yes| Yes| Yes| 8                   |
| Vanderlelie, 2014      | Yes| Yes| Yes| Yes| Yes | Yes | Yes| Yes| Yes| 9                   |
| Li, 2013               | Yes| Yes| Yes| Yes| Yes | Yes | Yes| Yes| Yes| 9                   |
| Timmermans, 2011       | Yes| Yes| Yes| Yes| Yes | Yes | Yes| Yes| Yes| 9                   |
| Catov, 2011            | Yes| Yes| Yes| Yes| No  | Yes | Yes| Yes| Yes| 7                   |
| Catov, 2009            | Yes| Yes| Yes| Yes| Yes | Yes | Yes| Yes| Yes| 9                   |
| Bukowski, 2009         | Yes| Yes| No  | Yes| Yes | Yes | No | Yes| Yes| 7                   |
| Wen, 2008              | Yes| Yes| Yes| Yes| Yes | Yes | Yes| Yes| Yes| 9                   |
| Oken, 2007             | Yes| Yes| Yes| Yes| Yes | Yes | Yes| Yes| Yes| 9                   |
| Bodnar, 2006           | Yes| Yes| Yes| Yes| Yes | Yes | Yes| No | Yes| 9                   |
| Hernández-Díaz, 2002   | Yes| Yes| Yes| Yes| Yes | Yes | Yes| Yes| No | 8                   |

a: Indicates exposed cohort truly representative  
b: Non-exposed cohort drawn from the same community  
c: Ascertainment of exposure from the same community  
d: Outcome of interest not present at start of study  
e: Cohorts comparable on basis of site and etiology of infection  
f: Cohorts comparable on others factors  
g: Assessment of outcome of record linkage or independent blind assessment  
h: Follow-up long enough for outcomes to occur  
i: Complete accounting for cohort

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Appendix 3 Figure 1

Appendix 3 Figure 2
