Vitamin C and vitamin E in pregnant women at risk for pre-eclampsia (VIP trial): randomised placebo-controlled trial

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Summary

Background Oxidative stress could play a part in pre-eclampsia, and there is some evidence to suggest that vitamin C and vitamin E supplements could reduce the risk of the disorder. Our aim was to investigate the potential benefit of these antioxidants in a cohort of women with a range of clinical risk factors.

Methods We did a randomised, placebo-controlled trial to which we enrolled 2410 women identified as at increased risk of pre-eclampsia from 25 hospitals. We assigned the women 1000 mg vitamin C and 400 IU vitamin E (RRR α tocopherol; n=1199) or matched placebo (n=1205) daily from the second trimester of pregnancy until delivery. Our primary endpoint was pre-eclampsia, and our main secondary endpoints were low birthweight (<2·5 kg) and small size for gestational age (<5th customised birthweight centile). Analyses were by intention to treat. This study is registered as an International Standard Randomised Controlled Trial, number ISRCTN 62368611.

Findings Of 2404 patients treated, we analysed 2395 (99·6%). The incidence of pre-eclampsia was similar in treatment and placebo groups (15% [n=181] *vs* 16% [n=187], RR 0·97 [95% CI 0·80–1·17]). More low birthweight babies were born to women who took antioxidants than to controls (28% [n=387] *vs* 24% [n=335], 1·15 [1·02–1·30]), but small size for gestational age did not differ between groups (21% [n=294] *vs* 19% [n=259], 1·12 [0·96–1·31]).

Interpretation Concomitant supplementation with vitamin C and vitamin E does not prevent pre-eclampsia in women at risk, but does increase the rate of babies born with a low birthweight. As such, use of these high-dose antioxidants is not justified in pregnancy.

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Introduction

Pre-eclampsia affects 2–3% of all pregnancies, and every year is responsible for about 60 000 deaths worldwide.[1] In the UK, hypertensive disease in pregnancy is a major cause of maternal death.[2] Pre-eclampsia is a syndrome in which the placenta is implicated in the evolution of a generalised maternal inflammatory response, characterised by activation of maternal vascular endothelial cells and leucocytes.[3,4] Markers of oxidative stress are present in the placenta and maternal circulation of affected women, suggesting it as a cause of the disorder. As such, antioxidants might help to prevent pre-eclampsia, though this notion has yet to be tested in large randomised trials.[4,5]

In 1999, we published the results of a small, randomised controlled trial[6] in which we assigned women at risk of pre-eclampsia daily vitamin C (1000 mg) and vitamin E (400 IU) from 16–22 weeks' gestation. The primary outcome was a reduction in maternal concentrations of biomarkers of pre-eclampsia; the disorder itself was a secondary outcome. The trial was stopped early after an interim analysis showed a significant improvement in the primary outcome (plasminogen-activator inhibitor [PAI]1-to-PAI-2 ratio); rate of pre-eclampsia was also reduced. Women entered the trial mainly on the basis of an abnormal uterine artery doppler waveform (a recognised risk factor for pre-eclampsia). The findings of another, smaller study[7] of women judged at risk of pre-eclampsia on the basis of their clinical history indicated no evidence of benefit with the same antioxidants.

Neither of these previous trials was powered to assess clinical outcomes. Our aim, therefore, was to assess whether supplementation with vitamin C and vitamin E prevents pre-eclampsia in women at increased risk. We also looked at rate of low birthweight and babies born small for gestational age, since maternal and fetal disease can be affected independently.

Methods

Participants

Between Aug 6, 2003, and June 27, 2005, we did a randomised controlled trial (the Vitamins in Pre-eclampsia [VIP] trial) to which we enrolled women with clinical risk factors for pre-eclampsia from 25 UK hospitals in ten geographical areas. The last baby was delivered on Dec 3, 2005. Eligible women could be referred to trial centres from any location in the UK. 13 women were recruited in Amsterdam, Holland.

Our inclusion criteria were gestational age 14^{+0}–21^{+6} weeks plus one or more of the following risk factors: pre-eclampsia in the pregnancy preceding the index pregnancy, requiring delivery before 37 completed weeks' gestation, diagnosis of HELLP syndrome (haemolysis, elevated liver enzymes, and low platelets) in any previous pregnancy at any stage of gestation, eclampsia in any previous pregnancy at any stage of gestation; essential hypertension requiring medication, currently or previously; maternal diastolic blood pressure of 90 mm Hg or more before 20 weeks' gestation in the current pregnancy; type 1 or type 2 diabetes, requiring insulin or oral hypoglycaemic therapy before the pregnancy; antiphospholipid syndrome;[8] chronic renal disease (creatinine ≥125 μmol/L pre-pregnancy or ≥100 μmol/L during pregnancy, or significant proteinuria [≥500 mg per 24 h]); multiple pregnancy; abnormal uterine artery doppler waveform (18–22 weeks' gestation, mean resistance index >0·67 or pulsatility index >1·65 with or without the presence of unilateral or bilateral diastolic notches); primiparity with body-mass index (BMI) at first antenatal appointment of 30 kg/m² or more.

We excluded eligible women if they were unable or unwilling to give written informed consent or were being treated with warfarin. We only excluded women who were taking vitamin supplements if they contained doses of vitamin C of 200 mg or more or of vitamin E of 40 IU or more daily.

