Calcium and vitamin D supplementation for prevention of preeclampsia: A systematic review and network meta-analysis

WIN KHAING\textsuperscript{1,2}, MBBS MMedSc, SAKDA ARJ-ONG VALLIPAKORN\textsuperscript{1*}, MD, PhD
VISASIRI TANTRAKUL\textsuperscript{3}, MD, MARK MCEVOY\textsuperscript{4}, PhD, JOHN ATTIA\textsuperscript{4}, MD, PhD,
AMMARIN THAKKINSTIAN\textsuperscript{1}, PhD

\textsuperscript{1}Section for Clinical Epidemiology and Biostatistics, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
\textsuperscript{2}Department of Preventive and Social Medicine, University of Medicine, Mandalay, Myanmar
\textsuperscript{3}Sleep Disorder Centre, Division of Pulmonary and Critical Care, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
\textsuperscript{4}School of Medicine and Public Health, Faculty of Health and Medicine, The University of Newcastle, Australia

*Corresponding Author:
Sakda Arj-Ong Vallipakorn, MD, MSIT., MA.IS, PhD
Section for Clinical Epidemiology and Biostatistics, 3rd Floor, Research Center Building, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.
270 RAMA VI Road. Rachatevi, Bangkok 10400, Thailand.
Tel. (+66)2-201-1269 Fax. (+66)2-201-1284
e-mail: dr.sakda@gmail.com

Word Count: Abstract = 243 words, Manuscript = 3231 words
Number of references = 49
Number of tables = 4
Number of figures = 8
ABSTRACT

Background: Effect of calcium supplementation for reducing risk of preeclampsia has been established but effects of vitamin D with or without calcium are controversial. We aim to conduct a systematic review and a network meta-analysis comparing supplementation effects of calcium, vitamin D, both or neither on preeclampsia.

Methods: We searched Medline and Scopus databases from inception to December 2015. A randomised controlled trials (RCTs) was selected if they studied in pregnancy, had any pair of interventions (calcium, vitamin D, both, or placebo) and preeclampsia as the outcome. Contingency-data between interventions and outcome were extracted. A two-step network-meta-analysis was used to indirectly estimated supplement effects.

Results: Twenty RCTs (26942 women) were eligible. Direct pooled Risk Ratios (RRs) for calcium vs placebo, vitamin D vs placebo and calcium plus vitamin D vs placebo were 0.52 (95%CI: 0.39, 0.68), 0.33 (95%CI: 0.01, 7.84) and 0.49 (95%CI: 0.31, 0.77), respectively. A network meta-analysis provided placebo-control effects of vitamin D, calcium plus vitamin D, and calcium alone with the pooled RRs of 0.33 (95% CI: 0.01, 8.94); 0.43 (0.22, 0.83); 0.49 (0.34, 0.69), respectively. None of active-controls was significant. Estimated ranking probabilities were: vitamin D (50.2%), calcium plus vitamin D (29.4%) and calcium (19.7%).

Conclusions: Vitamin D supplementation alone might be best for prevention of preeclampsia, followed by calcium plus vitamin D and calcium alone. The evidences were based on a few RCTs, larger-well-designed RCTs are still required, or update this network meta-analysis once more RCTs are available.

Keywords: calcium, network meta-analysis, preeclampsia, prevention, systematic review, vitamin D
INTRODUCTION

Preeclampsia is a new onset of high blood pressure with proteinuria with/without end-organ or utero-placental dysfunction after 20 weeks of gestation. It is one of the major contributing causes of maternal-foetal morbidity and mortality worldwide [1]. Its incidence is similar in South-East Asia and North African regions (i.e., 1.51% and 1.56%, respectively) [2] but higher in South Africa (i.e., 1.8% to 7.1%) [3].

Approximately 10% to 15% of maternal death is directly associated with preeclampsia or eclampsia in low- and middle-income countries [4]. It also related to unfavourable outcomes in both mother (e.g., placental abruption, preterm birth and haemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome, etc.) and foetus (e.g., stillbirth, low birth weight, and small for gestational age, etc.) [5, 6].

