

Review Article

Periconceptional folic acid fortification for the risk of gestational hypertension and pre-eclampsia: a meta-analysis of prospective studies

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Abstract

Published literatures report controversial results about the association of folic acid-containing multivitamins with gestational hypertension and pre-eclampsia. A comprehensive search was performed to identify related prospective studies to assess the effect of folic acid fortification on gestational hypertension and pre-eclampsia. The Q test and I^2 statistic were used to examine between-study heterogeneity. Fixed or random effects models were selected based on study heterogeneity. A funnel plot and modified Egger linear regression test were used to estimate publication bias. Eleven studies conformed to the criteria. Pooled results indicated that folic acid fortification alone was not associated with the occurrence of gestational hypertension [relative risk (RR) = 1.03, 95% confidence interval (CI): 0.98–1.09, $P = 0.267$] and pre-eclampsia (RR = 0.99, 95% CI: 0.90–1.08, $P = 0.738$). However, supplementation of multivitamins containing folic acid could prevent gestational hypertension (RR = 0.57, 95% CI: 0.43–0.76, $P < 0.001$) and pre-eclampsia (RR = 0.64, 95% CI: 0.48–0.84, $P = 0.001$). The difference between folic acid fortification alone and multivitamins containing folic acid was significant. This meta-analysis suggests that periconceptional multivitamin supplementation with appropriate dose, not folic acid alone, is an appropriate recommendation for pregnant women. The effect should be further confirmed by conducting large-scale randomised controlled trials.

Keywords: folic acid, multivitamins, gestational hypertension, pre-eclampsia, meta-analysis.

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Introduction

Gestational hypertension and pre-eclampsia are the most common hypertensive disorders associated with pregnancy. Ten per cent of women have high blood pressure during pregnancy, and pre-eclampsia complicates 2–8% of pregnancies (Duley 2009). They are also major causes of maternal and perinatal morbidity and mortality worldwide (World Health Organization 2005). Hypertension in pregnancy is also associated with an increased risk for subsequent cardiovascular disease or diabetes mellitus later in life (Feig *et al.* 2013; Charlton *et al.* 2014; Cusimano

et al. 2014). Identifying and characterising modifiable risk factors of gestational hypertension and pre-eclampsia remain important for public health and clinical medicine.

In the past decades, many research studies have focused on periconceptional folic acid fortification as a gestational hypertension or pre-eclampsia preventive factor. Further findings from these studies showed that there is a significant elevation in plasma or serum homocysteine among women with gestational hypertension or pre-eclampsia in pregnancy; thus, homocysteine may be an independent risk factor for these disorders (Cotter *et al.* 2003; Makedos *et al.*

2007; Dodds *et al.* 2008; Murphy & Fernandez-Ballart 2011; Bergen *et al.* 2012). Hyperhomocysteinaemia can usually be ameliorated by folic acid supplementation (Maron & Loscalzo 2009; Scorsatto *et al.* 2011; Sudchada *et al.* 2012; Yamamoto *et al.* 2012). Thus, a research hypothesis was formed on that administering of folic acid-containing multivitamins can help prevent the occurrence of gestational hypertension or pre-eclampsia. In addition, a number of extensive evidence have demonstrated that adequate folate intake protects against neural tube defects; hence, folic acid fortification became a general recommendation for pregnant women (Ray & Mamdani 2002; Ray *et al.* 2002; De Wals *et al.* 2007). The associations between folic acid fortification and other pregnancy outcomes, such as gestational hypertension and pre-eclampsia, has become a research hot spot. Numerous epidemiological studies have explored the influence of folic acid supplementation on gestational hypertension and pre-eclampsia. However, their reported results are inconsistent (Bukowski *et al.* 2009; Catov *et al.* 2009; Timmermans *et al.* 2011; Li *et al.* 2013; Vanderlelie *et al.* 2014). Individual studies could not draw solid conclusions, and no meta-analysis to explore the effect of folic acid fortification on gestational hypertension and pre-eclampsia has been conducted. Thus, we performed this meta-analysis of prospective studies to assess whether periconceptional folic acid fortification for pregnant women is associated with a decreased risk of subsequent gestational hypertension and pre-eclampsia.

