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Calcium supplementation commencing before or early in pregnancy, or food fortification with calcium, for preventing hypertensive disorders of pregnancy (Review)

Hofmeyr GJ, Manyame S

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[Intervention Review]

Calcium supplementation commencing before or early in pregnancy, or food fortification with calcium, for preventing hypertensive disorders of pregnancy

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ABSTRACT

Background

Pre-eclampsia is considerably more prevalent in low- than high-income countries. One possible explanation for this discrepancy is dietary differences, particularly calcium deficiency. Calcium supplementation in the second half of pregnancy reduces the serious consequences of pre-eclampsia and is recommended by the World Health Organization (WHO) for women with low dietary calcium intake, but has limited effect on the overall risk of pre-eclampsia. It is important to establish whether calcium supplementation before and in early pregnancy has added benefit. Such evidence would be justification for population-level fortification of staple foods with calcium.

Objectives

To determine the effect of calcium supplementation or food fortification with calcium, commenced before or early in pregnancy and continued at least until mid-pregnancy, on pre-eclampsia and other hypertensive disorders, maternal morbidity and mortality, as well as fetal and neonatal outcomes.

Search methods

We searched the Cochrane Pregnancy and Childbirth Trials Register (10 August 2017), PubMed (29 June 2017), ClinicalTrials.gov, the WHO International Clinical Trials Registry Platform (ICTRP) (10 August 2017) and reference lists of retrieved studies.

Selection criteria

Randomised controlled trials of calcium supplementation or food fortification which include women of child bearing age not yet pregnant, or in early pregnancy. Cluster-RCTs, quasi-RCTs and trials published in abstract form only would have been eligible for inclusion in this review but none were identified. Cross-over designs are not appropriate for this intervention.

The scope of this review is to consider interventions including calcium supplementation with or without additional supplements or treatments, compared with placebo or no intervention.

Data collection and analysis

Two review authors independently assessed trials for inclusion and risk of bias, extracted data and checked them for accuracy.

Main results

This review is based on one RCT (involving 60 women) which looked at calcium plus additional supplements versus control. The women (who had low antioxidant status) were in the early stages of pregnancy. We did not identify any studies where supplementation commenced pre-pregnancy. Another RCT comparing calcium versus placebo is ongoing but not yet complete. We did not identify any studies looking at any of our other planned comparisons.

Calcium plus antioxidants and other supplements versus placebo

We included one small study (involving 60 women with low antioxidant levels) which was conducted in an academic hospital in Indondesia. The study was at low risk of bias for all domains with the exception of selective reporting, for which it was unclear. Women in the intervention group received calcium (800 mg) plus N-acetylcysteine (200 mg), Cu (2 mg), Zn (15 mg), Mn (0.5 mg) and selenium (100 mcg) and vitamins A (1000 IU), B6 (2.2 mg), B12 (2.2 mcg), C (200 mg), and E (400 IU) versus the placebo control group of women who received similar looking tablets containing iron and folic acid. Both groups received iron (30 mg) and folic acid (400 mcg). Tablets were taken twice daily from eight to 12 weeks of gestation and then throughout pregnancy.

The included study found that calcium supplementation plus antioxidants and other supplements may slightly reduce **pre-eclampsia (gestational hypertension and proteinuria)** (risk ratio (RR) 0.24, 95% confidence interval (CI) 0.06 to 1.01; *low-quality evidence*), but this is uncertain due to wide confidence intervals just crossing the line of no effect, and small sample size. It appears that early**pregnancy loss before 20 weeks' gestation** (RR 0.06, 95% CI 0.00 to 1.04; *moderate-quality evidence*) may be slightly reduced by calcium plus antioxidants and other supplements, but this outcome also has wide confidence intervals, which just cross the line of no effect. Very few events were reported under the composite outcome, **severe maternal morbidity and mortality index** and no clear difference was seen between groups (RR 0.36, 95% CI 0.04 to 3.23; *low-quality evidence*). However, the included study observed a reduction in the composite outcome **pre-eclampsia and/or pregnancy loss at any gestational age** (RR 0.13, 95% CI 0.03 to 0.50; *moderate-quality evidence*), and **pregnancy loss/stillbirth at any gestational age** (RR 0.06, 95% CI 0.00 to 0.92; *moderate-quality evidence*) in the calcium plus antioxidant/supplement group.

Other outcomes reported (**placental abruption**, **severe pre-eclampsia** and **preterm birth (less than 37 weeks' gestation)**) were too infrequent for meaningful analysis. No data were reported for the outcomes caesarean section, birthweight < 2500 g, Apgar score less than seven at five minutes, death or admission to neonatal intensive care unit (ICU), or pregnancy loss, stillbirth or neonatal death before discharge from hospital.

Authors' conclusions

The results of this review are based on one small study in which the calcium intervention group also received antioxidants and other supplements. Therefore, we are uncertain whether any of the effects observed in the study were due to calcium supplementation or not. The evidence in this review was graded low to moderate due to imprecision. There is insufficient evidence on the effectiveness or otherwise of pre- or early-pregnancy calcium supplementation, or food fortification for preventing hypertensive disorders of pregnancy.

Further research is needed to determine whether pre- or early-pregnancy supplementation, or food fortification with calcium is associated with a reduction in adverse pregnancy outcomes such as pre-eclampsia and pregnancy loss. Such studies should be adequately powered, limited to calcium supplementation, placebo-controlled, and include relevant outcomes such as those chosen for this review.

There is one ongoing study of calcium supplementation alone versus placebo and this may provide additional evidence in future updates.

PLAIN LANGUAGE SUMMARY

Extra calcium in food or tablets before pregnancy, or in early pregnancy, for preventing high blood pressure complications of pregnancy

What is the issue?

This review's aim is to find out whether calcium supplementation or food fortification with calcium taken before or early in pregnancy and continued at least until mid-pregnancy, will reduce the number of women developing pre-eclampsia, high blood pressure, other serious health problems and death, as well as fetal and neonatal outcomes.

Why is this important?

Women can develop high blood pressure with protein in the urine after the 20th week of pregnancy, known as pre-eclampsia. Many women, particularly those in low-income countries, do not have enough calcium in their diets. Giving these women extra calcium during the second half of pregnancy has been shown to reduce their risk of serious consequences from developing high blood pressure and protein in the urine, such as convulsions (eclampsia), stroke, clotting disorders, fluid in the lungs, kidney failure and death. Taking extra calcium in the second half of pregnancy does not however appear to greatly reduce the number of women developing pre-eclampsia. It is important to know if taking extra calcium before pregnancy and in early pregnancy can reduce the number of women who develop blood pressure complications.

We searched for randomised controlled studies that looked at the effect of giving women extra calcium before or early in pregnancy on the number of women who developed pre-eclampsia.

What evidence did we find?

We searched the medical literature on 29 June 2017 and 10 August 2017 and found one randomised controlled study. Women with low antioxidant levels were given calcium, antioxidants and other supplements, starting within the first 12 weeks of pregnancy, or a dummy tablet. The tablets for both groups contained folic acid and iron supplements. Only 60 women took part in this study and the study was carried out in a hospital in Indonesia.

Women taking calcium plus antioxidants and other supplements were at reduced risk of experiencing pre-eclampsia, miscarriage or stillbirth when measured together compared with the women in the control group. For women taking the calcium supplement, miscarriage or stillbirth at any stage of pregnancy was also reduced (moderate-quality evidence). It is possible that pre-eclampsia alone (low-quality evidence), and early pregnancy losses before 20 weeks, might be reduced for women taking calcium plus antioxidants but we cannot be sure of this. Calcium supplementation did not make a clear difference to the number of women who developed severe pre-eclampsia or had a placental abruption - very few women developed these issues (low-quality evidence). Other outcomes were infrequent or not reported.

What does this mean?

The women who received calcium also received antioxidants and other supplements. This means that we cannot be certain that the reduction in pre-eclampsia and miscarriage or stillbirth, or any of the other results, were due to calcium or not. More research is needed to confirm this, and whether or not calcium reduces other outcomes such as preterm birth, caesarean section, low birthweight babies, and stillbirth or neonatal death before discharge from hospital. Only 60 women were involved in the included study so the quality of the evidence is not high, and future studies would need to be large enough to produce results that are more certain.

One trial of calcium supplementation given before pregnancy is currently underway but not yet complete.

SUMMARY OF FINDINGS

Summary of findings for the main comparison. Calcium +/- other supplements compared to no calcium or placebo for pregnancy, or food fortification with calcium, for preventing hypertensive disorders of pregnancy

Calcium +/- other supplements compared to no calcium or placebo for pregnancy, or food fortification with calcium, for preventing hypertensive disorders of pregnancy

Patient or population: pregnant women with confirmed low antioxidant levels
Setting: academic hospital in Indonesia
Intervention: supplementation with calcium (800 mg), folic acid (400 mcg), N-acetylcysteine (200 mg), Cu (2 mg), Zn (15 mg), Mn (0.5 mg), Fe (30 mg), and selenium (100 mcg) and vitamins A (1000 IU), B6 (2.2 mg), B12 (2.2 mg), C (200 mg), and E (400 IU) given as two tablets daily, from 8 to 12 weeks of gestation throughout pregnancy.

Comparison: supplementation with Fe (30 mg) and folic acid (400 mcg), given as two tablets similar in appearance to intervention tablets, from 8 to 12 weeks of gestation throughout pregnancy.

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with no calcium or placebo	Risk with calcium +/- other supplements				
Pre-eclampsia (gestational hypertension and proteinuria)	Study population		RR 0.24 (0.06 to 1.01)	60 (1 RCT) [1]	⊕⊕⊖⊖ LOW [2]	
	290 per 1,000	70 per 1000 (17 to 293)				
Pre-eclampsia and/or pregnancy loss at any gestational age	Study population		RR 0.13 (0.03 to 0.50)	60 (1 RCT)	⊕⊕⊕⊖ MODERATE [3]	
	548 per 1000	71 per 1000 (16 to 274)				
Severe maternal morbidity and mortality index	Study population		RR 0.36 (0.04 to 3.23)	60 (1 RCT)	⊕⊕⊖⊖ LOW [2]	Includes data for severe pre-eclampsia and placental abruption. There were 0 cases of placental abruption. No other outcomes from severe maternal morbidity/mortality index were reported in this study.
	97 per 1000	35 per 1000 (4 to 313)				
Pregnancy loss/stillbirth at any gestational age	Study population		RR 0.06 (0.00 to 0.92)	60 (1 RCT)	⊕⊕⊕⊖ MODERATE [3]	
	290 per 1000	17 per 1000 (0 to 267)				

Caesarean section	Study population		-	(0 studies)	-	No data reported for this outcome.
	see comment	see comment				
Birthweight < 2500 g	Study population		-	(0 studies)	-	No data reported for this outcome.
	see comment	see comment				
Apgar score less than 7 at 5 minutes	Study population		-	(0 studies)	-	No data reported for this outcome.
	see comment	see comment				
Death or admission to neonatal ICU for 24 hours or more	Study population		-	(0 studies)	-	No data reported for this outcome.
	see comment	see comment				
Pregnancy loss, stillbirth or neonatal death before discharge	Study population		-	(0 studies)	-	No data reported for this outcome.
	see comment	see comment				

*__The risk in the intervention group__ (and its 95% confidence interval) is based on the assumed risk in the comparison group and the __relative effect__ of the intervention (and its 95% CI).