Some evidences showed an inverse relationship between high blood pressure and calcium intake [7]. Numerous epidemiological and clinical studies,[7-9] and later a series of systematic reviews [10-13] also demonstrated this association. Their results suggested that calcium supplements (≥1 g/day) could lower the risk of preeclampsia [12]. As a result, the World Health Organization (WHO) has recommended to supplement calcium for pregnant women [14].

Vitamin D involved in regulating bone metabolism, absorption of calcium and phosphate, and maintenance of muscle function [15]. Therefore, there might be a benefit of vitamin D supplementation in prevention of preeclampsia. However, systematic reviews [16, 17] of randomised controlled trials (RCTs) did not show any benefit in prevention of preeclampsia whereas other two systematic reviews [18, 19] of observational studies did. This discrepancy results could be due to confounding bias in the latter or insufficient power in the former.
Although this evidence suggests benefits from both calcium and vitamin D supplements, it is still unclear which supplement or a combination of them is most beneficial for preventing preeclampsia. We therefore conducted a systematic review and a network meta-analysis of RCTs with the aims of directly and indirectly comparing the effect of supplementations of calcium, vitamin D, both, and neither on preeclampsia.

METHODS

This systematic review was conducted according to the preferred reporting items for systematic reviews and meta-Analyses (PRISMA), extension of network meta-analyses [20]. The review protocol has been registered with the international prospective register of systematic review (PROSPERO number CRD42015025389).

Search strategy

Studies were located from Medline via PubMed and Scopus databases. The search terms and strategies were constructed based on PICO (i.e., patient, intervention, comparator, and outcome) as described in detail in Appendix 1. These strategies were modified to suit each search engine where appropriate.

Study identification was done in two steps. First, all previous systematic reviews of calcium and vitamin D supplementations in pregnant women published since inception of each database to June 2015 were identified. Then only RCTs included in these previous reviews were selected. Second, all individual RCTs on the same topic published since the last systematic review (i.e., January 2014) until December 2015 were identified. The reference lists of the retrieved studies were also checked to identify more relevant publications. Where there were multiple publications from the same author(s) on the same topic, the most complete and recent study was included.
**Study selection**

Identified studies from Medline and Scopus were imported into EndNote X7 and duplicate studies were removed. The selected studies were independently screened by title and abstract by two reviewers (WK and VT). Full texts were retrieved if decisions could not be reached from information provided in the abstract. Disagreements regarding selection were resolved by consensus or discussion with a third reviewer (SAV). We contacted authors by email up to three times if data was insufficient. If there was no response after three attempts, then the study was excluded.

All RCTs conducted in humans and published in English were included if they met all of the following criteria: (1) included pregnant women of any gestational age; (2) compared outcomes of interest between any pair of the following supplementation groups: calcium, vitamin D, combined calcium and vitamin D, placebo/no supplementation; (3) had at least one of the outcomes of interest including preeclampsia, preterm birth, low birth weight, small for gestational age, still birth, maternal death, perinatal death, placental abruption or HELLP syndrome. Studies were excluded from the review if they were crossover trials, included multiple pregnancies, or after three unsuccessful attempts requesting data from authors in the case of insufficient data.

**Interventions**

Interventions were any of following supplements regardless of dosage and duration of supplements: Calcium, vitamin D, combined calcium and vitamin D. The control group could be placebo, a standard supplementation (e.g., folic acid), or no supplementation.
Outcomes of interest

The primary outcome of interest was preeclampsia, defined as per the original studies. Generally, it was a new onset hypertension (i.e., systolic blood pressure \( \geq 140 \) mmHg and/or diastolic blood pressure \( \geq 90 \) mmHg for two occasions at least 4 hours apart) and any of the following: proteinuria (dipstick urine 2+ or \( \geq 300 \) mg/24 hours), end-organ dysfunction, or utero-placental dysfunction after 20 weeks of gestation [21]. An early-onset occurred before 34 weeks of gestation, otherwise it was a late-onset preeclampsia [21].