Materials and methods

The meta-analysis of observational studies in epidemiology (MOOSE) guidelines were followed (Stroup *et al.* 2000).

Search strategy

Studies were identified using a search strategy in the PubMed, Scopus, Web of Science and Cochrane library to 8 December 2014. Search items were listed as follows: (gestational hypertension OR pregnancy-induced hypertension OR hypertension of pregnancy OR preeclampsia OR pre-eclampsia OR eclampsia) AND (folic acid OR folate OR micronutrient* OR vitamin* OR multivitamin*). Moreover, we reviewed the reference lists from retrieved articles to search for further relevant studies. When same data were reported in more than one publication, only studies with more complete data and more extensive interval of enrolment were included in the study.

Eligibility criteria

Each selected study was independently reviewed by two investigators (Yang and Chen) to determine whether it was eligible for inclusion in this meta-analysis. The inclusion criteria were as follows: (1) prospective study; (2) exposure of interest was folic acid or multivitamins containing folic acid; (3) outcome of interest was gestational hypertension or pre-eclampsia; (4) results with 95% confidence interval (CI; or data to calculate them) adjusted for confounding factors were provided; (5) no restriction in publication date, publication language and publication status; and (6) animal studies, reviews, comments and editorials were excluded. Any disagreement was settled by discussion.

Data extraction and quality assessment

A data extraction sheet was developed that included first author, publication year, country where the study was conducted, characteristics of study population,

Key messages

- Folic acid fortification alone did not prevent gestational hypertension.
- Multivitamins containing folic acid were associated with reduced risk of gestational hypertension.
- Folic acid fortification alone did not prevent pre-eclampsia.
- Multivitamins containing folic acid were associated with reduced risk of pre-eclampsia.
- The stable result and optimal dose for multivitamins should be further estimated.

number of total participants, number of total events, the folic acid fortification start time, study results with 95% CI for folic acid on gestational hypertension or pre-eclampsia, and adjusted confounding factors. All data were extracted independently by two reviewers (Yang and Chen). In cases of disagreement between the two reviewers, a consensus was achieved through discussion among all of the authors. The 9-star Newcastle–Ottawa Scale (Wells *et al.* 2000) was used to estimate the quality of each selected prospective study.

Statistical analysis

The type of folic acid fortification was categorised into two groups, namely folic acid fortification alone and multivitamins containing folic acid. Pooled measure was calculated as the inverse variance-weighted mean of the logarithm of relative risks (RRs) with 95% CI to assess the effect of folic acid fortification on gestational hypertension and pre-eclampsia. The Q test and I^2 were used to assess heterogeneity among the included studies (Higgins & Thompson 2002). In the presence of substantial heterogeneity ($I^2 > 50\%$) (Higgins *et al.* 2003), the DerSimonian and Laird random effect model (REM) was adopted as the pooling method; otherwise, the fixed effect model (FEM) was used as the pooling method. Meta-regression with restricted maximum likelihood estimation was performed to assess the potentially important covariates exerting substantial impact on between-study heterogeneity. Subgroup analysis for the type of folic acid fortification was further explored. The ‘leave one out’ sensitive analysis was carried out using $I^2 > 50\%$ as the criteria to evaluate the key studies with substantial impact on between-study heterogeneity (Patsopoulos *et al.* 2008). An influence analysis was conducted to describe how robust the pooled estimator was (Tobias 1999). Publication bias was estimated using Egger’s regression asymmetry test (Egger *et al.* 1997). All statistical analyses were performed with STATA version 13.1 (Stata Corporation, College Station, TX, USA). All reported probabilities (P -value) were two-sided, and less than 0.05 was considered statistically significant.