We employed study-specific research midwives in each geographical area, and each midwife covered up to three maternity units. The midwives instigated and maintained strategies to identify potentially eligible women at each centre from referral letters, high-risk antenatal clinics, and ultrasound departments, as well as from referrals from midwifery and medical colleagues. Recruitment occurred in various settings, including ultrasound departments, and, most frequently, antenatal clinics of participating centres, but also occasionally by telephone. We used a secure internet-based trial management system, which permitted both data collection and quality assurance from any computer terminal in all geographical locations (MedSciNet, AB Stockholm, Sweden). This system facilitated contemporaneous data management by the central trial team. The database was held on a server with backup at a remote site, and fulfilled all criteria of the Data Protection Act (1998). We kept identifying characteristics of participants on a secure and separate database.

The South East Multi Ethics Research Committee provided ethics approval (number 00/01/027), and we acquired site-specific approval from each participating centre. All participants gave written informed consent. Whenever possible trial visits coincided with routine antenatal appointments.

Procedures

Custom Pharmaceuticals (Hove, East Sussex, UK) manufactured the vitamin C (1000 mg) and identical placebo tablets (microcrystalline cellulose with addition of tartaric and citric acid to provide similar acidic taste), and Banner Pharmacaps (Europe BV, Tilburg, Netherlands) provided identical gelatin capsules containing 400 IU natural source vitamin E (RRR α tocopherol) or placebo (sunflower seed oil). DHP Investigational Medicinal Products Clinical Trial Supplies (Crickhowell, Powys, Wales, UK) packaged the tablets and capsules sealed in blister strips each with 1 week's supply, according to the randomisation sequence provided.

The randomisation sequence was blocked—ie, balanced—by centre in groups of two to ten individuals (mean size 6·7). The trial statistician (PTS) wrote the computer program that generated the sequence and a statistician not involved with the trial ran it with a new random number sequence. We gave copies of the sequence to MedSciNet and DHP. A password was available that enabled the treatment of any woman to be revealed in the event of a clinical emergency and after discussion with the trial team who were contactable at all times. This facility was not used; none of the trial staff or any other person involved in the trial knew the allocated treatment of any woman until after completion of the study.

Having gained informed written consent from all women, the trial midwives entered demographic and eligibility data (anonymously) onto the database in the presence of the participant. Once obligatory fields had been completed to establish eligibility, randomisation was undertaken online and the participant allocated a locally-stored pack of trial medication identified by a centre-specific and participant-specific number. Each pack contained a 7-month supply of trial medication. The midwives told women to take one tablet and one capsule daily, and asked participants to leave unused tablets or capsules in the blister strip. Postage prepaid envelopes were provided for return of blister strips to the research midwife at intervals of 4 weeks. If not received, the computer program generated a prompt to remind the women (by telephone) to return that month's packs. Participants were also given a postage prepaid postcard to notify the research midwife of delivery.

At trial entry, midwives asked women to provide a 30 mL venous blood sample, used to ascertain pre-randomisation plasma concentrations of vitamin C (ascorbic acid), vitamin E (RRR α tocopherol), and total cholesterol. We did not exclude women who refused to provide this blood sample. Women in two of the centres provided samples on at least one other occasion (20–22, 30–32, and 34–36 weeks' gestation) for analysis of the longitudinal biochemical profiles of these variables.

We ascertained concentrations of ascorbic acid by reverse phase high pressure liquid chromatography (HPLC) with electrochemical detection.[9] The lower limit of detection was 1 μmol/L and the intra-assay and interassay coefficients of variation were 2·2% and 3·5%, respectively. We measured RRR α tocopherol concentrations by reverse phase HPLC with detection at A292 nm[10] and normalised for plasma cholesterol concentration. The lower limit of detection was 0·3 μmol/L and the intra-assay and interassay coefficients of variation were 2·1% and 3·9%, respectively. We quantified plasma cholesterol concentrations by enzymatic colorimetric test with CHOD/PAP methods and Ultimate 5 Chol kits (Roche Diagnostic, East Sussex, UK). VWR International (Leicester, UK), Rathburn Chemicals (Peebleshire, UK), Sigma Chemical (Dorset, UK), Merck (Dorset, UK), and Fisher Scientific (Leicester, UK) supplied the chemicals needed.

Our primary outcome was pre-eclampsia, defined as gestational or severe gestational hypertension with proteinuria.[11] We defined gestational hypertension as two or more readings of a diastolic blood pressure of 90 mm Hg or more taken at least 4 h and up to 168 h apart and occurring after 20 weeks of pregnancy or in the early postnatal period (up to 48 h) and excluding labour. Severe gestational hypertension was defined as above, but with diastolic blood-pressure readings of 110 mm Hg or more on two occasions or a single reading of 120 mm Hg or more. We defined proteinuria as excretion of 300 mg protein or more over 24 h or two readings of 2+ or more on dipstick analysis of mid-stream urine (MSU)/catheter-specimen of urine (CSU) if 24-h collection result was not available. Severe proteinuria was defined as excretion of 5000 mg or more of protein over 24 h. Cases of HELLP and eclampsia had to fulfil these criteria to be included.

For women previously hypertensive—ie, on antihypertensive treatment pre-pregnancy or as defined above, but occurring before 20 weeks' gestation—we defined pre-eclampsia by the new development of proteinuria. For women with pre-existing proteinuria, the diagnosis of pre-eclampsia was based on development of hypertension, as defined above, or after identification of clinical or biochemical markers or at least one additional feature of pre-eclampsia—eg, HELLP syndrome, eclampsia. For women with pre-existing hypertension and proteinuria, the trial management team reviewed each case of hypertensive pregnancy, and diagnosis was confirmed by two senior clinical staff, acting independently.

With respect to secondary outcomes, our main endpoints were low birthweight (<2·5 kg) and small size for gestational age (<5th centile), assessed against customised birthweight centile charts.[12] Other prespecified secondary outcomes, for which our study was powered to detect important effects, were preterm birth ($\leq 37^{+0}$ weeks' gestation), gestational age at delivery, smaller than 10th centile for gestation, use of health-care resources (antenatal inpatient nights), and analyses of pre-randomisation and longitudinal biochemical indices in plasma.