CI: Confidence interval; **RR:** Risk ratio; **OR:** Odds ratio;

GRADE Working Group grades of evidence
High quality: We are very confident that the true effect lies close to that of the estimate of the effect
Moderate quality: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low quality: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect
Very low quality: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

[1] Unclear risk of bias for selective outcome reporting as no protocol available (no outcome downgraded).
[2] Small sample size, small number of events, single study with wide confidence intervals crossing the line of no effect indicates imprecision (-2).
[3] Small sample size, small number of events and single study contributing data indicates imprecision (-1).

BACKGROUND

Hypertension has been estimated to complicate 3% to 10% of all pregnancies (Mol 2016) and 11% of first pregnancies, half of these being associated with pre-eclampsia, and accounting for up to 30,000 maternal deaths annually (von Dadelszen 2016). Pre-eclampsia is defined as high blood pressure and proteinuria occurring after the 20th week of pregnancy.

In general, pre-eclampsia is considerably more prevalent in low-income than in high-income communities. Two striking exceptions have been identified. More than 50 years ago, a low prevalence of pre-eclampsia was reported from Ethiopia where the diet, among other features, contained high levels of calcium (Hamlin 1952). The observation in 1980 that Mayan Indians in Guatemala, who traditionally soaked their corn in lime before cooking, had a low incidence of pre-eclampsia and eclampsia (Belizan 1980), stimulated interest in the concept that the link between poverty and pre-eclampsia might be dietary calcium deficiency.

Subsequent epidemiological, clinical and laboratory studies linking pre-eclampsia to calcium deficiency have been outlined in a Cochrane review (Hofmeyr 2014).

Low dietary calcium intake is also associated with hypertension in the general population (Centeno 2009). A systematic review of randomised trials showed a small reduction in systolic and diastolic blood pressure with dietary and non-dietary calcium supplementation (Griffith 1999). Systolic blood pressure was reduced by -1.44 mm Hg (95% confidence interval (CI) -2.20 to -0.68; P < .001) and diastolic blood pressure by -0.84 mm Hg (95% CI -1.44 to -0.24; P < .001). Low dietary calcium intake is also considered a risk factor for osteoporosis, renal stones, increased body mass index, insulin resistance and colorectal cancer (Centeno 2009).

The hypothesis that calcium supplementation during pregnancy might reduce the incidence of pre-eclampsia was tested in several randomised trials commencing in the late 1980s.

The World Health Organization (WHO) conducted a randomised trial of calcium supplementation among low calcium intake pregnant women from 2001 to 2003 (Villar 2006). Results from this trial showed that although 1.5 g calcium/day supplement did not prevent pre-eclampsia, it reduced its severity, maternal morbidity, and neonatal mortality. Supplementation in this trial was only during later pregnancy, starting before the 20th week of pregnancy. This trial was included (along with other randomised trials of calcium supplementation during pregnancy) in the Cochrane review by Hofmeyr 2014. The results showed that calcium supplementation of at least 1 g daily, commencing around mid-pregnancy, was associated with a modest reduction in pre-eclampsia, and notably a reduction in its severe manifestations, particularly among women at increased risk, or with low dietary calcium intake. A review of lower-dose calcium supplementation (mainly 500 mg/day in the second half of pregnancy), with or without other supplements, including small trials of variable quality also found a reduction in pre-eclampsia (nine trials, 2234 women, risk ratio (RR) 0·38, 95% CI 0·28 to 0.52) (Hofmeyr 2014a).

WHO has recommended that in populations where dietary calcium intake is low, pregnant women receive 1.5 g to 2 g elemental calcium daily, particularly those at increased risk of pre-eclampsia (women with one or more of the following risk factors: obesity, previous pre-eclampsia, diabetes, chronic hypertension, renal disease, autoimmune disease, nulliparity, advanced maternal age, adolescent pregnancy and conditions leading to hyperplacentation and large placentas such as in twin pregnancy) (http://www.who.int/nutrition/publications/micronutrients/guidelines/calcium_supplementation/en/index.html).

Other related Cochrane reviews include Buppasiri 2015, De-Regil 2016 and Hofmeyr 2014.

Description of the condition

The hypertensive disorders of pregnancy include chronic hypertension, gestational hypertension, pre-eclampsia/eclampsia and unclassified hypertension (von Dadelszen 2016).

Pre-eclampsia is defined as high blood pressure and proteinuria occurring for the first time after 20 weeks' gestation. It resolves by three months after delivery (Magee 2014).

Gestational hypertension is defined as diastolic blood pressure ≥ 90 mmHg on two occasions four hours apart, or ≥ 110 mmHg once, and/or systolic blood pressure ≥ 140 mmHg on two occasions four hours apart, or ≥ 160 mmHg once, after 20 weeks' gestation.

Gestational proteinuria is defined as 2+ or more on urine dipstix, or > 300 mg/24 hours, or urinary protein/creatinine ratio > 30 g/mol, after 20 weeks' gestation (von Dadelszen 2016).

Early pregnancy events affecting placentation are thought to contribute to the development of pre-eclampsia (Lyall 2013; Palei 2013) via the following sequence:

1. failure of cytotrophoblast invasion to remodel uterine spiral arterioles to low-resistance vessels;
2. impaired uteroplacental blood flow;
3. syncytiotrophoblast oxidative stress and oversecretion of anti-angiogenic and pro-inflammatory factors from the ischaemic placenta;
4. widespread maternal endothelial dysfunction with vasoconstriction and renal dysfunction.

This sequence of events has been suggested to be a precursor particularly of early onset pre-eclampsia (Redman 2014).

Description of the intervention

Previous studies and reviews have focused on calcium supplementation during pregnancy (Hofmeyr 2014). In most studies, calcium supplementation was administered from around 20 weeks of pregnancy, the rationale being to cover the period during which pre-eclampsia is manifest. As set out below, this may be too late to interrupt early pregnancy events which are precursors of pre-eclampsia. This review will focus on interventions to improve calcium intake in early pregnancy. This may be achieved by means of calcium supplementation given to women before or very early in pregnancy and during at least the first half of pregnancy, or food fortification with calcium at an individual or community level. Fortification might be targeted by supplementation of staple foods typically consumed by communities with generally low dietary calcium intake.

The possibility of harm from calcium supplementation needs to be considered. Calcium supplementation (but not dietary calcium) has been associated with myocardial infarction risk in the Heidelberg study, an observation at risk of confounding (Li 2012); 1.5 g calcium/day during pregnancy may cause rebound postnatal bone demineralisation (an unexpected finding among multiple trial outcomes assessed) (Jarjou 2010); and an earlier review identified an unexpected increase in the syndrome of haemolysis, elevated liver enzymes and low platelets (HELLP) following calcium supplementation (Hofmeyr 2014), perhaps through the antihypertensive effect of calcium masking the evolution of mild pre-eclampsia into HELLP syndrome (Hofmeyr 2007).

Calcium may be administered in the form of carbonate, citrate, lactate or gluconate, which have good bioavailability. The *19th Expert Committee on the Selection and Use of Essential Medicines* recommended the listing of oral solid dosage forms of calcium, providing 500 mg of elemental calcium per dose (http://www.who.int/medicines/EC19uneditedReport.pdf).

Food fortification would involve the addition of calcium to staple foods that are low in calcium, such as maize or wheat.

How the intervention might work

Hofmeyr 2008 conducted a randomised trial nested within the large WHO trial of calcium supplementation (1.5 g daily from at least 20 weeks' gestation) in pregnant women with low dietary calcium intake (Villar 2006) and the nested trial failed to demonstrate an effect of calcium supplementation on biochemical measures commonly elevated in pre-eclampsia: serum urate, platelet count, and urine protein/creatinine ratio.

The lack of effect on proteinuria is consistent with the findings of the main WHO trial (Villar 2006), in which there was a statistically non-significant reduction in pre-eclampsia (8312 women, RR 0.92, 95% CI 0.75 to 1.13) and severe pre-eclampsia (8302 women, RR 0.74, 95% CI 0.48 to 1.15), but no reduction in proteinuria (8312 women, RR for proteinuria 1.01, 95% CI 0.88 to 1.15). Proteinuria is a hallmark of pre-eclampsia, and a predictor of adverse maternal outcome (von Dadelzsen 2004).

To reconcile the evidence from the systematic review for reduced pre-eclampsia with calcium supplementation (Hofmeyr 2014), with the absence of evidence of an effect on proteinuria and other markers for pre-eclampsia, we proposed the hypothesis that calcium supplementation in the second half of pregnancy reduces blood pressure and thus the diagnosis and severe manifestations of pre-eclampsia, without a significant effect on the underlying pathology (Hofmeyr 2008).

This hypothesis also serves to explain another anomaly identified in the systematic review: whereas pre-eclampsia was reduced overall by 22% (12 trials, 15,206 women, RR 0.78, 95% CI 0.68 to 0.89) and the composite outcome 'maternal death or severe morbidity' was reduced by 20% (five trials, 9734 women, RR 0.80, 95% CI 0.65 to 0.97), HELLP syndrome was increased 2.7 times with calcium supplementation (two trials, 12,901 women, RR 2.67, 95% CI 1.05 to 6.82) (Hofmeyr 2014). If calcium supplementation in the second half of pregnancy reduces only blood pressure, this would reduce the diagnosis and some of the hypertension-related complications of pre-eclampsia, while the effects on other organ systems, such as the endothelium, platelets and liver might continue for a longer time in the calcium supplementation group in which fewer early deliveries for hypertension would take place.

The second anomaly requiring explanation is the modest effect of calcium supplementation in late pregnancy on pre-eclampsia, in contrast to the striking epidemiological differences in populations with good and poor dietary calcium. Deficient dietary calcium before and during early pregnancy may place populations at risk for pre-eclampsia, and the potential to reverse this effect by supplementation in later pregnancy may be limited (Hofmeyr 2008).

Based on the epidemiological association of pre-eclampsia with low dietary calcium and the current understanding of pre-eclampsia as having its origins in early pregnancy events, it is hypothesised that calcium supplementation in early pregnancy may reduce the risk of pre-eclampsia (Hofmeyr 2008).

Why it is important to do this review

The benefits of calcium supplementation in the second half of pregnancy in the prevention of severe morbidity/mortality associated with pre-eclampsia a have been documented in a separate Cochrane review (Hofmeyr 2014). However, there is no systematic evidence to prove or disprove the potential benefits of pre- and early pregnancy calcium supplementation or food fortification in preventing pre-eclampsia (Hofmeyr 2008). Evidence for such an effect would create the opportunity to have a major impact on pre-eclampsia at a population level, for example, by food fortification with calcium among communities at risk. There has not to our knowledge been a previous systematic review on this subject.

OBJECTIVES

To determine the effect of calcium supplementation or food fortification with calcium, given before or very early in pregnancy and at least for the first half of pregnancy, on pre-eclampsia and other hypertensive disorders, maternal morbidity and mortality, as well as fetal and neonatal outcomes.

METHODS

Criteria for considering studies for this review

Types of studies

We included one randomised trial. In future updates, if identified, cluster-randomised trials will be included. Quasi-randomised trials will also be included with due caution use of and sensitivity analysis. Abstract reports will be included if sufficient information is given to assess trial quality and results. Cross-over designs are not appropriate for this intervention.

Types of participants

Women in the early stages of pregnancy (eight to 12 weeks' gestation). In future updates, we will include women of child bearing age not yet pregnant, or in early pregnancy. Women may be at low/average risk of pre-eclampsia, or high risk as predicted by their previous pregnancies, nulliparity or being from a high-risk population.

Types of interventions

We considered interventions including calcium supplementation with or without additional supplements or treatments, compared with placebo or no intervention, specifically:

A calcium supplementation;

B calcium plus additional supplements or treatments;

C calcium food fortification;

D different doses of calcium supplementation or food fortification.

Types of comparators:

E placebo;

F no supplementation;

G dummy food fortification;

H no food fortification.