Data extraction

Two reviewers (WK and VT) independently extracted the relevant data (participants, interventions and outcome characteristics) and these were recorded using a standardized data extraction form (Appendix 3). Co-variables such as mean age, gestational age at enrolment and delivery, gravida, parity, body mass index (BMI), smoking and diabetes mellitus were also extracted. Data entry, cleaning and checking were performed separately for each reviewer. The two datasets were compared and validated, and any disagreement resolved by consensus.

Risk of bias assessment

Study quality was independently assessed by two reviewers (WK and VT) using the Cochrane Collaboration tool for assessing risk of bias in RCTs version 5.1.0 [22], see (Appendix 4). The following seven domains were evaluated: selection bias (sequence generation and concealment), performance bias (blinding of participants and assessors), detection bias (blinding of outcome assessment), attrition bias (incomplete outcome data), selective outcome reporting, and other bias. Each item was classified as low, high, or an unclear risk of bias (if there was insufficient information).
**Statistical Analysis**

**Direct meta-analysis**

For studies reporting frequency data of supplementation and preeclampsia, log risk ratio (RR) along with its variance and the 95% confidence interval (CI) were estimated for each study. The RRs were then pooled across studies using fixed-effect model (i.e., inverse variance method) if heterogeneity was absent, otherwise a random-effect model (i.e., DerSimonian and Laird method) was used.

Heterogeneity was assessed by Cochrane’s Q test and $I^2$ statistic, respectively. If it was present ($p < 0.1$ or $I^2 \geq 25\%$), a source of heterogeneity was explored by fitting characteristics of subjects (i.e., mean age, mean gestational age), clinical data (i.e., dosage and duration of supplementation), and methodologic characteristics (i.e., definition of outcome measurements, setting of the study) in a meta-regression model. A sensitivity analysis by excluding the outlier studies and/or a subgroup analysis according to that factor were performed.

**Network meta-analysis**

Network meta-analysis was applied to indirectly compare effects of supplementation. A two-stage multivariate meta-analysis was applied as follows: Coefficients (i.e., lnRR) and variance-covariance of treatment comparisons were estimated for each study using a Poisson model. These parameters were then pooled across studies using a multivariate meta-analysis with maximum likelihood function [23]. Between-study variance and covariance of comparisons were taken into account using unstructured method. Effects between active versus active supplementation were then compared using a linear combination of the multivariate meta-analysis model.
The inconsistency assumption (i.e., whether direct effects agree with the indirect effects) was checked and explored using a design-treatment interaction model, and an inconsistency factor (IF, i.e., ln(RRdirect)-ln(RRindirect)) was then estimated. Violation of consistency was assumed if the IF was significantly different from 0. All pairwise comparisons between direct and indirect, were estimated and displayed. In addition, small study effect for the whole network was assessed by constructing a comparison-adjusted funnel plot taking into account different comparisons [24]. This plots the difference of each study's i observed ln(RR) of newer versus older supplement (yiXY) vs the comparison's mean ln(RR, μXY) against its variance. Supplementation were coded from older to newer as 1, 2, 3, 4 for placebo, calcium, vitamin D, and calcium plus vitamin D, respectively. In the absence of small-study effects, we expected the studies to form an inverted funnel centred at zero, i.e., the comparison-adjusted funnel plot should be symmetric around the zero line. Finally, a predictive probability of best intervention was estimated using surface under a cumulative ranking curve (SUCRA). Efficacy of supplementation was then ranked by predicting probability.

All analyses were performed using STATA version 14.0 [25]. P-values <0.05 were considered as statistically significant, except for the test of heterogeneity where p <0.10 was used.

RESULTS

Study selection and characteristics

The schema for selection of studies is displayed in Figure 1. Examination of the systematic reviews identified 172 review studies after removing duplicates. Among these, 157 review studies were excluded for reasons described in Figure 1, leaving 15 review studies with 68
RCTs that were eligible to for further assessment. Of these, only 27 studies met our inclusion criteria and were considered for pooling.