Results

Study characteristics

The detailed selection process was illustrated in Fig. 1 and we ultimately identified 11 eligible articles (Hernandez-Diaz *et al.* 2002; Ray & Mamdani 2002; Merchant *et al.* 2005; Oken *et al.* 2007; Wen *et al.* 2008; Bukowski *et al.* 2009; Catov *et al.* 2009; Timmermans *et al.* 2011; Li *et al.* 2013; Vanderlelie *et al.* 2014) out of 3942 articles for this meta-analysis. Among them, one was a prospective clinical trial (Merchant *et al.* 2005) and the rest were prospective observational studies. The total eligible pregnant women numbered 1 276 063 persons, out of which 78 105 cases of gestational hypertension and 44 608 cases of pre-eclampsia occurred during pregnancy. Two studies (Catov *et al.* 2009; Timmermans *et al.* 2011) reported separate outcomes for folic acid fortification start time. General characteristics of the included studies were shown in Table 1. Of these studies, six were conducted in North America, two in Europe, one in China, one in Australia and one in

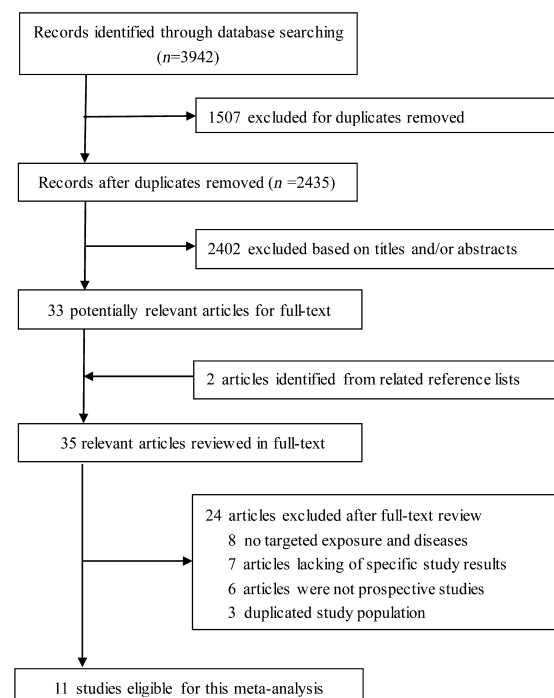


Fig. 1. Flow diagram of studies selection in the meta-analysis.