We also identified the following prespecified maternal and neonatal outcomes, for which our study was not powered to detect important effects: death of mother before discharge from hospital, eclampsia, HELLP syndrome, severe pre-eclampsia (defined as severe gestational hypertension plus proteinuria), delivery for pre-eclampsia at or before 34 weeks' and 37 weeks' gestation, premature rupture of the membranes, severe gestational hypertension, gestational hypertension, and pre-eclampsia by risk group; and death of the child (intrauterine or neonatal deaths by 28 days), admission to neonatal intensive care unit or the special-care baby unit for more than 7 days, very low birthweight (<1·5 kg), preterm birth (≤34 and ≤37 weeks' gestation), fetal abnormality, need for supplementary oxygen at discharge, retinopathy of prematurity, necrotising enterocolitis, intraventricular haemorrhage, mechanical ventilation, hypoxic ischaemic encephalopathy, Apgar score (<7 and <4 at 5 min), arterial cord blood pH of less than 7, and the main neonatal outcomes by risk group.

Statistical analysis

We planned to recruit 1200 women to each group. Based on the published incidence of pre-eclampsia in the component at-risk groups at the time of study design, we expected an incidence of pre-eclampsia in the placebo group of at least 15% and, based on our previous findings,[6] the relative reduction in the treatment group to be at least 30% (leading to an incidence of 10·5%). With 2400 women recruited, our study would have a 90% power (β) at the 5% significance level (α) to detect this difference. The main neonatal outcomes were low birthweight and birthweight centile. We planned our study to provide 80% power (β) to detect a 25% relative reduction in the number of children born with a low birthweight from 16·2% to 12·1% (based on incidence reported by Chappell and colleagues[6]) and 97% power (β) to detect a 20% to 14% difference (30% relative reduction) in the number born small for gestational age at the 5% significance level (α).

Our stopping rule was based on the Peto guidance for stopping rules—namely, that the trial should only stop if the effect seen would alter clinical practice. An asymmetric stopping rule was agreed by the trial steering committee based on both the primary endpoint and perinatal deaths. The "favourable for treatment" rule involved stopping of the trial only if both tests were significant—the primary endpoint at p<0·003 and perinatal death at p<0·01. The "unfavourable for treatment" rule involved stopping of the trial if perinatal death favoured the placebo group at

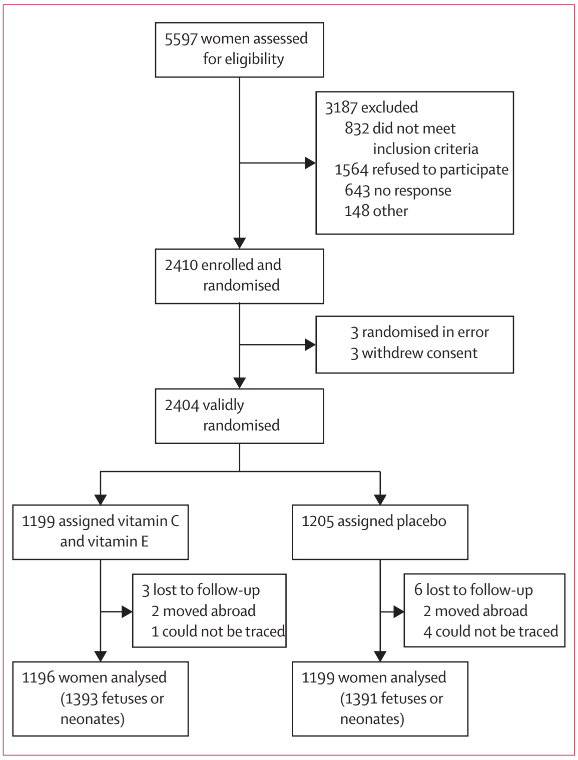

Figure 1: **Trial profile**

	Vitamin C and vitamin E (n=1199)	Placebo (n=1205)
Age (years)	31·0 (5·8)	30·9 (5·8)
Height (cm)	163·8 (6·6)	164·2 (6·9)
Weight (kg)	81·4 (20·0)	82·0 (20·1)
Body-mass index (kg/m²)	30·3 (7·1)	30·3 (7·0)
Parity 0	600 (50%)	613 (51%)
Employed	852 (71%)	863 (72%)
Higher education	570 (48%)	546 (45%)
Current smoker	155 (13%)	135 (11%)
Ethnic origin		
White European*	999 (83%)	989 (82%)
Gestational age (weeks)	18·6 (2·5)	18·5(2·5)
Blood pressure (mm Hg)		
Systolic	122 (15)	123 (15)
Diastolic	74 (12)	74 (11)
Dipstick proteinuria		
Normal or trace	1138 (95%)	1140 (95%)
+	34 (3%)	38 (3%)
>2+	27 (2%)	27 (2%)
Use of drugs and supplements		
Aspirin	278 (23%)	285 (24%)
Low molecular weight heparin or heparin	45 (4%)	45 (4%)
Folate	344 (29%)	330 (27%)
Multivitamins	337 (28%)	289 (24%)
Risk group		
Chronic hypertension	435 (36%)	426 (35%)
BMI >30 kg/m² in first pregnancy	396 (33%)	408 (34%)
Previous pre-eclampsia, HELLP, or eclampsia	274 (23%)	273 (23%)
Multiple pregnancy	192 (16%)	187 (16%)
Diabetes	97 (8%)	102 (8%)
Abnormal uterine artery doppler waveform (18–22 weeks' gestation)	49 (4%)	31 (3%)
Antiphospholipid syndrome	29 (2%)	23 (2%)
Chronic renal disease	23 (2%)	24 (2%)
Multiple risk factors†	260 (22%)	243 (20%)

Data are mean (SD) or number (%). *As defined by UK Office of National Statistics. †Women with more than one risk factor included in each relevant risk category.

