

# Respiratory outcomes in early childhood following antenatal vitamin C and E supplementation

Anne Greenough,<sup>1</sup> Seif O Shaheen,<sup>2</sup> Andrew Shennan,<sup>3</sup> Paul T Seed,<sup>4</sup> Lucilla Poston<sup>5</sup>

► Additional tables are published online only. To view these files please visit the journal online (<http://thorax.bmj.com>).

<sup>1</sup>Division of Asthma, Allergy and Lung Biology, MRC and Asthma UK Centre in Allergic Mechanisms of Asthma, King's College London, London, UK

<sup>2</sup>Centre for Health Sciences, Barts and The London School of Medicine and Dentistry, London, UK

<sup>3</sup>Maternal and Fetal Research Unit, Department of Women's Health, King's College London, UK

<sup>4</sup>Department of Women's Health, Medical Statistics, King's College London, London, UK

<sup>5</sup>Maternal and Fetal Research Unit - Division of Reproduction and Endocrinology, King's College London, London, UK

## Correspondence to

Professor Anne Greenough, Regional Neonatal Intensive Care Centre, 4th Floor Golden Jubilee Wing, King's College Hospital, London SE5 9RS, UK; [anne.greenough@kcl.ac.uk](mailto:anne.greenough@kcl.ac.uk)

Received 1 April 2010

Accepted 5 August 2010

Published Online First

1 October 2010

## ABSTRACT

**Background** Prenatal antioxidant supplementation might influence fetal lung growth and development and reduce infant respiratory morbidity. The aim of this study was to test the hypothesis that infants of mothers at risk of pre-eclampsia who were randomised to receive high-dose vitamins C and E (1000 mg vitamin C and 400 IU RRR  $\alpha$ -tocopherol daily) during pregnancy would have better respiratory outcomes than infants whose mothers were randomised to receive placebo.

**Methods** Respiratory outcomes to 2 years of age were documented using questionnaires and, in a subset, by recording their healthcare utilisation and calculating the cost of care data.

**Results** 330 women who had taken vitamin supplementation and 313 who had taken placebo completed the respiratory questionnaire (386 and 366 infants, respectively). There were no significant differences between the two groups in the proportions diagnosed with asthma. 54 women who had taken vitamin supplementation and 45 who had taken placebo took part in the healthcare utilisation study (65 and 53 infants, respectively). On average, infants of mothers receiving vitamin supplementation had 2.6 (99% CI 0.8 to 5.1) times more A&E/outpatient visits and 3.2 (99% CI 0.2 to 6.9) times more GP visits than infants of mothers receiving placebo, and their costs of care were £226 (99% CI £27 to £488) more for outpatient admissions, £57 (99% CI £3 to £123) more for GP visits and £22 (99% CI £3 to £50) more for medications.

**Conclusions** High-dose antenatal vitamin C and E supplementation does not improve infant respiratory outcome and is associated with increased healthcare utilisation and cost of care.

## INTRODUCTION

Infants born prematurely and/or of low birth weight suffer increased respiratory morbidity at follow-up. Approximately 50% of prematurely born infants suffer troublesome wheeze and cough in the first year after birth and one-third continue to have such problems during the preschool years.<sup>1 2</sup> Affected infants and children do respond to anti-asthma treatment<sup>3 4</sup> but the respiratory morbidity results in high healthcare utilisation.<sup>5</sup> Children who are of low birth weight because they were born small for gestational age are also at increased risk of wheezing, respiratory infection<sup>6</sup> and lung function abnormalities<sup>7 8</sup> at follow-up. Diminished airway function in adults born of lower birth weight led to the speculation that prenatal nutrition may programme fetal lung growth.<sup>8</sup>

Prematurely born infants most likely to suffer respiratory morbidity at follow-up are those who had developed bronchopulmonary dysplasia