Table 1. Description of the prospective studies included in the meta-analysis

Author (year)	Country	Total samples	Mean age	Exposure	Time	Disease	Cases	RR (95% CI)	Quality	Adjusted factors
Hernandez-Diaz <i>et al.</i> (2002)	United States and Canada	2 151	29.3	MvFa	Precon	GH PE	157 47	0.53 (0.36–0.80) 0.63 (0.28–1.45)	7	Geographic region, family income, age, prepregnancy weight, parity, twin pregnancy, diabetes, smoking and education Age and year of delivery
Ray <i>et al.</i> (2002)	Canada	1 001 441	28.9	Fa	Precon	GH PE	58965 37880	0.98 (0.95–1.01) 0.94 (0.91–0.97)	7	
Merchant <i>et al.</i> (2005)	Tanzania	1 078	24.7	MvFa	Postcon	GH	89	0.62 (0.40–0.94)	8	Randomisation, adjusted for all possible confounders
Bodnar <i>et al.</i> (2006)	United States	1 835	24.7	MvFa	Pericon	PE	75	0.55 (0.32–0.95)	8	Race, marital status, parity, preconceptional physical activity, and poverty index ratio
Oken <i>et al.</i> (2007)	United States	1 718	31.2	Fa	Postcon	GH PE	119 59	1.02 (0.99–1.06) 0.96 (0.89–1.03)	8	Age, BMI, first-trimester systolic blood pressure, race, education, and parity, total energy intake
Wen <i>et al.</i> (2008)	Canada	2 951	32.2	Fa MvFa	Postcon	PE	71	0.46 (0.16–1.31) 0.37 (0.18–0.75)	8	Age, ethnicity, education, parity, history of pre-eclampsia, chronic hypertension, diabetes, BMI, household income, gestational age at recruitment and smoking
Bukowski <i>et al.</i> (2009)	United States	34 480	30.0	Fa	Precon	PE	883	1.04 (0.90–1.19)	9	Age, BMI, race and ethnicity, education, marital status, smoking, parity and history of prior preterm birth
Catov <i>et al.</i> (2009)	Denmark	28 601	29.4	MvFa	Precon Postcon	PE Postcon	668	0.88 (0.70–1.10) 0.74 (0.56–0.98)	9	BMI, smoking, parity, chronic hypertension, gestational age at recruitment and partial multivitamin use
Timmermans <i>et al.</i> (2011)	The Netherlands	5 993	29.9	Fa	Postcon Precon Postcon	GH PE Postcon	301 125	1.40 (0.90–2.10) 1.10 (0.70–1.70) 0.90 (0.50–1.30)	8	Age, gestational age at time of measurement, BMI, parity, ethnicity, education, smoking, alcohol antenatal care
Li <i>et al.</i> (2013)	China	193 554	24.9	Fa	Postcon	GH	18474	1.08 (1.04–1.11)	9	Age, BMI, education, occupation, parity and multiple births
Vanderliele <i>et al.</i> (2014)	Australia	2 261	28.2	Fa MvFa	Postcon	PE	44	1.11 (1.04–1.18) 0.42 (0.13–0.98) 0.33 (0.14–0.75)	8	Age, parity, gestational diabetes, indigenous status and maternal smoking

BMI, body mass index; CI, confidence interval; Fa, folic acid fortification; GH, gestational hypertension; MvFa, multivitamins containing folic acid; PE, pre-eclampsia; Pericon, periconception; Postcon, post-conception; Precon, preconception; RR, relative risk; Time, the starting time of folic acid fortification.

Tanzania. All included studies met the quality criteria with scores ranging from 7 to 9 stars.

Quantitative synthesis

Folic acid fortification on gestational hypertension

The association of folic acid fortification with gestational hypertension was presented in Fig. 2. Pooled results indicated that the improvement of folic acid alone was not associated with the occurrence of gestational hypertension (REM: RR = 1.03, 95% CI: 0.98–1.09, $P = 0.267$), but supplementation of multivitamins containing folic acid was associated with a decreased risk of gestational hypertension (FEM:

RR = 0.57, 95% CI: 0.43–0.76, $P < 0.001$). The categorised difference was statistically significant ($P = 0.012$).

Folic acid fortification on pre-eclampsia

The REM was applied because I^2 of between-study heterogeneity was more than 50%. The association between folic acid fortification and pre-eclampsia was presented in Fig. 3. Pooled results indicated that the improvement of folic acid alone was not associated with pre-eclampsia (REM: RR = 0.99, 95% CI: 0.90–1.08, $P = 0.738$), but supplementation of multivitamins containing folic acid could decrease the risk of pre-eclampsia (REM: RR = 0.64, 95% CI: 0.48–0.84,