Table 1: **Baseline characteristics**

p<0·01. The primary endpoint would not be relevant if this scenario arose.

The data monitoring and ethics committee and the trial steering committee agreed the analysis plan at the beginning of the study. All analyses were by intention to treat and done with Stata (version 9.1). We present summary data by group as number (%) or mean (SD) as appropriate. We present analyses of the primary outcome (occurrence of pre-eclampsia) and maternal endpoints as simple risk ratios with 95% CIs. We assessed twin and triplet babies as if cluster randomised, the clusters being the mothers.[13] As such, we adjusted all CIs for neonatal outcomes (other than preterm birth rates) for multiplicity (clustering by mother) with multiple binomial regression, using robust standard errors.[14] Since the main neonatal outcomes were strongly affected by multiple births, we added a correction for confounding. The multiplicity of the pregnancy was not changed if a twin or triplet died. We analysed other adverse events in the same way. Since all triplets were born before 37 weeks' gestation, we excluded them from the analysis of this endpoint. We analysed continuous outcome measures by linear regression with robust standard errors.[14]

We analysed subgroups with the same approach used for the main analysis. Where necessary to achieve convergence, we used odds ratios rather then risk ratios. We analysed the longitudinal blood measurements with generalised estimating equations to correct for missing data and repeated measurements.[15] Vitamin E-to-cholesterol ratios were log transformed.

This study is registered as an International Standard Randomised Controlled Trial, number ISRCTN 62368611.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Figure 1 shows the trial profile. Of 2404 women enrolled and treated, we analysed 2395 (99·6%). Based on counts of returned pills from 2070 women, 80% (n=1653) of women took at least 50% of their tablets, 65% (n=1345) took 80% or more, and 32% (n=661) took all of their tablets; 6% (n=125) did not take any tablets. Neither compliance nor baseline characteristics differed greatly between groups (table 1) or subgroups. More than a quarter of women were taking multivitamin supplements at enrolment. Table 1 also shows the proportion of women with each identified risk factor for pre-eclampsia at baseline; 21% of women had more than one risk factor. Although only 3% of women entered the study with an abnormal uterine artery doppler waveform, 274 (11%) had a recorded abnormal waveform during the course of the study. We did not do this test systematically in trial participants nor is it routinely undertaken in clinical practice. As such, the true prevalence of abnormal waveforms is not known in this population.

Table 2 shows the primary and secondary maternal outcomes and table 3 the primary outcome, pre-eclampsia, stratified by risk at enrolment. Overall, 15% (n=368) of women developed pre-eclampsia. Treatment with vitamin C and vitamin E did not reduce this risk (table 2), irrespective of risk at enrolment (table 3). There was, however, a variation in the rate of pre-eclampsia, ranging from 11% in primiparous obese women and women with a multiple pregnancy to 32% in women with chronic renal disease (table 3). Women with multiple risk factors had a rate of pre-eclampsia of 26%. There was no difference in the risk of severe pre-eclampsia (pre-eclampsia with severe hypertension) or early onset pre-eclampsia (delivery for

	Vitamin C and vitamin E (n=1196)	Placebo (n=1199)	Risk ratio (95% CI)	p*
Hypertensive disorders				
Pre-eclampsia	181 (15%)	187 (16%)	0·97 (0·80–1·17)	0·754
Severe pre-eclampsia	62 (5%)	53 (4%)	1·17 (0·82–1·68)	
Gestational hypertension	84 (7%)	55 (5%)	1·53 (1·10–2·13)	
Severe gestational hypertension	7 (1%)	5 (0·4%)	1·40 (0·45–4·41)	
Antenatal onset of pre-eclampsia	170 (14%)	173 (14%)	0·99 (0·81–1·20)	
Postpartum onset of pre-eclampsia	11 (1%)	14 (1%)	0·79 (0·36–1·73)	
Delivery for pre-eclampsia before 37 weeks' gestation	96 (8%)	86 (7%)	1·12 (0·85–1·48)	
Delivery for pre-eclampsia before 34 weeks' gestation	43 (4%)	35 (3%)	1·23 (0·79–1·91)	
HELLP syndrome	6 (0·5%)	1 (0·1%)	6·02 (0·73–49·9)	
Eclampsia	3 (0·3%)	1 (0·1%)	3·01 (0·31–28·9)	
Severe proteinuria (>5 g in 24 h)	20 (2%)	13 (1%)	1·54 (0·77–3·09)	
Magnesium sulphate for pre-eclampsia	47 (4%)	26 (2%)	1·81 (1·13–2·91)	
Intravenous antihypertensive therapy	31 (3%)	16 (1%)	1·94 (1·07–3·53)	
Other outcomes				
Antenatal steroids	180 (15%)	134 (11%)	1·35 (1·09 to 1·66)	
Maternal death	1 (0·1%)	1 (0·1%)	NA	
Antenatal inpatient nights (mean, SD)	7 (8)	6 (6)	0·80 (0·22–1·37)	

Data are number (%) unless otherwise indicated. *p value presented only for primary outcome.