Comparisons

In future updates, comparisons will include A or B versus E or F, C versus G or H, A versus C and D versus D.

Studies of calcium plus other supplements or treatments will be included and subjected to subgroup analysis.

For this review, we included one study that compared calcium, antioxidants and other supplements versus placebo.

Types of outcome measures

We considered both maternal and fetal outcomes which might be related to the effects of the calcium supplements or food fortification.

Primary outcomes

1. Pre-eclampsia (gestational hypertension and proteinuria, as defined below)
2. Pre-elampsia and/or pregnancy loss/stillbirth at any gestational age
3. Severe maternal morbidity and mortality index: one or more of secondary outcomes marked # below

Secondary outcomes

Maternal

1. No conception during study period
2. Pregnancy loss before 20 weeks' gestational age
3. Pregnancy loss/stillbirth at any gestational age
4. Gestational hypertension (diastolic blood pressure $\geq$ 90 mmHg on two occasions four hours apart, or $\geq$ 110 mmHg once, and/or systolic blood pressure $\geq$ 140 mmHg on two occasions four hours apart, or $\geq$ 160 mmHg once, appearing after 20 weeks' gestation)
5. Gestational proteinuria (2+ or more on urine dipstix, or > 300 mg/24 hours, or > 500 mg/L or urinary protein/creatinine ratio > 0.034, appearing after 20 weeks' gestation)
6. * Severe gestational hypertension (systolic blood pressure $\geq$ 160 mmHg on two occasions four hours apart, or once followed by antihypertensive therapy, and/or diastolic blood pressure $\geq$ 110 mmHg on two occasions four hours apart, or once followed by antihypertensive therapy, appearing after 20 weeks' gestation)
7. * Early onset pre-eclampsia (< 32 weeks' gestation)
8. * # Severe pre-eclampsia (proteinuria plus severe diastolic and/or systolic hypertension)
9. Moderately severe thrombocytopenia (< 100 x 10^9/L or as defined by trial authors)
10. Uric acid $\geq$ reference values for gestational age
11. # Renal failure (serum creatinine $\geq$ 120 mmol/L or as defined by trial authors)
12. # Pulmonary oedema
13. # Cerebrovascular accident
14. Liver failure (serum aspartate aminotransferase (AST) $\geq$ 70 U/L or as defined by trial authors)
15. # Intensive care unit (ICU) admission > 24 hours
16. * # Eclampsia
17. * # HELLP (haemolysis, elevated liver enzymes and low platelets) syndrome (haemolysis (lactate dehydrogenase (LDH) > 600 U/L or bilirubin $\geq$ 1.2 mg/dL), elevated liver enzymes (AST $\geq$ 70 U/L), and low platelet count (< 100 x 10^9/L))
18. * # Placental abruption
19. # Maternal death
20. Mother's hospital stay seven days or more after birth
21. Caesarean section
22. Severe pre-eclamptic complications index (Villar 2006): one or more of outcomes marked ***** above

Neonatal

1. Birthweight < 2500 g
2. Preterm birth (< 37 weeks' gestation)
3. Early preterm birth (< 32 weeks' gestation)
4. Apgar score less than seven at five minutes
5. Death or admission to neonatal ICU for 24 hours or more
6. Stillbirth
7. Pregnancy loss, stillbirth or neonatal death before discharge
8. Neonate small-for-gestational age (non-pre-specified)

Search methods for identification of studies

The following methods section of this review is based on a standard template used by the Cochrane Pregnancy and Childbirth Group.

Electronic searches

We searched Cochrane Pregnancy and Childbirth's Trials Register by contacting their Information Specialist (10 August 2017).

The Register is a database containing over 23,000 reports of controlled trials in the field of pregnancy and childbirth. For full search methods used to populate Pregnancy and Childbirth's Trials Register including the detailed search strategies for CENTRAL, MEDLINE, Embase and CINAHL; the list of handsearched journals and conference proceedings, and the list of journals reviewed via the current awareness service, please follow this link to the editorial information about the Cochrane Pregnancy and Childbirth in the Cochrane Library and select the '***Specialized Register***' section from the options on the left side of the screen.

Briefly, Cochrane Pregnancy and Childbirth's Trials Register is maintained by their Information Specialist and contains trials identified from:

1. monthly searches of the Cochrane Central Register of Controlled Trials (CENTRAL);
2. weekly searches of MEDLINE (Ovid);
3. weekly searches of Embase (Ovid);
4. monthly searches of CINAHL (EBSCO);
5. handsearches of 30 journals and the proceedings of major conferences;
6. weekly current awareness alerts for a further 44 journals plus monthly BioMed Central email alerts.

Search results are screened by two people and the full text of all relevant trial reports identified through the searching activities described above is reviewed. Based on the intervention described, each trial report is assigned a number that corresponds to a specific Pregnancy and Childbirth review topic (or topics), and is then added to the Register. The Information Specialist searches the Register for each review using this topic number rather than keywords. This results in a more specific search set that has been fully accounted for in the relevant review sections (Included studies; Ongoing studies).

In addition, we carried out a supplementary search of PubMed (inception to current) using the strategy given in Appendix 1. Date of last search 29 June 2017.

We also searched ClinicalTrials.gov and the WHO International Clinical Trials Registry Platform (ICTRP) (10 August 2017) for unpublished, planned and ongoing trial reports using the terms given in Appendix 2.

Searching other resources

We searched the reference lists of retrieved papers.

We did not apply any language or date restrictions.

Data collection and analysis

The following methods section of this review is based on a standard template used by the Cochrane Pregnancy and Childbirth Group.

Selection of studies

Both review authors independently assessed for inclusion all the potential studies identified as a result of the search strategy. We resolved any disagreement through discussion or, if required, we planned to involve a third assessor.

Data extraction and management

We designed a form to extract data. For the one eligible study, both review authors extracted the data using the agreed form. We resolved discrepancies through discussion, if required, we would have consulted a third assessor. Data were entered into Review Manager software (RevMan 2014) and checked for accuracy.

When information regarding any of the above was unclear, we planned to contact authors of the original reports to provide further details.

Assessment of risk of bias in included studies

Both review authors independently assessed risk of bias for the study using the criteria outlined in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011). Any disagreement was resolved by discussion, if necessary we would have involved a third assessor.

(1) Random sequence generation (checking for possible selection bias)

We described for the included study the method used to generate the allocation sequence in sufficient detail to allow an assessment of whether it should produce comparable groups.

We assessed the method as:

- low risk of bias (any truly random process, e.g. random number table; computer random number generator);
- high risk of bias (any non-random process, e.g. odd or even date of birth; hospital or clinic record number);
- unclear risk of bias.

(2) Allocation concealment (checking for possible selection bias)

We described for the included study the method used to conceal allocation to interventions prior to assignment and assessed whether intervention allocation could have been foreseen in advance of, or during recruitment, or changed after assignment.

We assessed the methods as:

- low risk of bias (e.g. telephone or central randomisation; consecutively numbered sealed opaque envelopes);
- high risk of bias (open random allocation; unsealed or non-opaque envelopes, alternation; date of birth);
- unclear risk of bias.

(3.1) Blinding of participants and personnel (checking for possible performance bias)

We described for the included study the methods used, if any, to blind study participants and personnel from knowledge of which intervention a participant received. We considered that studies were at low risk of bias if they were blinded, or if we judged that the lack of blinding unlikely to affect results. We assessed blinding separately for different outcomes or classes of outcomes.

We assessed the methods as:

- low, high or unclear risk of bias for participants;
- low, high or unclear risk of bias for personnel.

(3.2) Blinding of outcome assessment (checking for possible detection bias)

We described for the included study the methods used, if any, to blind outcome assessors from knowledge of which intervention a participant received. We assessed blinding separately for different outcomes or classes of outcomes.

We assessed the methods used to blind outcome assessment as:

- low, high or unclear risk of bias.

(4) Incomplete outcome data (checking for possible attrition bias due to the amount, nature and handling of incomplete outcome data)

We described for the included study, and for each outcome or class of outcomes, the completeness of data including attrition and exclusions from the analysis. We stated whether attrition and exclusions were reported and the numbers included in the analysis at each stage (compared with the total randomised participants), reasons for attrition or exclusion where reported, and whether missing data were balanced across groups or were related to outcomes. Where sufficient information was reported, or could be supplied by the trial authors, we planned to re-include missing data in the analyses which we undertook.

We assessed methods as:

- low risk of bias (e.g. no missing outcome data; missing outcome data balanced across groups);
- high risk of bias (e.g. numbers or reasons for missing data imbalanced across groups; 'as treated' analysis done with substantial departure of intervention received from that assigned at randomisation);
- unclear risk of bias.

(5) Selective reporting (checking for reporting bias)

We described for the included study how we investigated the possibility of selective outcome reporting bias and what we found.

We assessed the methods as:

- low risk of bias (where it is clear that all of the study's pre-specified outcomes and all expected outcomes of interest to the review have been reported);
- high risk of bias (where not all the study's pre-specified outcomes have been reported; one or more reported primary outcomes were not pre-specified; outcomes of interest are reported incompletely and so cannot be used; study fails to include results of a key outcome that would have been expected to have been reported);
- unclear risk of bias.

(6) Other bias (checking for bias due to problems not covered by (1) to (5) above)

We described for the included study any important concerns we had about other possible sources of bias.

(7) Overall risk of bias

We made explicit judgements about whether the study was at high risk of bias, according to the criteria given in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011). With reference to (1) to (6) above, we planned to assess the likely magnitude and direction of the bias and whether we considered it is likely to impact on the findings. In future updates, we will explore the impact of the level of bias through undertaking sensitivity analyses - *see* Sensitivity analysis.

Assessing the quality of the body of evidence using the GRADE approach

We assessed the quality of the evidence using the GRADE approach as outlined in the GRADE handbook in order to assess the quality of the body of evidence relating to the following outcomes for the main comparisons (calcium with or without additional supplements or treatments versus placebo or no treatment).

1. Pre-eclampsia (gestational hypertension and proteinuria, as defined below)
2. Pre-eclampsia and/or pregnancy loss/stillbirth at any gestational age
3. Severe maternal morbidity and mortality index
4. Pregnancy loss/stillbirth at any gestational age
5. Caesarean section
6. Birthweight < 2500 g
7. Apgar score less than seven at five minutes
8. Death or admission to neonatal ICU for 24 hours or more
9. Pregnancy loss, stillbirth or neonatal death before discharge

We used the GRADEpro Guideline Development Tool to import data from Review Manager 5.3 (RevMan 2014) in order to create a 'Summary of findings' table. A summary of the intervention effect and a measure of quality for each of the above outcomes was produced using the GRADE approach. The GRADE approach uses five considerations (study limitations, consistency of effect, imprecision, indirectness and publication bias) to assess the quality of the body of evidence for each outcome. The evidence can be downgraded from 'high quality' by one level for serious (or by two levels for very serious) limitations, depending on assessments for risk of bias, indirectness of evidence, serious inconsistency, imprecision of effect estimates or potential publication bias.

Measures of treatment effect

Dichotomous data

For dichotomous data, we presented results as summary risk ratio with 95% confidence intervals.

Continuous data

We did not report any continuous data, however, in future review updates, we will use the mean difference if outcomes are measured in the same way between trials. We will use the standardised mean difference to combine trials that measure the same outcome, but use different methods.