In searching for additional individual studies between January 2014 to December 2015, 428 studies were identified but 6 studies met inclusion criteria. After removing duplicates with finding from systematic reviews, 29 RCTs were eligible for inclusion in a network meta-analysis. Among these, the outcomes were preeclampsia (n=20), eclampsia (n=1), preterm birth (n=14), neonatal death (n=7), low-birth weight (n=8), small for gestational age (n=6), still birth (n=11), maternal death (n=2), placental abruption (n=3), and HELLP syndrome (n=1) (see Figure 1). Due to space constraints, we present only the results for preeclampsia in this manuscript.

The characteristics of the 20 RCTs with preeclampsia outcome are described in Table 1. Among these, 15 studies (n=25593) [26-40] compared calcium vs placebo, 1 study (n=54) [41] compared vitamin D vs placebo, 3 studies (n=1120) [42-44] compared calcium plus vitamin D vs placebo, and 1 study (n=175) [45] compared calcium plus vitamin D vs calcium.

Cross-tabulated data for these interventions and preeclampsia are described in Table S1. Individual sample sizes ranged from 30 to 9178 with a median of 225. The types of pregnant women varied, 55% (11/20) of RCTs studies in general pregnancies and 45% (9/20) RCTs studied in high risk pregnancies, e.g., adolescent pregnancy, elderly pregnancy, and nulliparity. The mean age ranged from 16 to 37.2 years; mean gestational age at enrolment and at delivery ranged from 15.1 to 29.7 and 37.5 to 39.1 weeks, respectively.

**Risk of bias assessment**

Risk of bias assessment was performed for each RCT (see Table S2) and summarised in Figure S1. Among 20 RCTs, two studies [34, 39] were conference abstracts, thus could not assess the risk of bias because authors did not publish full articles. In the remaining 18
studies, sequence generation was clearly described in 13 trials (72.2%), whereas 5 trials (27.8%) were unclear. Allocation concealment was adequately performed in 10 trials (55.6%). Most studies (15/18) reported about blinding of participants and blinding of outcome assessors, whereas 12 trials (66.7%) reported incomplete outcome data. Most RCTs (11/18) had low risk of bias for selective outcome reports, intention-to-treat (ITT) analysis was used in 11/18 trials.

**Direct meta-analysis**

Direct comparisons for calcium vs placebo, vitamin D vs placebo and calcium plus vitamin D vs placebo were pooled across 15 (n = 12797 vs 12796), 1 (n = 27) and 3 RCTs (n = 560 vs 560), respectively. These corresponding pooled effects were 0.52 (95%CI: 0.39, 0.68), 0.33 (95%CI: 0.01, 7.84) and 0.49 (95%CI: 0.31, 0.77), (see Figure S2a,b). This indicated that calcium and calcium plus vitamin D supplementations could reduce preeclampsia risk by approximately 48% and 51% when compared with placebo.

Sources of heterogeneity for the pooled calcium vs placebo effect were explored using a meta-regression as mentioned in the method. Only type of pregnancy (general versus high risk pregnancy) and duration of calcium supplementation (>18 versus ≤18 weeks) could reduce the degree of heterogeneity from 73.45% to 56.72% and 44.03%, respectively. Subgroup analysis was therefore performed. The protective effect of calcium supplementation was greater in high risk pregnancies than general pregnancies with a pooled RR of 0.38 (95% CI: 0.27, 0.53) and 0.70 (95%CI: 0.53, 0.91), respectively (see Figure S2a). The calcium supplement effect was also statistically significant in pregnant women who received it for 18 weeks or less (pooled RR=0.36, 95%CI: 0.26, 0.49) but not for women who supplemented for >18 weeks (pooled RR=0.80, 95%CI: 0.63, 1.01).
Network meta-analysis

Data from 20 RCTs were used in a network meta-analysis (see Table S1), and all interventions were mapped in a network plot (see Figure S3). The size of each node is proportional to the number of included studies whereas the edge of each comparison is weighted by the number of pregnant women for that comparison. Two indirect comparisons were performed by “borrowing” data from common comparators in the network, i.e., vitamin D vs calcium and calcium plus vitamin D vs vitamin D, respectively.