Table 2: **Maternal pregnancy outcomes by treatment allocation**

pre-eclampsia <34 weeks' gestation) between groups, although significantly more women in the treatment group than in the control group developed gestational hypertension and received intravenous antihypertensive therapy (table 2), antenatal steroids, or magnesium sulphate. Six women taking supplements developed HELLP syndrome compared with one on placebo (not significant). Other indicators of serious morbidity were similar in antioxidant versus placebo groups: stroke (0 *vs* 1), cerebral haemorrhage (none), pulmonary oedema (3 *vs* 2), disseminated intravascular coagulation (1 *vs* 1), epigastric pain (16 *vs* 21), liver haematoma or rupture (none), and admission to intensive-care unit (6 *vs* 9). Neither of the women who died did so because of pre-eclampsia (table 2); one woman committed suicide 5 days postnatally and the second died 6 months postpartum after an infection.

Table 4 shows all neonatal outcomes, and figure 2 effect of treatment on number of children born with a low birthweight or small for gestational age. Babies of women who took supplements were significantly more likely to be born with a low birthweight than those of controls (table 4, figure 2); small size for gestational age did not differ between groups. Within the nine risk subgroups, there were significantly more small and growth-restricted singleton babies born to women with diabetes who were taking antioxidants (low birthweight 20% [n=19] *vs* 10% [n=6], risk ratio [RR] 3·26 [95% CI 1·36–7·84]; small for gestational age 12% [n=12] *vs* 3% [n=3], 4·12 [1·20–14·21]; figure 2).

18% (n=367) of singletons were delivered preterm, and 14% (n=284) were small for gestational age. There was no difference in rate of preterm birth between groups, but babies born to mothers taking antioxidants had a higher incidence of arterial cord pH of less than 7. Although more babies died in the antioxidant group than in the placebo group, this statistic was not significant, and the

	Vitamin C and vitamin E	Placebo	Risk ratio (95% CI)
Chronic hypertension	100/435 (23%)	92/422 (22%)	1·05 (0·82–1·35)
BMI >30 kg/m² (primiparous)	40/394 (10%)	47/405 (12%)	0·87 (0·59–1·30)
Previous pre-eclampsia, HELLP, or eclampsia	62/273 (23%)	62/273 (23%)	1·00 (0·73–1·36)
Multiple pregnancy	23/191 (12%)	18/187 (10%)	1·25 (0·70–2·24)
Diabetes	18/97 (19%)	16/102 (16%)	1·18 (0·64–2·18)
Abnormal uterine artery doppler waveform (18–22 weeks' gestation)	10/42 (24%)	6/24 (25%)	0·95 (0·40–2·29)
Antiphospholipid syndrome	2/29 (7%)	4/23 (17%)	0·40 (0·08–1·98)
Chronic renal disease	6/23 (26%)	9/24 (38%)	0·70 (0·29–1·64)
Multiple risk factors*	71/254 (28%)	56/237 (24%)	1·18 (0·87–1·60)

*Women with more than one risk factor included in each relevant risk category.

Table 3: **Pre-eclampsia stratified by risk at enrolment**

causes of death were balanced (table 4). The trial steering committee requested further analysis of the cause of death after allocation was revealed; the findings of this analysis showed significantly more unexplained stillbirths (antepartum deaths from 24 weeks' gestation), but fewer deaths due to immaturity, in women who took vitamins, even after correction for multiple births. The rate of congenital malformations was similar in both groups (table 4).

With respect to plasma analyses, 1355 women had their concentrations of vitamin C measured at recruitment. Neither baseline concentrations of vitamin C (mean 68 µmol/L, SD 26) nor vitamin E (corrected for plasma cholesterol 5·6 µmol/mmol, SD 1·2) differed between groups (figure 3). In the 208 women who provided longitudinal blood samples throughout pregnancy, however, there was a significant increase in concentration of both vitamins at all follow-up points in those assigned antioxidants. This finding was independent of development of pre-eclampsia (figure 3). In this subgroup, treatment compliance was better than in the group as a whole (85% [169 of 200] taking at least 80% of tablets, compared with 65% [1345 of 2070]). Plasma concentrations of vitamin C were lower throughout the gestational period studied in those women who took placebo and developed pre-eclampsia (n=20) than in women who did not (n=81; p=0·043). Similar analyses showed no statistical differences between these groups in corrected plasma concentrations of vitamin E.

In view of the results for the main outcomes we did some additional analyses that were not in the predefined analysis plan for additional insight. Similar rates of pre-eclampsia