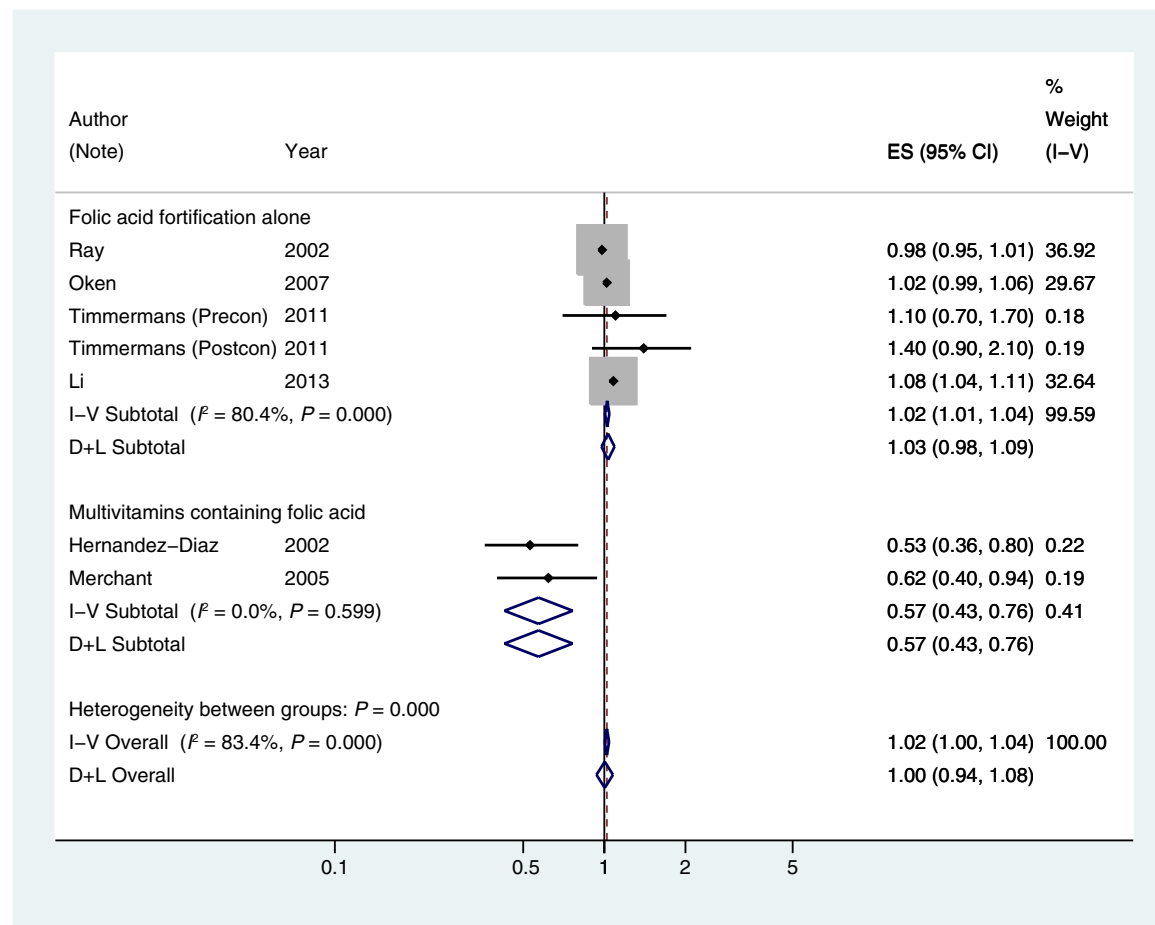


Fig. 2. Forest plot of RR of the association between folic acid fortification and gestational hypertension. Postcon, post-conception; Precon, preconception; RR, relative risk.