The network meta-analysis indicated significant intervention effects for calcium and calcium plus vitamin D when compared to placebo with pooled RRs of 0.49 (95% CI: 0.34, 0.69) and 0.43 (95% CI: 0.22, 0.83), respectively (see Figure 2). Vitamin D alone also seemed to be effective, but it was not statistically significant when compared to placebo with a pooled RR of 0.33 (95% CI: 0.01, 8.94). All multiple comparisons were further estimated (see Table 2) suggesting vitamin D and calcium plus vitamin D seemed to be better than calcium supplement alone but these were not statistically significantly different with pooled RRs of 0.68 (95% CI: 0.02, 18.61) and 0.87 (95% CI: 0.43, 1.77), respectively.

Ranking of all interventions was performed using the method of SUCRA and probability of ranking (see Table 2 and Figure S4), which suggested that vitamin D was the most effective supplement, followed by calcium plus vitamin D, and then calcium. The estimated ranking probabilities for these corresponding supplements were 50.2%, 29.4%, and 19.7%, respectively. Furthermore, a design-by-treatment inconsistency model was applied which suggested that there was no evidence of inconsistency between direct and indirect effects (Chi-square test = 0.40, p = 0.526).

A comparison-adjusted funnel plot was constructed indicating asymmetry of the funnel, i.e., there might be small study-effects, particularly from studies with calcium versus placebo (see Figure S5). Sample sizes of all studies ranged from 30 to 9178, with a median of
A sensitivity analysis was then performed by excluding studies whose sample sizes were small, i.e., those that were in the first quartile of smallest sample size. Five RCTs [26, 27, 30, 36, 40] comparing calcium vs placebo (n = 317), one [41] comparing vitamin D vs placebo (n = 54) and one [44] comparing calcium plus vitamin D vs placebo (n=60) were excluded, although the results did not show much difference from pooling all trials. The pooled RRs were 0.57 (95% CI: 0.39, 0.82) and 0.45 (95% CI: 0.23, 0.89) for calcium vs placebo and calcium plus vitamin D vs placebo, respectively. Thus, there was little effect of small study influence on our pooled estimates.

Finally, number needed to treat (NNT) for supplementations were estimated. We found that a total of 15, 18, and 20 pregnant women needed to receive supplements with vitamin D, calcium plus vitamin D, and calcium in order to prevent one episode of preeclampsia.

DISCUSSION

We have performed a systematic review and network meta-analysis of calcium and vitamin D supplementation effects on preeclampsia risk. Our finding from direct meta-analysis suggested that calcium supplementation could reduce the risk of preeclampsia by approximately half when compared with placebo. Supplementation appeared more effective in high risk pregnancies than in general pregnancies, and in those who consumed the supplement for 18 weeks or less. In addition, the network meta-analysis indicated that vitamin D supplementation could potentially be the most effective supplementation, reducing the risk of preeclampsia by 67%, followed by calcium plus vitamin D by 57%, and calcium alone by 51%. The NNTs for these corresponding supplements would be 15, 18, and 20.

Although our diagnostics do not indicate any heterogeneity or little effect of small study effects, these results are based on very small numbers of participants. Our results are
consistent with the updated Cochrane review [46] which found significant preventive effect of calcium supplementation on preeclampsia especially in high risk women. Tang et al also found the same effect of calcium supplementation in high risk, but not for general pregnancy [47].

Strengths and limitations
Our study has a number of strengths. In comparison with earlier systematic reviews of observational studies, our meta-analysis included only RCTs, thus selection bias and confounding biases should be minimized. We compared effects of all supplementations on preeclampsia using network meta-analysis to indirectly compare efficacy between supplementations by borrowing data from common comparators. Neither publication bias nor inconsistency was detected. A ranking of interventions with their NNTs has also been calculated.

However, our study also has some limitations. The number of included studies for vitamin D and calcium plus vitamin D were very small, and thus estimation of supplementation effects were imprecise. Different dosages of supplementations had been used, and given the small number of included studies we were unable to tease out a dosage effect.