Unit of analysis issues

Cluster-randomised trials

No cluster-randomised trials were identified during the search process. We will include cluster-randomised trials in the analyses along with individually-randomised trials if they are identified for future updates. We will adjust their standard errors using the methods described in the *Handbook [Section 16.3.4 or 16.3.6]* using an estimate of the intracluster correlation co-efficient (ICC) derived from the trial (if possible), from a similar trial or from a study of a similar population. If we use ICCs from other sources, we will report this and conduct sensitivity analyses to investigate the effect of variation in the ICC. If we identify both cluster-randomised trials and individually-randomised trials, we plan to synthesise the relevant information. We will consider it reasonable to combine the results from both if there is little heterogeneity between the study designs and the interaction between the effect of intervention and the choice of randomisation unit is considered to be unlikely.

We will also acknowledge heterogeneity in the randomisation unit and perform a sensitivity analysis to investigate the effects of the randomisation unit.

Cross-over trials

Cross-over trials are not appropriate for this intervention.

Studies with multiple arms

For multi-armed studies, in future updates, pairs of arms relevant to the review will be compared. Where one arm appears more than once on a meta-analysis, the outcomes and denominators will be divided by the number of times it appears to avoid multiple counting.

Dealing with missing data

For the included study, the level of attrition was noted. In future updates, if more eligible studies are identified, the impact of including studies with high levels of missing data in the overall assessment of treatment effect will be explored by using sensitivity analysis.

For all outcomes, analyses were carried out, as far as possible, on an intention-to-treat basis, i.e. we attempted to include all participants randomised to each group in the analyses. The denominator for each outcome in the trial was the number randomised minus any participants whose outcomes were known to be missing.

Assessment of heterogeneity

As only one study was included, it was not appropriate to assess statistical heterogeneity. However if more studies are included in future updates, heterogeneity will be assessed in each meta-analysis using the Tau^2, I^2 and Chi^2 statistics. Heterogeneity will be regarded as substantial if an I^2 is greater than 30% and either a Tau^2 is greater than zero, or there is a low P value (less than 0.10) in the Chi^2 test for heterogeneity. If we identify substantial heterogeneity (above 30%), we plan to explore it by pre-specified subgroup analysis.

Assessment of reporting biases

In future updates, if there are 10 or more studies in the meta-analysis we will investigate reporting biases (such as publication bias) using funnel plots. We will assess funnel plot asymmetry visually. If asymmetry is suggested by a visual assessment, we will perform exploratory analyses to investigate it.

Data synthesis

We carried out statistical analysis using the Review Manager software (RevMan 2014). We did not carry out meta-analysis as we included only one study. In future updates, we will use fixed-effect meta-analysis for combining data where it is reasonable to assume that studies are estimating the same underlying treatment effect: i.e. where trials are examining the same intervention, and the trials' populations and methods are judged to be sufficiently similar.

In future updates, if there is clinical heterogeneity sufficient to expect that the underlying treatment effects differs between trials, or if substantial statistical heterogeneity is detected, we will use random-effects meta-analysis to produce an overall summary, if an average treatment effect across trials is considered clinically meaningful. The random-effects summary will be treated as the average range of possible treatment effects and we will discuss the clinical implications of treatment effects differing between trials. If the average treatment effect is not clinically meaningful, we will not combine trials. If we used random-effects analyses, the results were presented as the average treatment effect with 95% confidence intervals, and the estimates of Tau^2 and I^2.

Subgroup analysis and investigation of heterogeneity

If we had identified substantial heterogeneity, we planned to investigate it using subgroup analyses and sensitivity analyses, however only one study was included in this review. We planned to consider whether an overall summary was meaningful, and if it was, use random-effects analysis to produce it.

We planned to carry out the following subgroup analyses.

1. Women at high risk of pre-eclampsia versus low risk versus risk unclear/mixed risk
2. Women with low dietary calcium versus adequate dietary calcium versus dietary calcium unclear/mixed
3. High-dose calcium supplementation (1 g daily or more) versus low-dose supplementation
4. Supplementation versus food fortification
5. Calcium alone versus calcium plus other supplements
6. Calcium commenced before pregnancy versus in early pregnancy (< 13 weeks)

In future updates of the review, the specified for the GRADE tables will be used in subgroup analysis.

We will assess subgroup differences by interaction tests available within RevMan (RevMan 2014). We will report the results of subgroup analyses quoting the Chi^2 statistic and P value, and the interaction test I^2 value.

Sensitivity analysis

In future updates, we will perform sensitivity analysis by examining the effect on results of excluding:

1. trials at high risk of bias based on allocation concealment;
2. trials with small sample sizes (less than 200);
3. trials with no pre-registered protocols.

We will also carry out sensitivity analysis to investigate the effect of the randomisation unit (where we analyse cluster-randomised controlled trial data along with the individually-randomised trials).

Sensitivity analysis will be limited to the outcomes specified for the GRADE tables.

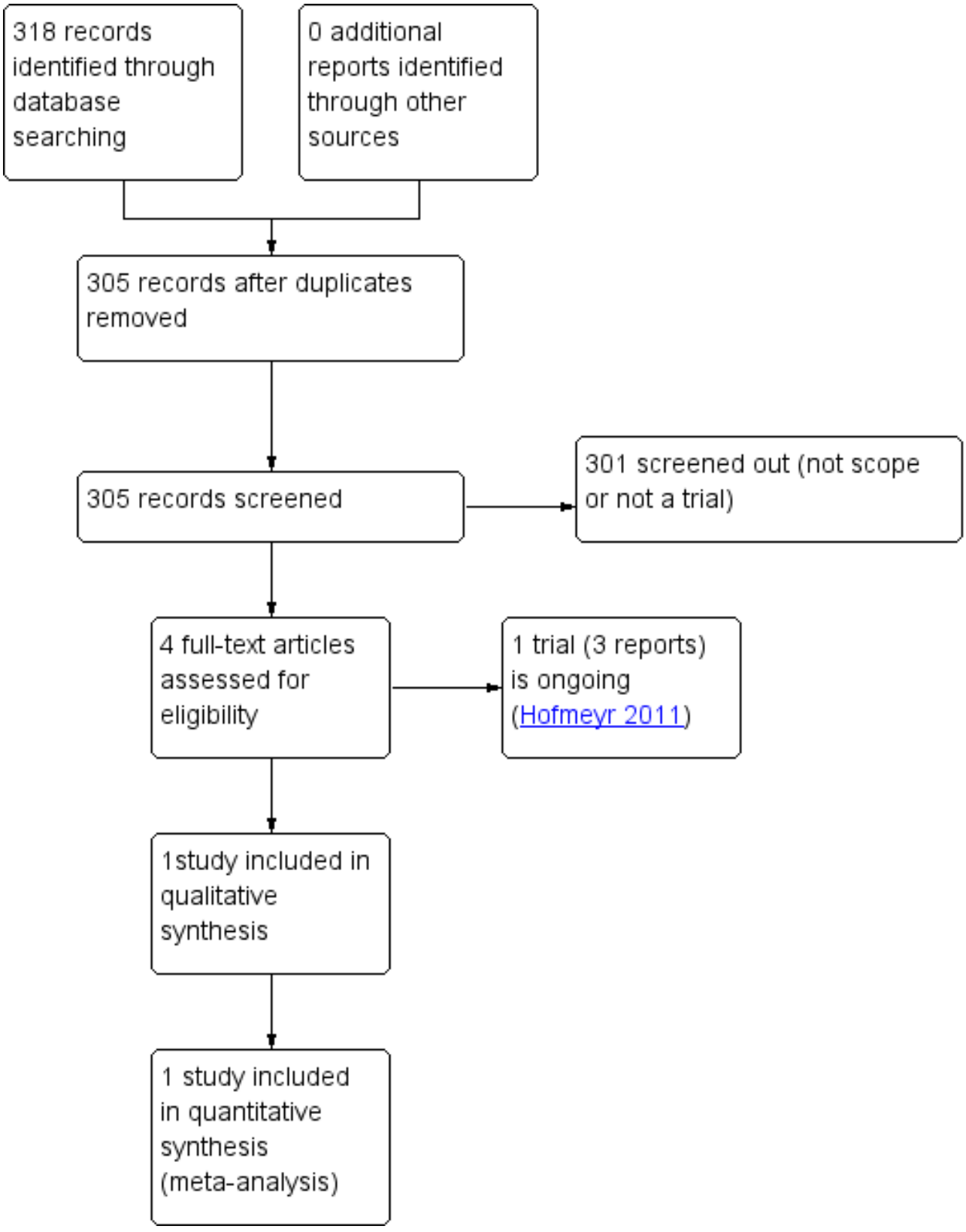
RESULTS

Description of studies

Results of the search

The search retrieved 318 hits in total. After screening, we found three reports relating to one ongoing study (Hofmeyr 2011 - see Ongoing studies section), and one report of a study which was included (Rumiris 2006).See: Figure 1 for search details.

Figure 1. Study flow diagram.



Included studies

No studies of calcium alone were found.

Design

We included one small double-blind, placebo-controlled trial (Rumiris 2006). The study start and end dates were not reported. The study looked at calcium (800 mg) plus antioxidant and other supplements (see Included studies section for further details) commencing in early pregnancy (eight to 12 weeks' gestation throughout pregnancy). Both groups received iron and folic acid.

Sample sizes

The included study (Rumiris 2006) was small (n = 60).

Settings

The Rumiris 2006 study was conducted in a university hospital in Indonesia.

Participants

Rumiris 2006 enrolled healthy pregnant women with low antioxidant status determined by blood tests.

Interventions and comparisons

The women in the intervention group received supplementation with calcium (800 mg) plus N-acetylcysteine (200 mg), Cu (2 mg), Zn (15 mg), Mn (0.5 mg) and selenium (100 mcg) and vitamins A (1000 IU), B6 (2.2 mg), B12 (2.2 mcg), C (200 mg), and E (400 IU) (see Characteristics of included studies for further details).

The control group was given tablets similar in appearance. Both groups received supplementation with Fe (30 mg) and folic acid (400 mcg).

Both groups received one tablet in the morning and evening, from randomisation at eight to 12 weeks' gestation throughout pregnancy.

Outcomes

Rumiris 2006 reported maternal outcomes including pre-eclampsia, hypertension, birthweight, placental abruption and (spontaneous) abortion. Perinatal outcomes included intrauterine growth restriction (IUGR), intrauterine fetal death, and gestational age at delivery.

Sources of trial funding

Rumiris 2006 did not mention sources of funding for the study.

Declarations of interest

This was not mentioned in Rumiris 2006.

Excluded studies

No studies were excluded.

Risk of bias in included studies

Allocation

Rumiris 2006 was assessed as being at low risk of bias (computer-generated random sequence implemented by an independent person).

Blinding

Rumiris 2006 was assessed as at low risk of bias (double-blind, placebo-controlled).

Incomplete outcome data

Rumiris 2006 was assessed as at low risk of bias (100% follow-up).

Selective reporting

Rumiris 2006 was assessed as at unclear risk of bias as a pre-published protocol was not available.

Other potential sources of bias

No other potential sources of bias were identified for the Rumiris 2006 study and it was assessed as at an unclear risk of bias.

Effects of interventions

See: **Summary of findings for the main comparison** Calcium +/- other supplements compared to no calcium or placebo for pregnancy, or food fortification with calcium, for preventing hypertensive disorders of pregnancy

One study was included, thus no meta-analysis was carried out.

Calcium versus placebo

We found no completed studies, but one study is ongoing (Hofmeyr 2011).

Calcium plus antioxidant and other supplements versus placebo

See: Summary of findings for the main comparison.

We included one study of tablets containing calcium (800 mg) plus N-acetylcysteine (200 mg), Cu (2 mg), Zn (15 mg), Mn (0.5 mg) and selenium (100 mcg) and vitamins A (1000 IU), B6 (2.2 mg), B12 (2.2 mcg), C (200 mg), and E (400 IU), versus placebo tablets similar in appearance, in women with low antioxidant levels. The tablets of both groups contained iron (30 mg) and folic acid (400 mcg).