(BPD).<sup>9</sup> Oxidative stress has been implicated in the development of BPD<sup>10</sup> and the antioxidant defences of prematurely born infants are impaired. As a consequence, prenatal antioxidant supplementation should theoretically reduce BPD and associated chronic respiratory morbidity. Prenatal antioxidant exposure might also influence fetal lung growth and development and hence reduce postnatal respiratory morbidity. In rats, prenatal vitamin E treatment improved fetal lung growth.<sup>11</sup> In birth cohort studies a lower maternal intake of vitamin E in pregnancy has been associated with a higher prevalence of early childhood wheezing<sup>12 13</sup> and later asthma.<sup>14</sup> In contrast, a higher maternal intake of vitamin C was associated with a higher prevalence of eczema.<sup>12</sup> The findings from these observational birth cohort studies,<sup>12–14</sup> however, could be biased or influenced by residual confounding factors. To more robustly determine the effect of prenatal antioxidant supplementation, a randomised controlled trial would be needed. Given, however, the paucity of epidemiological data and the conflicting findings for vitamins E and C, it would be difficult to justify a de novo intervention study in pregnancy.<sup>15</sup> We therefore devised a new study which involved following up the offspring of women who had participated in a randomised double-blind placebo controlled trial of vitamin C and vitamin E supplementation in pregnancy (Vitamins In Pre-eclampsia (VIP) trial).<sup>16</sup> Women were eligible for recruitment into that trial<sup>16</sup> if they had one or more recognised clinical risk factors for pre-eclampsia (see online supplement). Their offspring were at heightened risk of fetal growth restriction and premature delivery. The aim of our study was to test the hypothesis that infants of those high-risk mothers who were randomised to receive vitamins C and E (1000 mg vitamin C and 400 IU RRR  $\alpha$ -tocopherol daily) during pregnancy would have better respiratory outcomes (less wheeze, cough and need for anti-asthma therapy) and less healthcare utilisation than infants whose mothers were randomised to receive placebo.

## METHODS

### Protocol of the follow-up study

The VIP trial database was examined to identify all women who gave birth to a live infant. When the infants were predicted to be 22 months of age, the general practitioner (GP) was contacted to determine if the infant was still alive. If the answer was positive, the research nurse sent information to the parent(s) about the respiratory follow-up study. Researchers assessing the respiratory outcome of the infants were blind to the maternal allocation of treatment. All women who gave informed written

consent to the respiratory follow-up study were sent a questionnaire when their child was 2 years of age (see online supplement). If the parents did not send back the questionnaire the research nurse contacted them and, if possible, completed the questionnaire during a telephone interview.

### Respiratory questionnaire

Women were asked whether (or not) their infant had eczema, coughed and/or wheezed and the frequency of the cough and wheeze. They were asked whether their infant had taken medications for respiratory problems (in particular, bronchodilators and inhaled or oral corticosteroids) and/or been hospitalised for respiratory problems. Questions were also asked about factors that could influence respiratory morbidity including parental smoking, breast/bottle feeding, family history of atopy and the number of siblings aged <5 years.

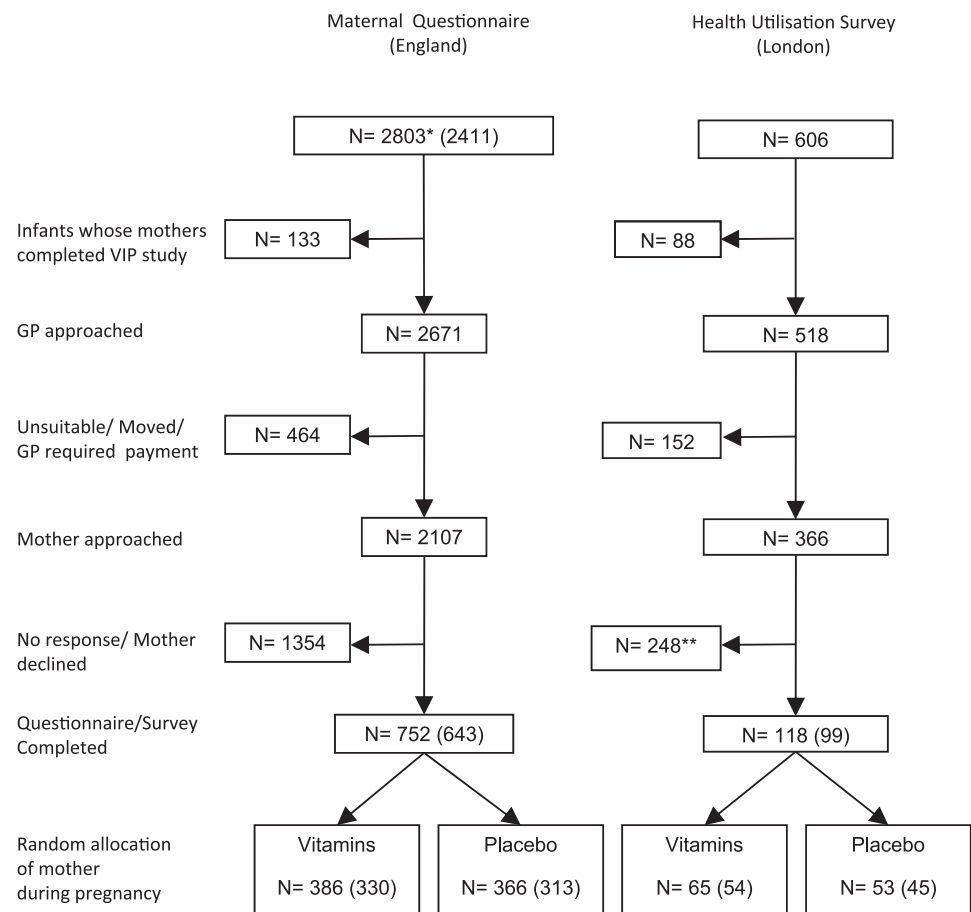
Women recruited to five London centres were also asked if they would take part in a healthcare utilisation study. If they gave informed written consent, the following data were collected from the GP records: venue of all hospital admissions, number of GP consultations, all medications prescribed, use and duration of home oxygen and number of referrals to community practitioners. For each hospital admission the following information was recorded: diagnosis, duration of stay, admission to a paediatric ward, high dependency (HDU) or intensive care (ICU) unit and all medication. The hospital records were