	Vitamin C and vitamin E (n=1393)	Placebo (n=1391)	Risk ratio or difference* (95% CI)	p†
Sex				
Boy	701 (50%)	685 (49%)	Reference	
Girl	683 (49%)	701 (50%)	0·98 (0·90 to 1·06)	
Unknown	9 (1%)	5 (0%)	1·67 (0·51 to 5·51)	
Gestational age at delivery (weeks)	37·4 (3·9)	37·6 (3·7)	–0·2 (–0·5 to 0.1)*	
Preterm birth				
<37 weeks' gestation (singletons and twins only)	400/1372 (29%)	373/1376 (27%)	1·07 (0·93 to 1·22)	
<34 weeks' gestation	174 (12%)	144 (10%)	1·23 (0·95 to 1·59)	
Birthweight (g)‡	2901 (891)	2967 (873)	–59 (–122 to 4)*	
Low birthweight				
<2·5 kg	387 (28%)	335 (24%)	1·15 (1·02 to 1·30)	0·023
<1·5 kg	111 (8%)	97 (7%)	1·16 (0·85 to 1·57)	
Small for gestational age				
<5th adjusted centile	294 (21%)	259 (19%)	1·12 (0·96 to 1·31)	0·161
<10th adjusted centile	403 (29%)	360 (26%)	1·10 (0·97 to 1·25)	
Outcome at 28 days				
Alive	1342 (96%)	1352 (97%)	Reference	
Antepartum deaths	38 (3%)	23 (2%)	1·62 (0·92 to 2·86)	
Intrapartum deaths	5 (0·4%)	4 (0·3%)	1·18 (0·30 to 4·57)	
Death after delivery	8 (0·6%)	12 (0·9%)	0·67 (0·25 to 1·79)	
Baby deaths				
Total	51 (4%)	39 (3%)	1·28 (0·80 to 2·05)	
Congenital defects (malformation, lethal, or severe)	5 (0·4%)	4 (0·3%)	1·27 (0·34 to 4·72)	
Unexplained antepartum fetal death from trial entry	35 (3%)	19 (1%)	1·79 (0·96 to 3·34)	
>24 weeks' gestation (stillbirth)§	19 (1%)	7 (0·5%)	2·70 (1·02 to 7·14)	
Intrapartum asphyxia, anoxia, or trauma	2 (0·1%)	0	NA	
Immaturity	2 (0·1%)	10 (0·7%)	0·20 (0·04 to 0·97)	
Infection	6 (0·4%)	2 (0·1%)	3·03 (0·61 to 14·9)	
Other specific				
Fetal	0	2 (0·1%)	NA	
Neonatal	0	2 (0·1%)	NA	
Sudden infant death	1 (0·03%)	0	NA	
Admission to NICU/SCBU for >7 days	193 (14%)	175 (13%)	1·07 (0·86 to 1·34)	
APGAR score (live births only)				
<7 at 5 min	31 (2%)	19 (1%)	1·63 (0·90 to 2·97)	
<4 at 5 min	8 (0·6%)	9 (0·7%)	0·88 (0·29 to 2·64)	

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Other indices of neonatal morbidity			
Arterial cord pH <7	20/675 (3%)	9/657 (1%)	2·18 (1·00 to 4·76)
Any admission to NICU/SCBU	280 (20%)	255 (18%)	1·08 (0·91 to 1·29)
Surfactant	64 (5%)	48 (4%)	1·32 (0·87 to 2·00)
Mechanical ventilation	74 (5%)	58 (4%)	1·25 (0·86 to 1·82)
Discharged home on oxygen	7 (0·5%)	9 (0·6%)	0·74 (0·26 to 2·16)
Respiratory distress syndrome	91 (7%)	89 (6%)	1·01 (0·73 to 1·39)
Retinopathy of prematurity	6 (0·4%)	6 (0·4%)	1·00 (0·32 to 3·10)
Necrotising enterocolitis	11 (0·8%)	4 (0·3%)	2·67 (0·69 to 10·31)
Hypoxic ischaemic encephalopathy	3 (0·2%)	0	NA
Intraventricular haemorrhage			
None	1383 (99%)	1375 (99%)	NA
Grade 1	5 (0·4%)	9 (1%)	NA
Grade 2	2 (0·1%)	1 (0·07%)	NA
Grade 3	3 (0·2%)	6 (0·4%)	NA
Grades 2 or 3	5 (0·4%)	7 (0·5%)	0·71 (0·21 to 2·40)

NICU=neonatal intensive care unit. SCBU=special-care baby unit. Data are number (%). *Difference shown for gestational age at delivery and birthweight, other values are risk ratios. †p value presented only for main neonatal outcomes. ‡Data available for 1385 in treatment and 1386 in control group. §Data available for 1369 and 1372, respectively .

***Table 4:* Neonatal outcomes by treatment allocation**

in the treatment (n=181) and placebo (n=187) groups meant we were able to meaningfully compare the women. The mean gestational age at diagnosis of pre-eclampsia was more than a week earlier (34^{+0} *vs* 35^{+1}, difference 8 days, 95% CI 2–14) in women taking antioxidants and the mean gestational age at delivery was significantly earlier (35^{+6} *vs* 36^{+4}, difference 5 days, 0–10). These women were also more likely than controls to have received magnesium sulphate (23% [n=41] *vs* 12% [n=22], RR 1·93, 1·2–3·1) and antenatal steroids (38% [n=69] *vs* 28% [n=53], 1·35, 1·0–1·8). The highest quartile of vitamin C at baseline after correction for risk group, degree of education, and housing status and smoking status was associated with fewer infants born small for gestational age (0·42, 0·26–0·67), a lower rate of low birthweight babies (0·39, 0·24–0·63), and a lower rate of pre-eclampsia (odds ratio [OR] 0·59, 0·38–0·93). Vitamin C concentrations were higher by 15 μmol/L (95% CI 11–18) in 313 women taking pregnancy-specific multivitamins at trial entry (mean 79 μmol/L, SD 25) than in 1005 women taking none (65 μmol/L, SD 26). We did not include women taking other multivitamins in this analysis. The use of pregnancy-specific multivitamins was associated with lower rates of low birthweight (RR 0·72, 95% CI 0·56–0·93) and better birthweight centiles, but after correction for risk group at baseline, degree of education, and housing and smoking status remained significant only for birthweight centiles (OR 0·68, 0·52–0·88).

Discussion

Our findings do not lend support to the hypothesis that vitamin C (1000 mg) and vitamin E (400 IU) supplements given prophylactically from the second trimester of pregnancy lead to a reduction in the rate of pre-eclampsia in women at risk for the condition. Instead, they indicate an association between supplementation with antioxidants and low birthweight, and an umbilical artery cord pH of less than 7·0, which could be attributable to the earlier onset of pre-eclampsia in this group than in controls and could explain why some women who took supplements delivered at an earlier gestational age.