Primary outcomes

Pre-eclampsia

One study (Rumiris 2006) found that calcium supplementation commenced in early pregnancy may be associated with reduction in pre-eclampsia (risk ratio (RR) 0.24, 95% confidence interval (CI) 0.06 to 1.01; one study, 60 women, *low-quality evidence* (Analysis 1.1)), although the quality of this evidence is low and the confidence intervals just cross the line of no effect.

Pre-elampsia and/or pregnancy loss/stillbirth at any gestational age

One study (Rumiris 2006) found a reduction in this composite outcome for women taking calcium supplementation (RR 0.13, 95% CI 0.03 to 0.50; one study, 60 women, *moderate-quality evidence* (Analysis 1.2)).

Severe maternal morbidity and mortality index

One study (Rumiris 2006) reported severe pre-eclampsia and placental abruption (RR 0.36, 95% CI 0.04 to 3.23; one study, 60 women; *low-quality evidence* (Analysis 1.3)) with no clear difference between the calcium supplementation and control groups. There

were no cases of placental abruption reported. Other outcomes in the index were not reported in this study including: renal failure, pulmonary oedema, cerebrovascular accident, intensive care unit (ICU) admission > 24 hours, eclampsia, HELLP syndrome and maternal death.

Secondary outcomes

Maternal outcomes

Pregnancy loss before 20 weeks' gestational age

One study (Rumiris 2006) found that calcium supplementation may be associated with a reduction in early pregnancy loss (RR 0.06, 95% CI 0.00 to 1.04; one study, 60 women, *moderate-quality evidence* (Analysis 1.4)), however, wide confidence intervals cross the line of no effect and we cannot be certain of this result.

Pregnancy loss/stillbirth at any gestational age

One study (Rumiris 2006) found calcium supplementation was associated with a reduction in pregnancy loss or stillbirth (RR 0.06, 95% CI 0.00 to 0.92; one study, 60 women; *moderate-quality evidence* (Analysis 1.5)).

Severe pre-eclampsia

One study (Rumiris 2006) had too few outcomes (1/29 versus 3/31) for meaningful statistical analysis (RR 0.36, 95% CI 0.04 to 3.23; one study, 60 women (Analysis 1.6)).

Placental abruption

One study (Rumiris 2006) reported no cases of placental abruption (Analysis 1.7).

The following maternal outcomes were not reported

- No conception during study period
- Gestational hypertension (diastolic blood pressure ≥ 90 mmHg on two occasions four hours apart, or ≥ 110 mmHg once, and/or systolic blood pressure ≥ 140 mmHg on two occasions four hours apart, or ≥ 160 mmHg once, appearing after 20 weeks' gestation)
- Gestational proteinuria (2+ or more on urine dipstix, or > 300 mg/24 hours, or > 500 mg/L or urinary protein/creatinine ratio > 0.034, appearing after 20 weeks' gestation)
- Severe gestational hypertension (systolic blood pressure ≥ 160 mmHg on two occasions four hours apart, or once followed by antihypertensive therapy, and/or diastolic blood pressure ≥ 110 mmHg on two occasions four hours apart, or once followed by antihypertensive therapy, appearing after 20 weeks' gestation)*
- Early onset pre-eclampsia (< 32 weeks' gestation)*
- Moderately severe thrombocytopenia (< 100 x 10^9/L or as defined by trial authors)
- Uric acid ≥ reference values for gestational age
- Renal failure (serum creatinine ≥ 120 mmol/L or as defined by trial authors)
- Pulmonary oedema
- Cerebrovascular accident
- Liver failure (serum AST ≥ 70 U/L or as defined by trial authors)
- ICU admission > 24 hours
- Eclampsia*
- HELLP syndrome (haemolysis (LDH > 600 U/L or bilirubin ≥ 1.2 mg/dl), elevated liver enzymes (AST ≥ 70 U/L), and low platelet count (< 100 x 10^9/L))*
- Maternal death
- Mother's hospital stay seven days or more after birth
- Caesarean section
- Severe pre-eclamptic complications index (Villar 2006): one or more of outcomes marked * above

Neonatal outcomes

Preterm birth (< 37 weeks' gestation)

One study (Rumiris 2006) had too few outcomes (1/29 versus 3/31) for meaningful statistical analysis (RR 0.36, 95% CI 0.04 to 3.23; one study, 60 women (Analysis 1.8)).

The following neonatal outcomes were not reported.

- Birthweight < 2500 g
- Early preterm birth (< 32 weeks' gestation)
- Apgar score less than seven at five minutes
- Death or admission to neonatal ICU for 24 hours or more
- Stillbirth
- Pregnancy loss, stillbirth or neonatal death before discharge
- Neonate small-for-gestational age (non-pre-specified)

DISCUSSION

Summary of main results

Calcium versus placebo

No data were available

Calcium plus antioxidant and other supplements versus placebo

One small study (Rumiris 2006) of calcium (800 mg) plus antioxidants and other supplements versus placebo in women with low antioxidant status found a reduction in the composite outcomes **pre-eclampsia and/or pregnancy loss at any gestational age**, and **pregnancy loss/stillbirth at any gestational age**. It appears that **pre-eclampsia** and **severe maternal morbidity and mortality index** including data for severe pre-eclampsia and placental abruption may be reduced by calcium plus antioxidants and other supplements, however both these outcomes have wide confidence intervals, which just cross the line of no effect, and are *low-quality evidence*. It appears that early**pregnancy loss before 20 weeks' gestation** may be slightly reduced by calcium plus antioxidants and other supplements, but this outcome also has wide confidence intervals, which just cross the line of no effect. Other outcomes reported were too infrequent for meaningful statistical analysis.

Overall completeness and applicability of evidence

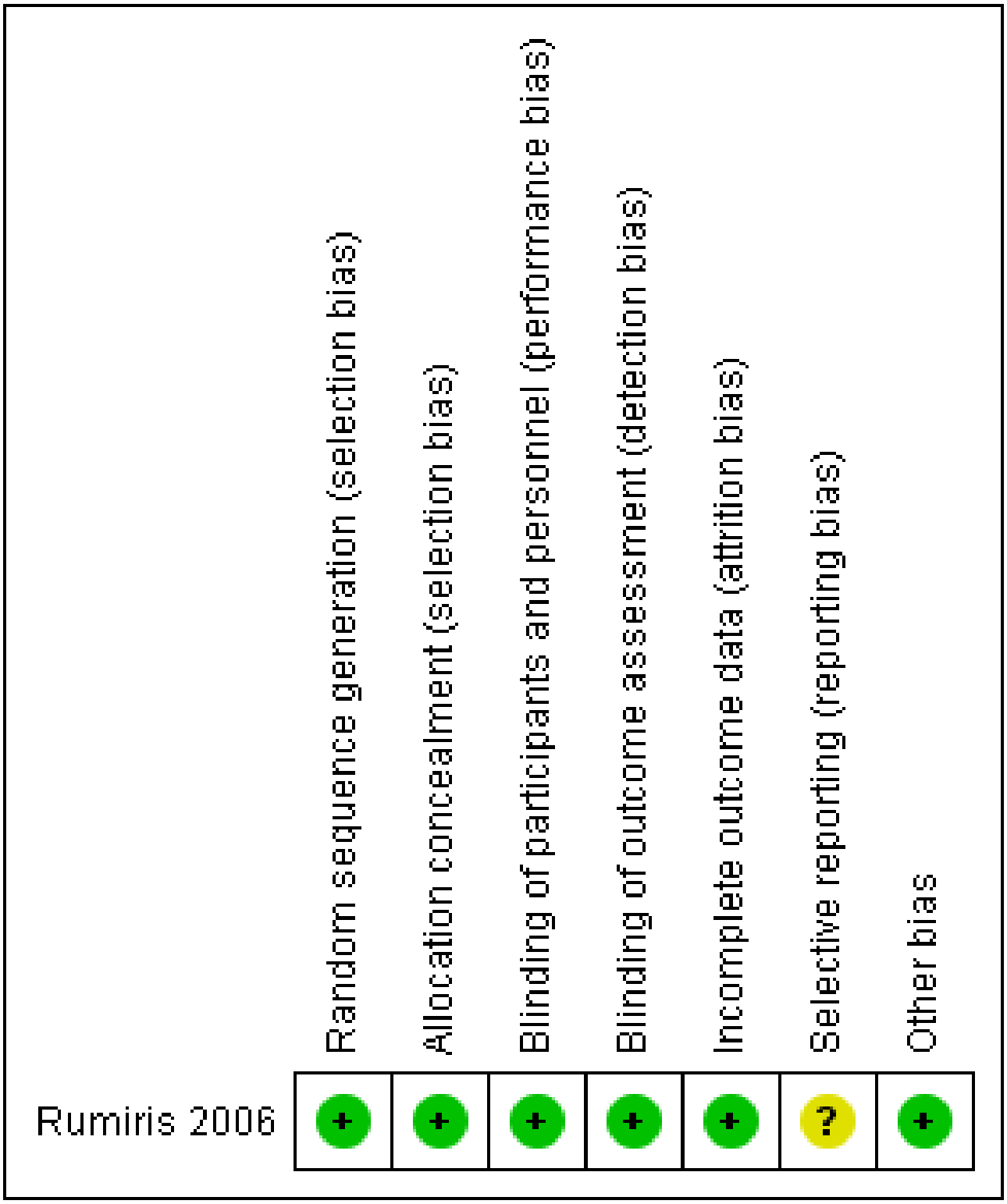
The evidence is limited to one small study of calcium plus antioxidants and other supplements versus placebo control. Therefore, we cannot be certain whether any of the effects found in this study were due to calcium supplementation alone. Elsewhere, evidence from one small study of antioxidants in women with low antioxidant levels found a reduction in pre-eclampsia risk (Wibowo 2012).

Several outcomes reported (**placental abruption**, **severe pre-eclampsia** and **preterm birth (less than 37 weeks' gestation)**) were too infrequent for meaningful analysis. No data were reported for the outcomes caesarean section, birthweight < 2500 g, Apgar score less than seven at five minutes, death or admission to neonatal ICU, or pregnancy loss, still birth or neonatal death before discharge from hospital.

Quality of the evidence

The single study included in this review was assessed to be at low risk of selection, performance, detection and attrition bias, and at unclear risk of reporting bias (Figure 2).

Figure 2. 'Risk of bias' summary: review authors' judgements about each risk of bias item for each included study.



We used GRADEpro software to grade the outcomes listed in Assessment of risk of bias in included studies. The evidence for two primary outcomes, **pre-eclampsia** and **severe maternal morbidity and mortality index,** was graded as *low quality.* This was due to the small sample size, number of events, and the wide confidence intervals which crossed the line of no effect, all of which contribute to imprecision of evidence. The level of evidence for the outcomes **pre-eclampsia and/or pregnancy loss at any gestational age** and **pregnancy loss/stillbirth at any gestational age** was graded as *moderate quality*. This was also downgraded for imprecision of effect estimates, because of the small sample size and few events.

Potential biases in the review process

The review authors are investigators in an ongoing study of pre-pregnancy calcium supplementation. They will not participate in decisions regarding the inclusion or data extraction of this study.

Agreements and disagreements with other studies or reviews

The effect on pre-eclampsia in Rumiris 2006 is consistent with the large reduction in pre-eclampsia in the smaller studies of calcium supplementation alone in later pregnancy reviewed elsewhere (see Cochrane review by Hofmeyr 2014), though the magnitude of this reduction was not confirmed in the largest study (Villar 2006).