examined to ascertain the number of outpatient attendances. The cost of care was calculated from the hospital costs per bed/day for HDU and ICU and the data displayed on the NHS website for the cost of admission to a general paediatric ward. Drug costs were calculated from the British National Formulary prices. The cost of outpatients was calculated using the mean of the costs from the hospitals and the cost of the GP time and other community practitioners based on their average net remuneration, allowing for capital costs, travel and overheads.<sup>17</sup>

### Statistical analysis

Comparisons between groups were made by regression models with adjustments made for twins and triplets, and infants grouped by mother as for the original study.<sup>16</sup> Twin and triplet infants were regarded as cluster randomised, the clusters being the mothers.<sup>18</sup> All CIs for neonatal outcomes (other than preterm birth rates) were therefore adjusted for multiplicity (clustering by mother) using multiple binomial regression with robust standard errors.<sup>19</sup> For economic data where there was strong evidence of non-normality, CIs were estimated by the bias corrected and accelerated bootstrap with 10 000 replications.<sup>20</sup> A large number of comparisons were made so the results are presented with 99% CIs and the significance level set at  $p < 0.01$ . As the main neonatal outcomes were strongly influenced by multiple births, a correction for confounding was added. The multiplicity of the pregnancy was not changed

**Figure 1** Flow diagram of recruitment. VIP, Vitamins In Pre-eclampsia trial.



\* Includes 19 infants of 17 mothers randomised too late for inclusion in original VIP paper. One maternal mortality excluded.

\*\* Includes 23 women who completed Questionnaire, but declined permission for Health Utilisation survey.

where a twin or triplet died. Linear regression was used for continuous outcome and logistic regression (to give ORs) for yes/no outcomes. Statistical analysis was performed using Stata Version 10.1. Analyses were by intention to treat.

**Sample size**

Prior to commencement of the study it was calculated that recruitment of 750 infants would provide 80% power at the 5% level to detect a 30% difference in symptoms between the two groups. We also calculated that recruitment of 100 infants into the healthcare utilisation study would provide 80% power at the 5% level to detect a difference between the two groups in the cost of care equivalent to 0.75 standard deviations. The latter was a conservative estimate as we assumed the data would be non-normally distributed.

**RESULTS**

Three hundred and thirty women randomised to vitamin supplementation and 313 randomised to placebo filled in the respiratory questionnaire (386 and 366 infants respectively, overall response rate 27%, figure 1). The 643 women who completed the respiratory questionnaire were slightly older, with a lower body mass index (BMI), more likely to be white than black, less likely to have had two or more previous pregnancies, more likely to be working and to have a college education, more likely to own their home and less likely to be current smokers than women who did not complete it (see table 1 in online supplement).