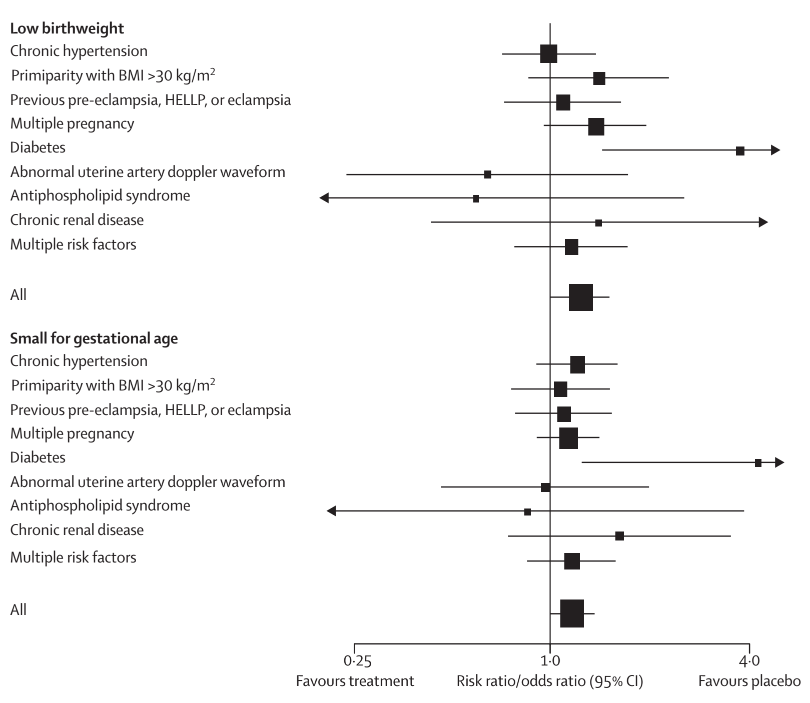

***Figure 2:* Main neonatal outcomes, according to mother's risk of pre-eclampsia at baseline**
*Odds ratios presented for low birthweight because of non-convergence of risk ratios.

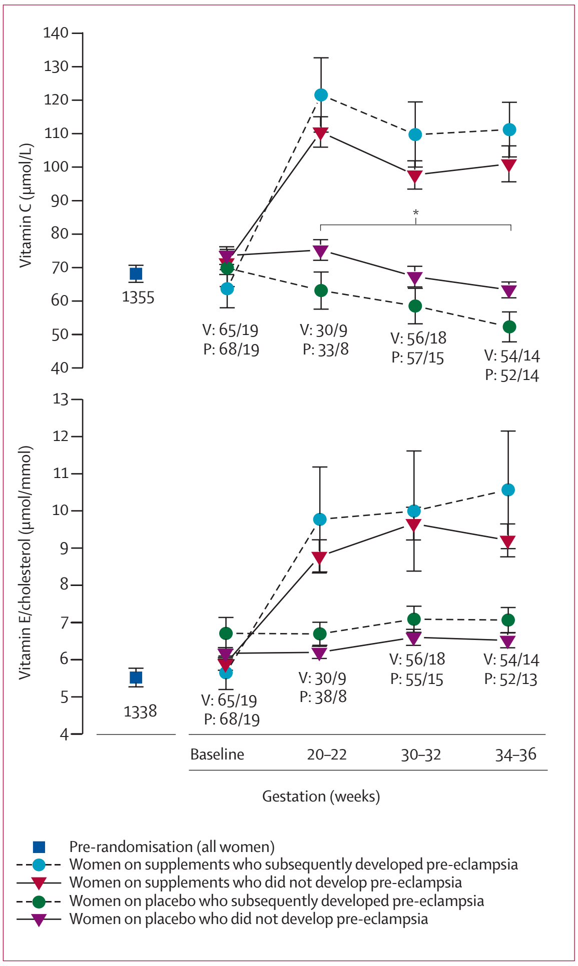

***Figure 3:* Plasma concentrations of vitamin C and vitamin E by weeks' gestation**

Numbers under each set of data points are numbers of women in treatment (V) and placebo (P) groups who did not develop pre-eclampsia/number of women who did. Bars=SE. *p=0·043 comparing vitamin C in the placebo group between women developing and not developing pre-eclampsia between 20 and 36 weeks' gestation.

Since early onset disease is usually severe, this explanation might also account for the mothers' increased need for treatment with magnesium sulphate, intravenous antihypertensive therapy, and antenatal steroids. This notion would not explain, however, the increased risk of gestational hypertension seen in the treated group.

Growth restriction, as assessed by birthweight centiles, was not increased in women who took antioxidants, but there was a significant increase in growth-restricted babies in the subgroup of women with diabetes who received antioxidants, and the trends in most other risk groups did not favour the supplements. The increase in unexplained stillbirths in women who received antioxidants is a concern, but might be due to chance, since this was a post-hoc analysis. There were fewer deaths from immaturity in the antioxidant group.

The rate of pre-eclampsia in our population was similar to that reported elsewhere;[16] treatment with antioxidants had no effect, irrespective of nature of risk at enrolment, although this analysis could have been underpowered in some groups.

Our population had higher plasma concentrations of vitamin C at baseline than previously reported by us in a similar cohort of women.[6,17] Improved ability to stabilise samples before assay, a higher dietary intake of vitamin C, or greater use of multivitamin supplements could explain this difference. The lower concentrations of vitamin C in plasma during gestation in those who did, than in those who did not, develop pre-eclampsia in the placebo group lends support to the findings of previous work,[3,4] which suggest that oxidative stress is associated with the disorder. Our present data also concur with those derived from our previous longitudinal study[17] of vitamin C in women taking placebo.