AUTHORS' CONCLUSIONS

Implications for practice

Calcium supplementation in the second half of pregnancy is currently recommended by the World Health Organization for women with low dietary calcium intake. There is insufficient evidence from RCTs on the effectiveness or otherwise of pre- or early-pregnancy supplementation, or food fortification, with calcium. The evidence in this review is limited to one small study in which women with low antioxidant status were given calcium plus antioxidants and other supplements or placebo, during early pregnancy. Therefore, we cannot be certain whether the effects observed in this study can be attributed to calcium supplementation.

Implications for research

Further research is needed to determine whether pre- or early-pregnancy supplementation, or food fortification, with calcium is associated with a reduction in adverse pregnancy outcomes such as pre-eclampsia and pregnancy loss. Such studies should be adequately powered, limited to calcium supplementation, placebo-controlled, and include the outcomes chosen for this review.

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As part of the pre-publication editorial process, this review has been commented on by four peers (an editor and three referees who are external to the editorial team), a member of Cochrane Pregnancy and Childbirth's international panel of consumers and the Group's Statistical Adviser.

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* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Rumiris 2006

Methods	Double-blind, placebo-controlled trial.
Participants	Healthy pregnant women with low antioxidant status (SOD concentration below 1102/U/gHb or 164 U/mL) at 8 to 12 weeks of gestation. "...women who consulted at the antenatal clinic of the Department of Obstetrics and Gynecology, University of Indonesia between March 2003 and June 2004 were candidates for inclusion in the study". Exclusion criteria: 1) history or current use of anti-hypertensive medication or diuretics; 2) use of vitamins C > 150 mg and/or E > 75 IU per day; 3) known placental abnormalities; 4) current pregnancy as a result of in vitro fertilisation; 5) regular use of platelet active drugs or non-steroidal anti-inflammatory drugs; 6) known fetal abnormalities; 7) documented uterine bleeding within a week of screening;

Rumiris 2006 *(Continued)*

	8) uterine malformations; 9) history of medical complications. Participants were randomised according to a computer-generated random number sequence by an independent third party who had no conflict of interest in the study. 29 assigned to supplementation and 31 to placebo.
Interventions	Intervention group: supplementation with calcium (800 mg), folic acid (400 mcg), N-acetylcysteine (200 mg), Cu (2 mg), Zn (15 mg), Mn (0.5 mg), Fe (30 mg), and selenium (100 mcg) and vitamins A (1000 IU), B6 (2.2 mg), B12 (2.2 mcg), C (200 mg), and E (400 IU). Control group: supplementation with Fe (30 mg) and folic acid (400 mcg). From 8 to 12 weeks of gestation throughout pregnancy. Placebo supplement's size and appearance were matched with those of antioxidants. "Subjects were asked to take one tablet in the morning and one tablet in the evening."
Outcomes	Maternal - pre-eclampsia (previously normotensive woman with hypertension and proteinuria after the 20th week of pregnancy and returned to normal levels by the 6th week postpartum), hypertension (blood pressure of at least 140/90 mm Hg on 2 or more occasions, separated by more than 4 hours) proteinuria (2+ on urinalysis, or at least 300 mg/L or 500 mg per 24 hours in a 24-hour collection of urine in the absence of a urinary tract infection) and abortion. Perinatal - intrauterine growth restriction, intrauterine fetal death, preterm delivery (before 37 weeks).
Notes	No additional information requested. The rate of pre-eclampsia in the control group was unexpectedly high (30%). In another study from the same group the rate in the control group of women with low antioxidant status was 14.5% (Wibowo 2012). **Sources of funding:** not stated. **Declarations of interest:** not stated. **Trial dates**: not stated.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer-generated random number sequence by an independent third party who had no conflict of interest in the study.
Allocation concealment (selection bias)	Low risk	Double-blind placebo controlled. Participants received similar-looking tablets twice daily.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Double-blind placebo controlled. Participants received similar-looking tablets twice daily.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Double-blind placebo controlled. Participants received similar-looking tablets twice daily.
Incomplete outcome data (attrition bias)	Low risk	No loss to follow-up.

Rumiris 2006 *(Continued)*
All outcomes

Selective reporting (reporting bias)	Unclear risk	No prospectively published protocol available.
Other bias	Low risk	None noted.

Hb:haemoglobin
Cu: copper
Fe: iron
Mn: manganese
IU: international units
mg: milligram
mcg: microgram
SOD: superoxidedismutase
Zn: zinc

Characteristics of ongoing studies *[ordered by study ID]*

Hofmeyr 2011

Trial name or title	The Calcium and Pre-eclampsia (CAP) Study.
Methods	Double-blind, placebo-controlled randomised clinical trial.
Participants	Non-pregnant women with pre-eclampsia in their most recent pregnancy.
Interventions	Elemental calcium 500 mg as calcium carbonate daily from enrolment until 20 weeks' gestation, versus placebo.
Outcomes	Primary: pre-eclampsia. Secondary: pregnancy loss or pre-eclampsia; severe maternal morbidity and mortality index; conception; pregnancy loss; no conception during study period; pregnancy loss before 24 weeks' gestational age; pregnancy loss/stillbirth; gestational hypertension; gestational proteinuria; severe gestational hypertension; early onset pre-eclampsia; severe pre-eclampsia; moderately severe thrombocytopenia; uric acid $\geq$ reference values for gestational age; renal failure; pulmonary oedema; cerebrovascular accident; liver failure; intensive care unit admission > 24 hour; eclampsia; HELLP syndrome; placental abruption; maternal death; mother's hospital stay 7 days or more after birth; caesarean section; severe pre-eclamptic complications index; birthweight < 2500 g; preterm birth; early preterm birth; Apgar score less than 7 at 5 minutes; death or admission to neonatal intensive care unit for 24 hours or more; stillbirth; pregnancy loss, stillbirth or neonatal death before discharge.
Starting date	
Contact information	GJ Hofmeyr, Effective Care REsearch Unit, University of the Witwatersrand/Fort Hare; Eastern Cape Department of Health. justhof@gmail.com
Notes	

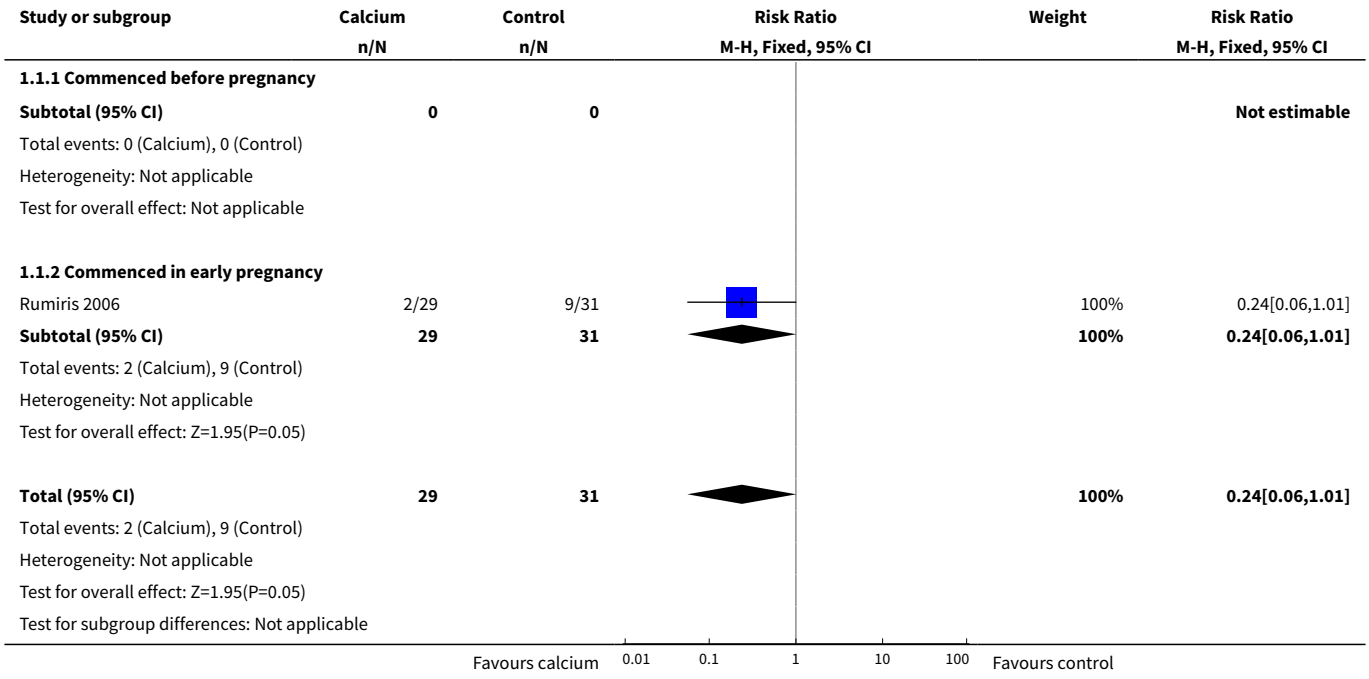
DATA AND ANALYSES

Comparison 1. Calcium +/- other supplements versus no calcium or placebo

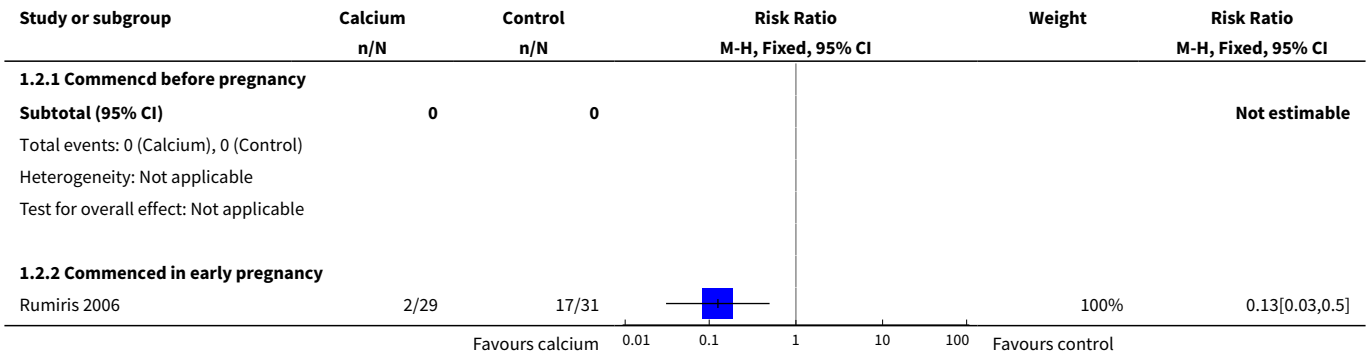
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Pre-eclampsia	1	60	Risk Ratio (M-H, Fixed, 95% CI)	0.24 [0.06, 1.01]
1.1 Commenced before pregnancy	0	0	Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
1.2 Commenced in early pregnancy	1	60	Risk Ratio (M-H, Fixed, 95% CI)	0.24 [0.06, 1.01]
2 Pre-eclampsia and/or pregnancy loss/stillbirth at any gestational age	1	60	Risk Ratio (M-H, Fixed, 95% CI)	0.13 [0.03, 0.50]
2.1 Commencd before pregnancy	0	0	Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
2.2 Commenced in early pregnancy	1	60	Risk Ratio (M-H, Fixed, 95% CI)	0.13 [0.03, 0.50]
3 Severe maternal morbidity and mortality index	1	60	Risk Ratio (M-H, Fixed, 95% CI)	0.36 [0.04, 3.23]
3.1 Commenced before pregnancy	0	0	Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
3.2 Commenced in early pregnancy	1	60	Risk Ratio (M-H, Fixed, 95% CI)	0.36 [0.04, 3.23]
4 Pregnancy loss before 20 weeks' gestation	1	60	Risk Ratio (M-H, Fixed, 95% CI)	0.06 [0.00, 1.04]
4.1 Commenced before pregnancy	0	0	Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
4.2 Commenced in early pregnancy	1	60	Risk Ratio (M-H, Fixed, 95% CI)	0.06 [0.00, 1.04]
5 Pregnancy loss/stillbirth at any gestational age	1	60	Risk Ratio (M-H, Fixed, 95% CI)	0.06 [0.00, 0.92]
5.1 Commenced before pregnancy	0	0	Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
5.2 Commenced in early pregnancy	1	60	Risk Ratio (M-H, Fixed, 95% CI)	0.06 [0.00, 0.92]
6 Severe pre-eclampsia	1	60	Risk Ratio (M-H, Fixed, 95% CI)	0.36 [0.04, 3.23]
6.1 Commenced before pregnancy	0	0	Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
6.2 Commenced in early pregnancy	1	60	Risk Ratio (M-H, Fixed, 95% CI)	0.36 [0.04, 3.23]
7 Placental abruption	1	60	Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
7.1 Commencd before pregnancy	0	0	Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
7.2 Commencd in early pregnancy	1	60	Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]