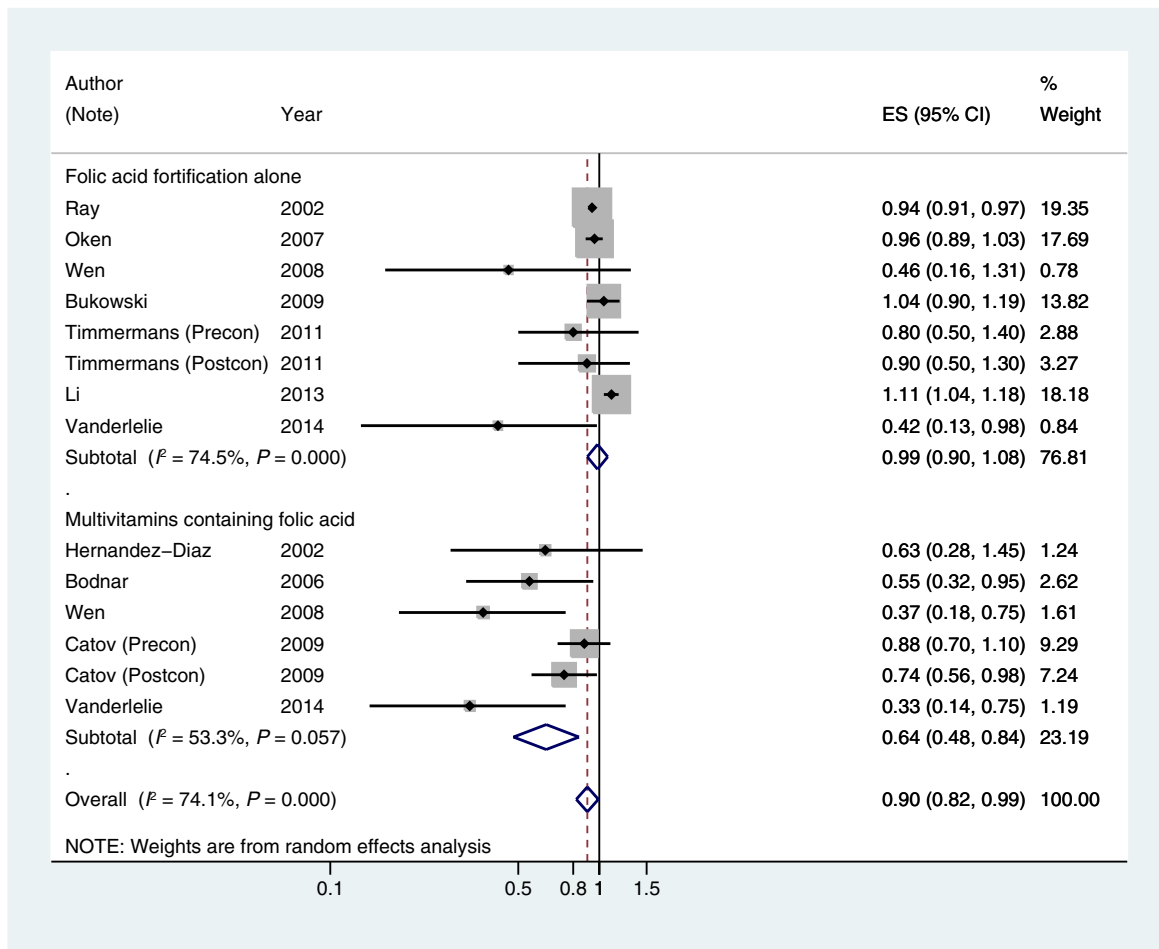


Fig. 3. Forest plot of RR of the association between folic acid fortification and pre-eclampsia. Postcon, post-conception; Precon, preconception; RR, relative risk.

$P = 0.001$). The categorised difference was significant ($P = 0.020$).

Sources of heterogeneity and sensitivity analysis

The between-study heterogeneity ($I^2 > 50\%$) could be found among nearly all analyses except for multivitamins containing folic acid and gestational hypertension. The univariate meta-regression, with the covariates of folic acid fortification start time (pre-conception and post-conception), published year, location (categorised as North America, European and Other), maternal body mass index (BMI)

(adjusted and non-adjusted), mean age, sample size and study quality, showed that no aforementioned covariates had a significant impact on between-study heterogeneity.

In the sensitivity analysis, Li *et al.*'s (2013) study was the key contributor to between-study heterogeneity for folic acid fortification alone on gestational hypertension and pre-eclampsia, and Catov *et al.*'s (2009) study for multivitamins containing folic acid on pre-eclampsia. After excluding the two studies, the meta-analysis showed a modest protective effect of folic acid intake on pre-eclampsia (FEM: RR = 0.95, 95% CI: 0.92–0.97, $P < 0.001$). The other two pooled results did not change substantially.