This study is different from our previous, smaller trial in women at high risk of pre-eclampsia, though the same intervention and dose were used in both. That study was powered on a biochemical endpoint (PAI-1-to-PAI-2 ratios) not pre-eclampsia and recruited women mainly on the basis of abnormal uterine artery doppler waveform. The two protocols were almost identical, except that in the previous investigation gestational age at trial entry was later (16–22 weeks' gestation compared with 14–22 weeks') and, previously, the women screened were a predominantly low-risk population. The reduction in pre-eclampsia that we noted in our earlier trial, however, is unlikely to be peculiar to the population chosen, since in the present study we noted no association between the condition and abnormal doppler waveforms. The source of medication in the two trials was different, but we confirmed adequate supplementation by biochemical analyses in both. We believe that our previous conclusion of reduced pre-eclampsia in the treatment group occurred through a type 1 statistical error based on only 35 events.

A Cochrane metanalysis[18] of antioxidant supplements for prevention of pre-eclampsia suggests a modest benefit, but includes studies in which micronutrients other than antioxidants were used. Two previous relevant, but small studies in high-risk women used vitamin C and vitamin E in the same doses as in this trial. Neither was powered to detect differences in pre-eclampsia, although that by Chappell and colleagues[6] showed a significant reduction. Both studies reported a slight, non-significant reduction in the weight of babies born to mothers taking antioxidant supplements. In another study,[19] vitamin C supplements or placebo were given to women at risk of preterm labour to investigate the hypothesis that gestation would be prolonged. The study was stopped early because of an increase in spontaneous preterm labour in the treatment group. We noted no such association. Finally, findings of a non-randomised study[20] indicate that high-dose vitamin E could lead to a reduction in the birthweight of babies.

Our trial will inevitably raise questions about the safety of use during pregnancy of multivitamins that contain vitamin C and vitamin E. It is noteworthy, therefore, that birthweight centiles after correction for potentially confounding variables were significantly higher in those women in the placebo group taking pregnancy-related multivitamin supplements than in those who were not. This finding suggests that such preparations are not associated with a detrimental effect, and does not exclude a benefit, but this observation should be treated with caution, since this investigation was an exploratory analysis of a subgroup and not a predefined outcome. These observational data from the placebo group concur with other data,[21] notably from developing countries, that suggest that multivitamin supplementation increases birthweight. Furthermore, the association we noted between higher plasma concentrations of vitamin C at baseline and higher birthweight, has also been reported in a cohort of Korean women.[22]

We do not know why supplementation with vitamin C and vitamin E to above physiological doses does not reduce the risk of pre-eclampsia, but increases the rate of low birthweight babies. A detrimental effect on placental function is possible, given the lower birthweight. Direct effects on fetal growth also cannot be excluded. Studies are underway to investigate the effect of the intervention on markers of oxidative stress and placental function.

The daily doses of vitamin C and vitamin E that we gave were below the maximum recommended intake in pregnant women. In the USA, the Institute of Medicine's Food and Nutrition Board have set an upper tolerable limit of vitamin C ingestion in pregnancy at 2000 mg per day and of vitamin E ingestion at 1000 mg (1600 IU).[23] Neither antioxidant is contraindicated in pregnancy, although there is limited evidence on which to assess their safety. Diarrhoea is the only consistent side-effect of vitamin C (120–6000 mg daily) in non-pregnant individuals.[24] In controlled trials[25] in preterm infants of vitamin E supplementation for the treatment of retinopathy of prematurity, the supplement was associated with increased risk of sepsis and necrotising enterocolitis. Cochrane reviews of vitamin E[26] and vitamin C[24] supplementation in pregnancy emphasise the need to establish the safety of their use in high doses in pregnant women. The absence of benefit and evidence of unfavourable outcomes in this study cannot be extrapolated to other antioxidants, including the same vitamins at lower doses. Additionally, our findings should not detract from the potential importance of oxidative stress in pre-eclampsia.

Our results provide another example of the lack of efficacy of high-dose antioxidants in prevention of disease despite consistent evidence for a state of oxidative stress.[3,4] Pre-eclampsia shares many of the characteristics of a proatherogenic state, including oxidative stress, dyslipidaemia, and endothelial cell activation, yet most of the evidence from studies of antioxidant supplementation in cardiovascular disease indicates no positive affect.[27] There is also little evidence of harm, although a recent meta-analysis[28] claimed that persistently high intake of vitamin E (>400 IU daily) increased mortality. It is noteworthy, however, that the methods used for analysis have been questioned.[29]

Careful consideration of underlying pathophysiology should accompany further investigation and direct ongoing clinical trials in this area. In the mean time, our findings of an increase in low birthweight and no benefit with respect to risk of pre-eclampsia suggest a contraindication of the studied doses of vitamin C and vitamin E in pregnancy.

Contributors

L Poston, A H Shennan, and A L Briley designed the study, promoted it, and sought ethical approval. F J Kelly gave scientific advice. A L Briley co-ordinated trial management, under the supervision of A H Shennan and L Poston. P T Seed provided statistical advice and managed and analysed the data. All authors helped to prepare the final report.

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Conflict of interest statement

A H Shennan and L Poston are principal investigators on the WHO trial of vitamin C and vitamin E supplementation for the prevention of pre-eclampsia (WHO A35048). A H Shennan, L Poston, and P T Seed hold patent EP01980705.6-PCTGB0104892, entitled *Diagnosis of pre-eclampsia*, and have filed patent P517376GB3, entitled *Detecting and predicting pre-eclampsia*. L Poston is a shareholder in CORRA Life Sciences, California, USA, a biotech company that develops biomarker tests to predict abnormal pregnancy outcomes. F J Kelly and A L Briley declare that they have no conflict of interest.

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