Summary of evidence
Our meta-analysis has advantages over previous systematic reviews by integrating both direct and indirect comparison of calcium, vitamin D, and calcium plus vitamin D supplementation in the entire network approach. Until now, there has been no RCTs directly assessing the efficacy of supplementation on preeclampsia comparing calcium vs vitamin D, and calcium plus vitamin D vs vitamin D, but our network meta-analysis extrapolates these results.
Vitamin D may be the best supplementation for prevention of preeclampsia. Possible explanations for this result might be as follows: First, adequate vitamin D intake might maintain calcium homeostasis, which in turn has an inverse relationship with blood pressure [7], or might directly suppress vascular smooth muscle cell proliferation [48]. Second, vitamin D might be a potent endocrine suppressor of renin biosynthesis and regulate the renin-angiotensin system, which plays a critical role in the regulation of blood pressure [48]. Third, vitamin D might have immune-modulatory effect by balancing T helpers cells [49].

Although supplementation of vitamin D with/without calcium ranked higher than calcium supplement alone, this needs to be confirmed in head to head trials. If proven, applying this in routine care of pregnancy might be more difficult particularly in developing countries because of its greater investigation cost when compared to calcium supplements. This suggest that, calcium supplementation may remain the standard of choice where accessibility to vitamin D is limited.

Further research should focus on the recommended daily allowance of vitamin D for pregnant women, minimally clinical effective dosage of vitamin D, safety of vitamin D with different dosages, timing of initiation of supplementation in pregnancy, supplementation regimen (daily or weekly or single dose), supplementation alone or combination with other nutrients and to which type of pregnancy (general or high risk).

**CONCLUSION**

Our evidence suggests that vitamin D supplementation alone may be best for prevention of preeclampsia, followed by calcium plus vitamin D and calcium alone. However, the evidence was based on only one study examining vitamin D with short term assessment. Therefore, larger, well-designed RCTs are still required to determine the efficacy of vitamin D supplementation alone or in combination with calcium to reduce the risk of preeclampsia. Or
this network meta-analysis is needed to update once more RCTs of vitamin D supplementation are available.

**List of abbreviations used**

BMI = body mass index
CI = confidence interval
HELLP = haemolysis, elevated liver enzymes, and low platelets
ITT = intention-to-treat
RCTs = randomised controlled trials
RR = risk ratio
WHO = World Health Organization

**Details of ethics approval**

There is no need for ethical approval for a systematic review.

**Availability of Data and Materials**

The data supporting the study findings and conclusions of this article is contained within the manuscript and also provided as an additional supplementation Microsoft Excel spreadsheet file.

**Competing interests**

The authors report no conflicts of interest.

**Funding sources:** no funding source or any sponsor was involved for this research or preparation of the manuscript
Authors’ Contribution

Study concept and design: WK, SAV, VT, AT. Study selection and risk of bias assessment: WK, VT. Data extraction: WK, SAV. Data analysis: WK, SAV, AT. Interpretation of data: WK, SAV, JA, MM, AT. Drafting the manuscript: WK, SAV, JA, MM, AT. Critical revision of the manuscript for important intellectual content: WK, SAV, JA, MM, AT. Final approval of the version to be published: all authors read and approved the final manuscript.

Acknowledgement

This study is a part of Dr. Win Khaing’s training in Ph.D. program for Clinical Epidemiology, Faculty of Medicine Ramathibodi Hospital and Faculty of Graduate Studies, Mahidol University, Bangkok, Thailand.
REFERENCES