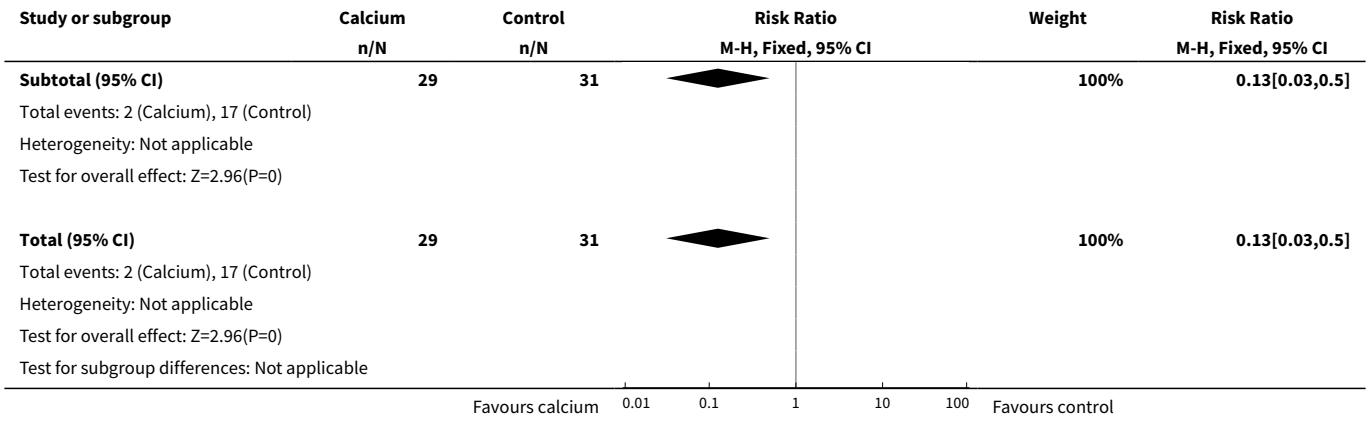
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
8 Preterm birth (< 37 weeks' gestation)	1	60	Risk Ratio (M-H, Fixed, 95% CI)	0.36 [0.04, 3.23]
8.1 Commenced before pregnancy	0	0	Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
8.2 Commenced in early pregnancy	1	60	Risk Ratio (M-H, Fixed, 95% CI)	0.36 [0.04, 3.23]

Analysis 1.1. Comparison 1 Calcium +/- other supplements versus no calcium or placebo, Outcome 1 Pre-eclampsia.

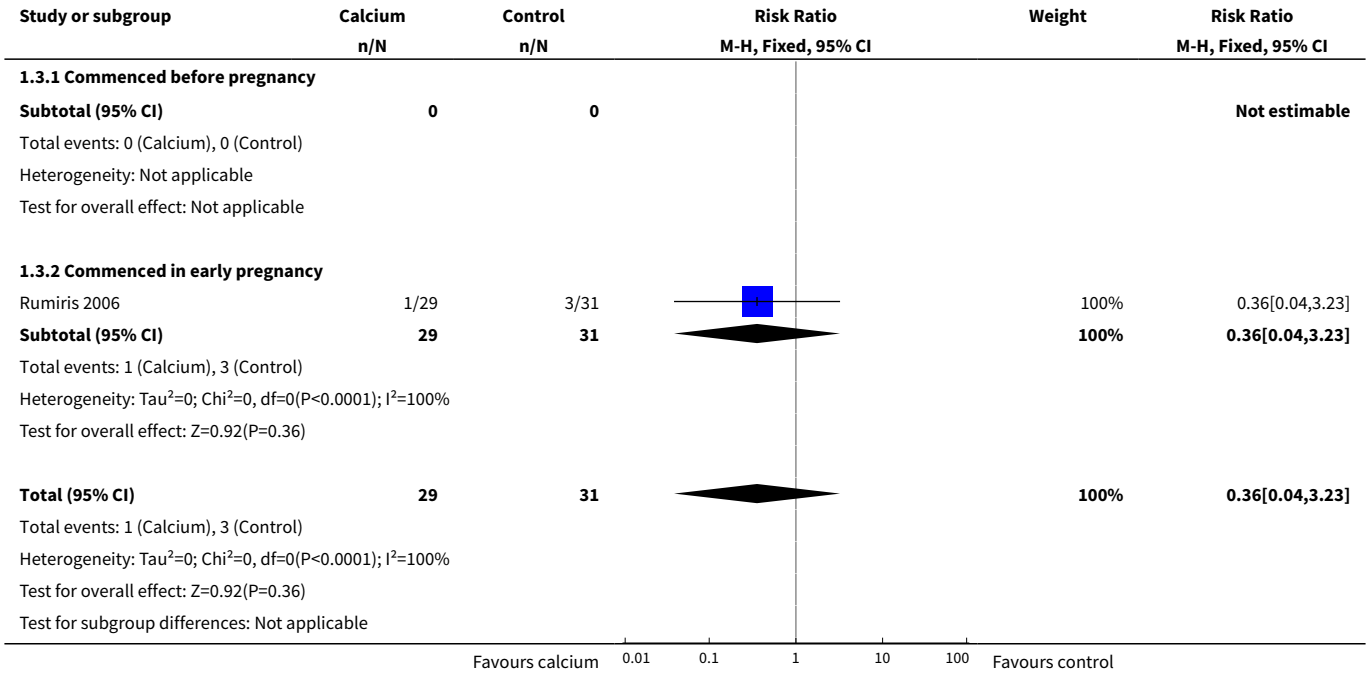


Analysis 1.2. Comparison 1 Calcium +/- other supplements versus no calcium or placebo, Outcome 2 Pre-eclampsia and/or pregnancy loss/stillbirth at any gestational age.

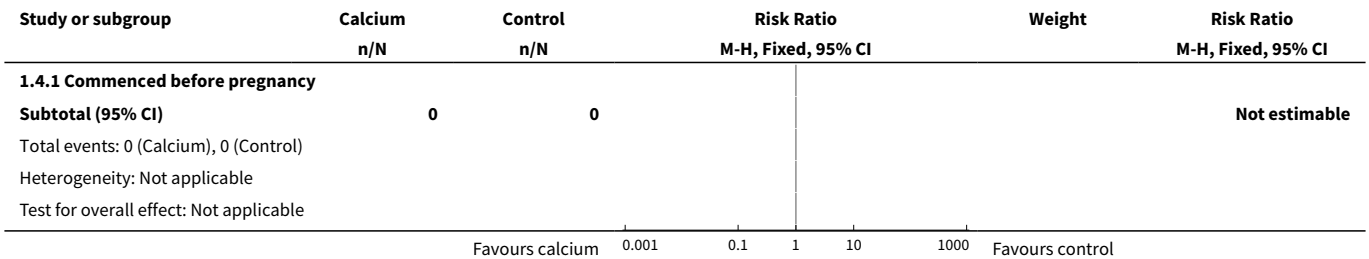




Analysis 1.3. Comparison 1 Calcium +/- other supplements versus no calcium or placebo, Outcome 3 Severe maternal morbidity and mortality index.



Analysis 1.4. Comparison 1 Calcium +/- other supplements versus no calcium or placebo, Outcome 4 Pregnancy loss before 20 weeks' gestation.



Study or subgroup	Calcium n/N	Control n/N	Risk Ratio M-H, Fixed, 95% CI	Weight	Risk Ratio M-H, Fixed, 95% CI
1.4.2 Commenced in early pregnancy					
Rumiris 2006	0/29	8/31		100%	0.06[0,1.04]
Subtotal (95% CI)	**29**	**31**		**100%**	**0.06[0,1.04]**
Total events: 0 (Calcium), 8 (Control)					
Heterogeneity: Not applicable					
Test for overall effect: Z=1.93(P=0.05)					
Total (95% CI)	**29**	**31**		**100%**	**0.06[0,1.04]**
Total events: 0 (Calcium), 8 (Control)					
Heterogeneity: Not applicable					
Test for overall effect: Z=1.93(P=0.05)					
Test for subgroup differences: Not applicable					

Favours calcium 0.001 0.1 1 10 1000 Favours control

Analysis 1.5. Comparison 1 Calcium +/- other supplements versus no calcium or placebo, Outcome 5 Pregnancy loss/stillbirth at any gestational age.

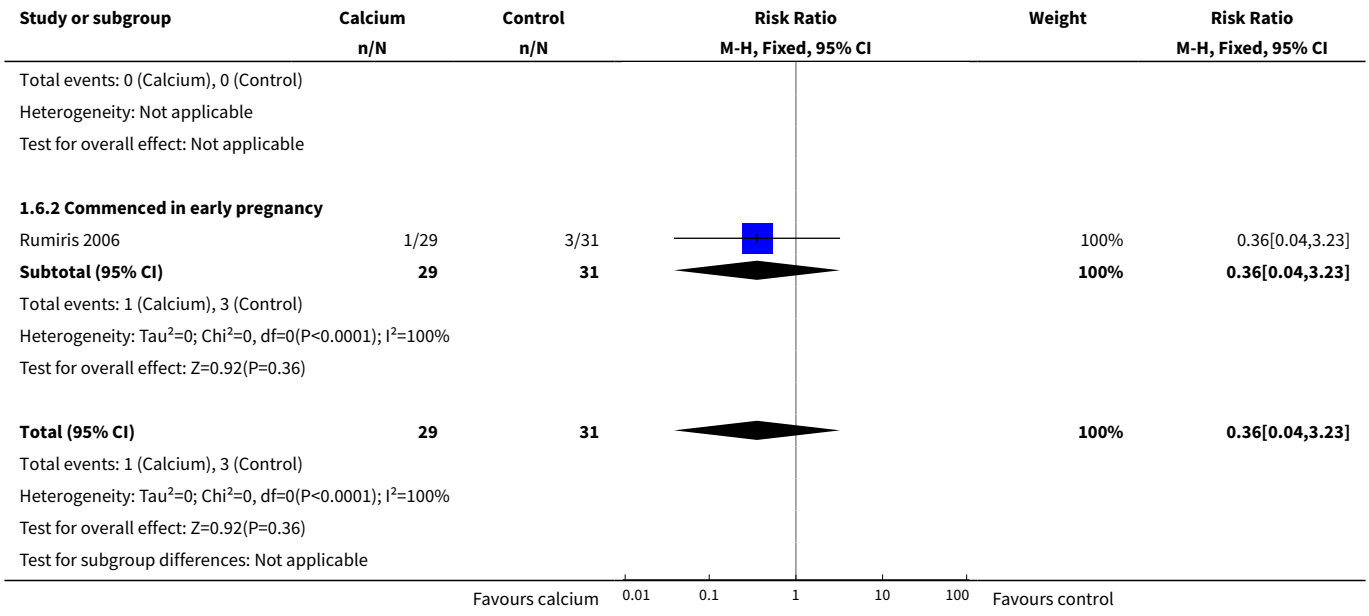
Study or subgroup	Calcium n/N	Control n/N	Risk Ratio M-H, Fixed, 95% CI	Weight	Risk Ratio M-H, Fixed, 95% CI
1.5.1 Commenced before pregnancy					
Subtotal (95% CI)	**0**	**0**			**Not estimable**
Total events: 0 (Calcium), 0 (Control)					
Heterogeneity: Not applicable					
Test for overall effect: Not applicable					
1.5.2 Commenced in early pregnancy					
Rumiris 2006	0/29	9/31		100%	0.06[0,0.92]
Subtotal (95% CI)	**29**	**31**		**100%**	**0.06[0,0.92]**
Total events: 0 (Calcium), 9 (Control)					
Heterogeneity: Not applicable					
Test for overall effect: Z=2.02(P=0.04)					
Total (95% CI)	**29**	**31**		**100%**	**0.06[0,0.92]**
Total events: 0 (Calcium), 9 (Control)					
Heterogeneity: Not applicable					
Test for overall effect: Z=2.02(P=0.04)					
Test for subgroup differences: Not applicable					