Influence and publication bias analyses

Both before and after excluding the two studies as the key contributors to between-study heterogeneity, no individual study was found to have excessive influence on the pooled effect. Before excluding the Catov's study, significant publication bias was found in the multivitamins containing folic acid on pre-eclampsia studies ($P = 0.014$). The trim and fill analysis showed that multivitamins containing folic acid still reduced the risk of pre-eclampsia (REM: RR = 0.67, 95% CI: 0.51–0.89, $P = 0.006$). No significant publication bias was observed in any other analyses, both before and after excluding the two studies identified as the key contributors to between-study heterogeneity.

Discussion

To the best of our knowledge, this study is the first meta-analysis conducted to specifically investigate the association of folic acid fortification with incidence of gestational hypertension and pre-eclampsia. The results of this study indicated that folic acid fortification alone did not prevent the occurrence of gestational hypertension and pre-eclampsia. However, the results suggested that supplementation of multivitamins containing folic acid could reduce the risk of gestational hypertension and pre-eclampsia.

In this meta-analysis, between-study heterogeneity was found among some groups based on the tentative categorisation of I^2 values (Higgins *et al.* 2003). An indeterminate number of characteristics that varied among studies (such as starting time of folic acid fortification, maternal BMI, published year, ethnicity, age, sample size and quality) could be the sources of between-study heterogeneity. Therefore, we applied meta-regression and 'leave one out' sensitivity analysis to explore the potential important causes of between-study heterogeneity for both covariates and studies. Our meta-analysis did not identify any of the aforementioned covariates as being an important contributor to between-study heterogeneity. The aetiology of gestational hypertension and pre-eclampsia is complex and unclear (Roberts *et al.*

2003). Although the aforementioned covariates were not found to be important sources of disease–effect heterogeneity across the studies in this meta-analysis, other variables, as well as their possible interaction, may well be potential contributors to this disease–effect unconformity. In this respect, the lack of relevant study-level covariates in the reported articles precluded a more robust assessment of sources of this heterogeneity.

In the 'leave one out' sensitivity analysis, two studies (Catov *et al.* 2009; Li *et al.* 2013) were identified as being possible key contributors to between-study heterogeneity. After carefully evaluating the two studies, they were entirely appropriate for the current meta-analysis, except for the potential ethnic difference. We did not advise excluding the two studies.

Although publication bias was detected in the multivitamins containing folic acid on pre-eclampsia studies, the trim and fill analysis showed that multivitamins containing folic acid still reduced the risk of pre-eclampsia. No significant influence and publication bias were observed in the other analyses.

The mechanism in folic acid preventing cardiovascular disease focuses on its critical role in lowering plasma homocysteine, which is found to be associated with a higher risk of cardiovascular disease from epidemiological studies (Moat *et al.* 2004). Many studies in the past decades show that women with gestational hypertension or pre-eclampsia have a significant elevation of homocysteine post-partum, in the third trimester, in the second trimester or even in early pregnancy (Cotter *et al.* 2003; Makedos *et al.* 2007; Dodds *et al.* 2008; Murphy & Fernandez-Ballart 2011; Bergen *et al.* 2012). Taking this emerging evidence into consideration, in combination with other data implicating hyperhomocysteinaemia as an independent risk factor for vascular disease, some researchers hypothesised that hyperhomocysteinaemia may be an independent risk factor for gestational hypertension (Roberts & Cooper 2001). However, recent trials have failed to demonstrate a benefit of lowering homocysteine for cardiovascular disease (Albert *et al.* 2008; Ebbing *et al.* 2010). Steegers-Theunissen *et al.* (2004) contend that, in most cases, hyperhomocysteinaemia may be a consequence rather than

a cause of hypertensive disorders in pregnancy. Folic acid fortification can effectively decrease homocysteine (Maron & Loscalzo 2009; Scorsatto *et al.* 2011; Sudchada *et al.* 2012; Yamamoto *et al.* 2012). Thus, theoretically, if elevated homocysteine is a cause of gestational hypertension or pre-eclampsia, periconceptional folic acid fortification may significantly decrease the incidence of these outcomes. However, our study results did not find a protective effect of folic acid supplementation alone on gestational hypertension or pre-eclampsia, but rather, multivitamins containing folic acid could reduce the risk of gestational hypertension and pre-eclampsia.