Table 1. Characteristics of included studies on preeclampsia

<table>
<thead>
<tr>
<th>Author (Year)</th>
<th>Country</th>
<th>Study period (months)</th>
<th>Type of pregnancy</th>
<th>n Intervention</th>
<th>n Control</th>
<th>Duration (weeks)</th>
<th>Mean Age (years)</th>
<th>Mean Gestational Age at Enrolment (weeks)</th>
<th>Mean Gestational Age at Delivery (weeks)</th>
<th>SBP</th>
<th>DBP</th>
<th>BMI</th>
<th>Weight Gain</th>
<th>Nulliparity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium versus placebo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Villar et al. (1987)[26]</td>
<td>Baltimore, Argentina</td>
<td>36</td>
<td>General Women</td>
<td>27</td>
<td>25</td>
<td>12</td>
<td>21.10</td>
<td>-</td>
<td>-</td>
<td>63.81</td>
<td>100.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lopez-Jaramillo et al. (1989)[27]</td>
<td>Ecuador</td>
<td>30</td>
<td>General Women</td>
<td>43</td>
<td>49</td>
<td>15</td>
<td>18.47</td>
<td>23.00</td>
<td>-</td>
<td>430.80</td>
<td>100.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Villar et al. (1990)[28]</td>
<td>Baltimore</td>
<td>36</td>
<td>High Risk Women</td>
<td>88</td>
<td>90</td>
<td>18</td>
<td>16.25</td>
<td>23.55</td>
<td>38.55</td>
<td>102.75</td>
<td>61.10</td>
<td></td>
<td></td>
<td>85.26</td>
</tr>
<tr>
<td>Belizan et al. (1991)[29]</td>
<td>Argentina</td>
<td>33</td>
<td>High Risk Women</td>
<td>588</td>
<td>579</td>
<td>18</td>
<td>23.70</td>
<td>20.80</td>
<td>-</td>
<td>103.95</td>
<td>66.45</td>
<td></td>
<td></td>
<td>100.00</td>
</tr>
<tr>
<td>Sanchez-Ramos et al. (1994)[30]</td>
<td>Florida</td>
<td>55</td>
<td>High Risk Women</td>
<td>34</td>
<td>29</td>
<td>14</td>
<td>18.38</td>
<td>24.44</td>
<td>-</td>
<td>113.50</td>
<td>64.01</td>
<td></td>
<td></td>
<td>100.00</td>
</tr>
<tr>
<td>Puwar et al. (1996)[31]</td>
<td>India</td>
<td>15</td>
<td>General Women</td>
<td>93</td>
<td>97</td>
<td>18</td>
<td>21.93</td>
<td>18.07</td>
<td>37.50</td>
<td>103.02</td>
<td>63.32</td>
<td></td>
<td></td>
<td>100.00</td>
</tr>
<tr>
<td>Lopez-Jaramillo et al. (1997)[33]</td>
<td>Ecuador</td>
<td>56</td>
<td>High Risk Women</td>
<td>135</td>
<td>125</td>
<td>18</td>
<td>15.99</td>
<td>20.00</td>
<td>39.13</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td>414.19</td>
</tr>
<tr>
<td>Levine et al. (1997)[32]</td>
<td>US</td>
<td>36</td>
<td>General Women</td>
<td>2294</td>
<td>2295</td>
<td>21</td>
<td>21.00</td>
<td>17.15</td>
<td>38.90</td>
<td>106.50</td>
<td>59.70</td>
<td></td>
<td></td>
<td>100.00</td>
</tr>
<tr>
<td>Almirante et al. (1998)[34]</td>
<td>Philippines</td>
<td>-</td>
<td>High Risk Women</td>
<td>210</td>
<td>212</td>
<td>22</td>
<td>-</td>
<td>18.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td>100.00</td>
</tr>
<tr>
<td>Crowther et al. (1999)[35]</td>
<td>Australian</td>
<td>53</td>
<td>General Women</td>
<td>229</td>
<td>227</td>
<td>18</td>
<td>24.70</td>
<td>18.37</td>
<td>-</td>
<td>115.80</td>
<td>68.20</td>
<td>26.60</td>
<td></td>
<td>100.00</td>
</tr>
<tr>
<td>Niromanesh et al. (2001)[36]</td>
<td>Iran</td>
<td>-</td>
<td>High Risk Women</td>
<td>15</td>
<td>15</td>
<td>10</td>
<td>23.15</td>
<td>29.70</td>
<td>38.60</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Country</td>
<td>Gender</td>
<td>Sample Size</td>
<td>n</td>
<td>SBP Mean</td>
<td>DBP Mean</td>
<td>BMI Mean</td>
<td>SBP SD</td>
<td>DBP SD</td>
<td>BMI SD</td>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------------</td>
<td>----------------------------------</td>
<td>--------------</td>
<td>-------------</td>