Favours calcium 0.001 0.1 1 10 1000 Favours control

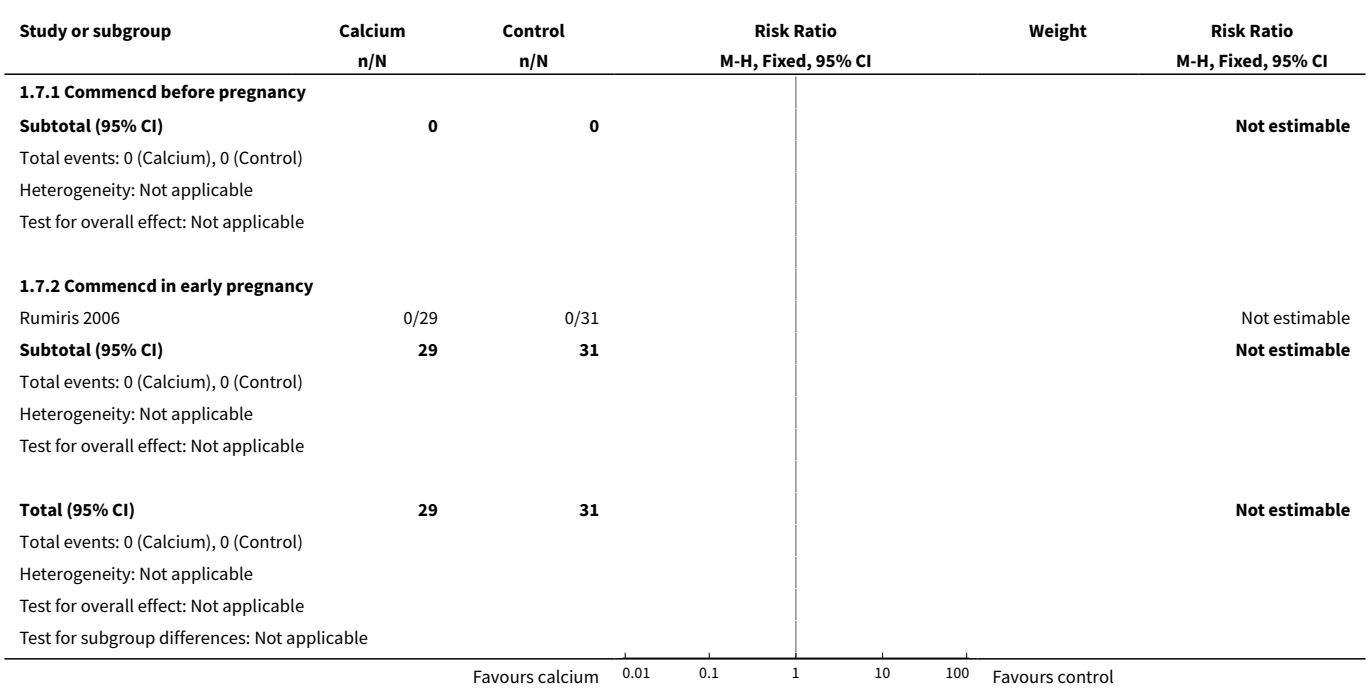
Analysis 1.6. Comparison 1 Calcium +/- other supplements versus no calcium or placebo, Outcome 6 Severe pre-eclampsia.

Study or subgroup	Calcium n/N	Control n/N	Risk Ratio M-H, Fixed, 95% CI	Weight	Risk Ratio M-H, Fixed, 95% CI
1.6.1 Commenced before pregnancy					
Subtotal (95% CI)	**0**	**0**			**Not estimable**

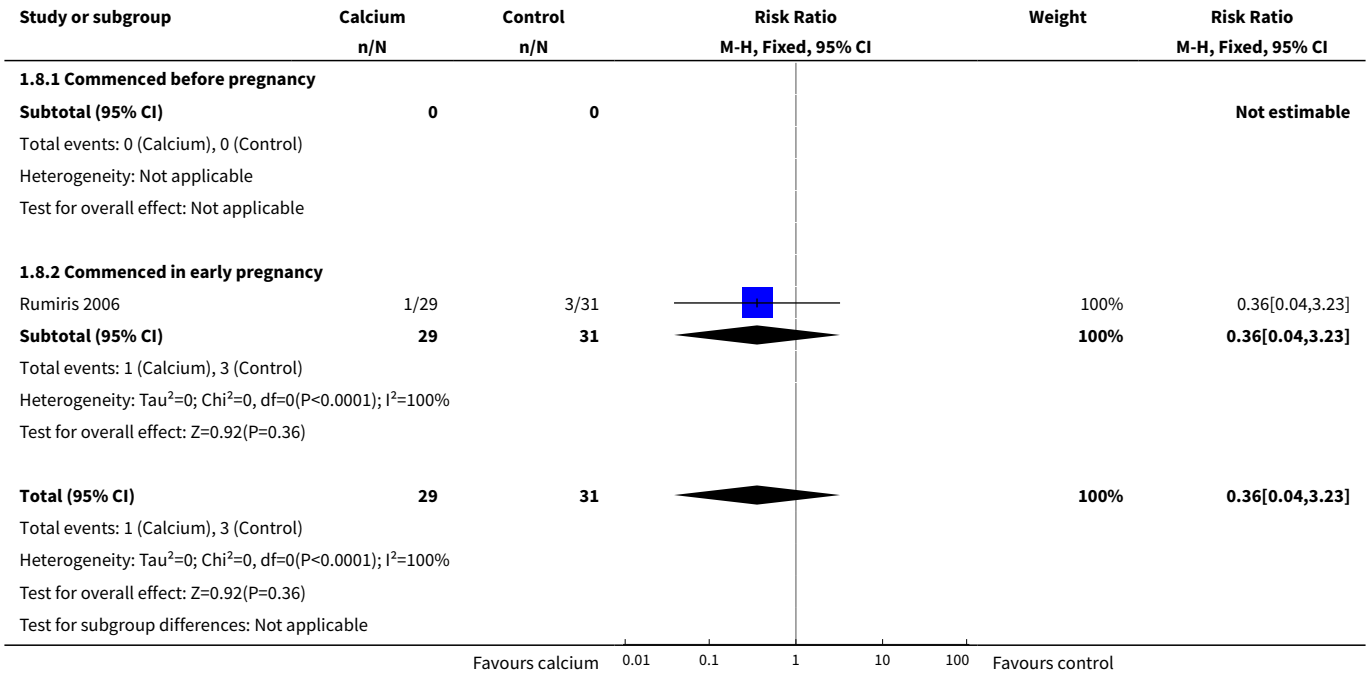
Favours calcium 0.01 0.1 1 10 100 Favours control



Analysis 1.7. Comparison 1 Calcium +/- other supplements versus no calcium or placebo, Outcome 7 Placental abruption.



Analysis 1.8. Comparison 1 Calcium +/- other supplements versus no calcium or placebo, Outcome 8 Preterm birth (< 37 weeks' gestation).



APPENDICES

Appendix 1. PubMed search strategy

(calcium) AND ((preeclampsia) OR (eclampsia)) AND ((pregnancy) OR (pregnant) OR (pregnancies)) AND ((random) OR (randomised) OR (randomized)) AND (trial)

Appendix 2. Search terms used in ICTRP and ClinicalTrials.gov

calcium AND pregnancy

CONTRIBUTIONS OF AUTHORS

GJ Hofmeyr (GJH) conceived the review, revised the protocol and the response to reviewers, and wrote the first draft of the review. S Manyame wrote the first draft of the protocol and revised the final review. Both review authors assessed studies for inclusion and extracted data.

DECLARATIONS OF INTEREST

Justus Hofmeyr: is an investigator on a trial could potentially be included in this review in future updates - however, he will not be involved in any decisions relating to the trial. Two external experienced systematic reviewers, who are not directly involved with the trial, will evaluate the trial for inclusion, assess risk of bias, and carry out data extraction.

Sarah Manyame is an investigator on a trial could potentially be included in this review in future updates - however, she will not be involved in any decisions relating to the trial. Two external experienced systematic reviewers, who are not directly involved with the trial, will evaluate the trial for inclusion, assess risk of bias, and carry out data extraction.

SOURCES OF SUPPORT

Internal sources

- Eastern Cape Department of Health, South Africa.

  Salary support (GJH)

External sources

- No sources of support supplied

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

There are differences between our published protocol (Hofmeyr 2014b) and the full review - please see below for further details.

- **Methods/subgroup analysis and investigation of heterogeneity** - we have added a further subgroup analysis: 'Calcium commenced before pregnancy or in early pregnancy (< 13 weeks').
- **Methods/outcomes** - we have changed the outcome 'Pregnancy loss before 24 weeks' gestational age' to 'Pregnancy loss before 20 weeks' gestational age' for consistency with generally accepted definition of early pregnancy loss. We have also added an additional secondary outcome 'Neonate small for gestational age' (but no data were available for this version of the review).
- **Methods/types of interventions** - We have broadened our 'Types of interventions' to also include calcium supplementation in combination with other supplements or treatments.

We have added an additional search of ClinicalTrials.gov and the WHO International Clinical Trials Registry Platform (ICTRP). We have updated our methods in line with the standard methods of Cochrane Pregnancy and Childbirth, including the use of GRADE and Summary of findings for the main comparison.

INDEX TERMS

Medical Subject Headings (MeSH)

*Dietary Supplements; *Food, Fortified; Abortion, Spontaneous [epidemiology]; Acetylcysteine [administration & dosage]; Antioxidants [administration & dosage]; Calcium [*administration & dosage]; Calcium, Dietary [*administration & dosage]; Copper [administration & dosage]; Hypertension, Pregnancy-Induced [*prevention & control]; Manganese [administration & dosage]; Maternal Mortality; Pre-Eclampsia [prevention & control]; Prenatal Care [*methods]; Randomized Controlled Trials as Topic; Selenium [administration & dosage]; Vitamins [administration & dosage]; Zinc [administration & dosage]

MeSH check words

Female; Humans; Pregnancy