Some possible reasons for the current outcome include the following. Firstly, some ingredients in multivitamins may play important roles in preventing gestational hypertension and pre-eclampsia. For instance, either maternal vitamin B6 or vitamin D deficiency is an independent risk factor for pre-eclampsia (Thaver *et al.* 2006; Hovdenak & Haram 2012; Bodnar *et al.* 2014; Brodowski *et al.* 2015). Secondly, the joint effect of multivitamins, such as folic acid, vitamins B6 and B12, may synergistically affect the development of hypertension. For example, a recent meta-analysis indicated that folic acid supplementation has no significant effect on revascularisation, but a combination of folic acid and vitamin B6 may be beneficial for revascularisation (Qin *et al.* 2014). Thirdly, with the development of modern society and the implementation of mandatory folic acid fortification since 1998, the folic acid demand during pregnancy may be supplied adequately from dietary intake of folic acid (Lawrence *et al.* 1999, Global Progress 2013). A dose-response study showed that the decrease of plasma homocysteine achieved with more than $600 \mu\text{g day}^{-1}$ folic acid was not obvious (Van Oort *et al.* 2003). Thus, when folic acid supplement is adequate, its effect for gestational hypertension and pre-eclampsia may be not significant. Although our study implied that periconceptional multivitamins containing folic acid supplementation could reduce the risk of gestational hypertension and pre-eclampsia, the potential adverse effects, such as early fetal death (Nohr *et al.* 2014), should be considered in clinical application for

multivitamin supplementation. Therefore, large-scale randomised controlled trials with appropriate dose at appropriate times during pregnancy are recommended to estimate the impact of multivitamins containing folic acid supplement on pregnant women.

Strengths and weaknesses

This meta-analysis had several strengths. This study included prospective studies to determine the risk of gestational hypertension and pre-eclampsia over time. It also had a large sample size and our included studies were adjusted for potential confounding, which increased the accuracy of the effect estimate. However, the potential limitations of this meta-analysis should be considered. Firstly, exposures cited by the included studies were mainly folic acid supplement rather than the real folic acid intake and the vitamins' dose varied in each study, which may influence the pooled estimated value. Secondly, although significant protective function of multivitamin containing folic acid on gestational hypertension and pre-eclampsia was found, the recurring result should be confirmed in the future, for a few samples included. Thirdly, strong heterogeneity was observed in our study. Although we applied meta-regression and sensitive analysis to explore it, the source of heterogeneity was not determined. Fourthly, due to our inability to obtain raw data, we could only perform a study-level but not a patient-level meta-analysis, which would have enabled us to control some confounders, such as maternal BMI, smoking, physical activity. Lastly, the different subtypes and definitions of gestational hypertension or pre-eclampsia may affect the pooled estimated result.

Conclusion

This meta-analysis suggested that periconceptional multivitamins containing folic acid supplementation could reduce the risk of gestational hypertension and pre-eclampsia, whereas folic acid fortification alone did not prevent the occurrence of gestational hypertension and pre-eclampsia. Large-scale randomised controlled trials are recommended to confirm the impact of multivitamins containing folic acid sup-

plement on the incidence rates of gestational hypertension and pre-eclampsia during pregnancy.

Source of funding

None.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Contributions

XY performed the literature searches, study selection, data extraction, data analyses, data interpretation and wrote the initial draft of the manuscript. HC involved in literature searches, study selection, data extraction, data analyses. YD and SW solved disagreements regarding study selection and risk of bias assessment, and critically revised the article. ZW contributed to the study design, interpreted the data and revised the initial draft. All authors contributed to the editing of the final version of the manuscript.

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