<td>----</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>----------------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Villar et al. (2006)[37]</td>
<td>Argentina, Egypt, India, Peru, South Africa, Viet Nam</td>
<td>General Women</td>
<td>4161</td>
<td>21</td>
<td>22.65</td>
<td>15.10</td>
<td>-</td>
<td>105.05</td>
<td>60.80</td>
<td>21.90</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kumar et al. (2009)[38]</td>
<td>New Delhi</td>
<td>General Women</td>
<td>251</td>
<td>36</td>
<td>21.85</td>
<td>17.83</td>
<td>38.44</td>
<td>113.19</td>
<td>74.00</td>
<td>23.35</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nenad et al. (2011)[39]</td>
<td>Serbia</td>
<td>General Women</td>
<td>4588</td>
<td></td>
<td>18.50</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aghamohammadi (2015)[40]</td>
<td>Iran</td>
<td>High Risk Women</td>
<td>40</td>
<td></td>
<td>37.15</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>26.8</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin D versus placebo</td>
<td>Asemi et al. (2013)[41]</td>
<td>Iran</td>
<td>High Risk Women</td>
<td>27</td>
<td>17.44</td>
<td>26</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>30.8</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium plus vitamin D versus placebo</td>
<td>Marya et al. (1987)[42]</td>
<td>India</td>
<td>General Women</td>
<td>200</td>
<td>22.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taherian et al. (2002)[43]</td>
<td>Iran</td>
<td>General Women</td>
<td>330</td>
<td>36</td>
<td>21.55</td>
<td>20.00</td>
<td>38.80</td>
<td>97.25</td>
<td>57.88</td>
<td>22.55</td>
<td>10.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Samimi (2015)[44]</td>
<td>Iran</td>
<td>High Risk Women</td>
<td>30</td>
<td></td>
<td>27.2</td>
<td>-</td>
<td>-</td>
<td>111.7</td>
<td>72.4</td>
<td>26.5</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium plus vitamin D versus calcium</td>
<td>Hussain et al. (2014)[45]</td>
<td>Pakistan</td>
<td>General Women</td>
<td>89</td>
<td>25.57</td>
<td>20.00</td>
<td>37.61</td>
<td>-</td>
<td>-</td>
<td>23.64</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n = number of subjects, SBP = Mean Systolic Blood Pressure (mmHg), DBP = Mean Diastolic Blood Pressure (mmHg), BMI = Mean Body Mass Index (kg/m²)
### Table 2. Estimation of multiple supplementation effects on preeclampsia

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Calcium</th>
<th>Vitamin D</th>
<th>Calcium + Vitamin D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>0.49 (0.34, 0.69) [59.0, 19.7]</td>
<td>0.68 (0.02, 18.61)</td>
<td>0.87 (0.43, 1.77)</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>0.33 (0.01, 8.94) [61.3, 50.2]</td>
<td>1.28 (0.04, 36.65)</td>
<td></td>
</tr>
<tr>
<td>Calcium + Vitamin D</td>
<td></td>
<td>0.43 (0.22, 0.83) [65.9, 29.4]</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as pooled RR along with 95% CIs in round parentheses; on diagonal line comparing supplement vs placebo, off the diagonal line comparing column vs row supplements; values < 1 indicates that the intervention listed in the column is more effective than the one in the row; Values in the diagonal in square parentheses indicate surface under the cumulative ranking curve and the probability of being the best treatment. The larger the surface under the cumulative ranking curve or probability of being the best treatment, the better the treatment.
Figure legends

Figure 1. Flow of selection of studies

Figure 2. Forest plot of intervention effects compared to placebo: a network meta-analysis