There were no statistically significant differences between the two randomised intervention groups who filled in the questionnaire with regard to maternal characteristics or risk group at trial entry (table 1). Approximately one-third of the infants were born small for gestational age (less than 10th customised centile) and more than 25% were delivered prematurely (<37 weeks of gestation); 19% were admitted to the neonatal unit or special care baby unit. The mean birth weights of the infants in the vitamin and placebo groups were similar, but a greater proportion of the vitamin group were born preterm (p=0.075, table 2). There were no significant differences with regard to risk factors for chronic respiratory morbidity between the two groups (table 3).

**Table 1** Characteristics of study women completing questionnaires by randomisation status

|   | Vitamins (n=330) | Placebo (n=313) | Comparison (99% CI)  |
|---|------------------|-----------------|----------------------|
| Mean (SD) maternal age (years)                        | 32.2 (5.1)       | 32.0 (5.2)      | 0.2 (-0.9 to 1.2)    |
| Mean (SD) BMI (kg/m <sup>2</sup> )                    | 29.3 (6.7)       | 29.3 (6.8)      | -0.04 (-1.4 to 1.3)  |
| Ethnicity (non-white)                                 | 12%              | 11%             | 1.10 (0.58 to 2.10)  |
| Primiparity   | 52%              | 52%             | 1.00 (0.67 to 1.50)  |
| Multiple pregnancy*                                   | 17%              | 17%             | 0.96 (0.56 to 1.64)  |
| In work (including self-employed and maternity leave) | 80%              | 75%             | 1.33 (0.82 to 2.17)  |
| Smoking status  |                  |                 |                      |
| Never   | 60%              | 61%             |                      |
| Current smoker ≥1/day                                 | 9%               | 7%              | 1.25 (0.59 to 2.68)  |
| Stopped before present pregnancy                      | 24%              | 24%             | 1.04 (0.63 to 1.569) |
| Stopped during pregnancy                              | 7%               | 8%              | 0.81 (0.37 to 1.79)  |
| Compliance with trial medication (took at least 80%)  | 77%              | 76%             | 1.02 (0.63 to 1.65)  |

Results shown are mean (SD) or percentages as appropriate. Comparisons are differences in means or ORs as appropriate with 99% CIs. \*Includes three mothers of triplets (one in vitamin group, two in placebo group). BMI, body mass index.

**Table 2** Neonatal outcome measures by maternal randomisation status for whom questionnaire data were provided

|  | Vitamins (n=386) | Placebo (n=366) | Comparison (99% CI)   | p Value |
|--|------------------|-----------------|-----------------------|---------|
| Mean (SD) birth weight (g)                 | 2989 (813)       | 3026 (818)      | -35 (-176 to 106)     | 0.524   |
| IUGR                                       |                  |                 |                       |         |
| <5th centile                               | 22.0%            | 24.3%           | 0.87 (0.53 to 1.43)   | 0.462   |
| <10th centile                              | 30.8%            | 30.6%           | 1.02 (0.65 to 1.62)   | 0.895   |
| Mean (SD) gestational age at birth (weeks) | 37.80 (2.96)     | 37.99 (2.93)    | -0.17 (-0.74 to 0.40) | 0.436   |
| Preterm birth                              | n=380            | n=363           |                       |         |
| <37 weeks (singletons and twins only)      | 28.9%            | 22.9%           | 1.47 (0.84 to 2.55)   | 0.075   |
| <34 weeks                                  | 9.8%             | 8.2%            | 1.19 (0.51 to 2.76)   | 0.600   |
| Admission to NNU/SCBU                      | 19.7%            | 18.9%           | 1.04 (0.60 to 1.83)   | 0.843   |
| Surfactant                                 | 4.7%             | 3.8%            | 1.16 (0.40 to 3.34)   | 0.726   |
| Mechanical ventilation                     | 4.9%             | 4.1%            | 1.17 (0.41 to 3.28)   | 0.702   |

Results shown are mean (SD) or percentages as appropriate. Comparisons are differences in means or ORs as appropriate with 99% CIs. IUGR, intrauterine growth restriction; NNU, neonatal unit; SCBU, special care baby unit.

Fewer infants in the vitamin group had required medication for chest problems in the second year (p=0.068), and there was weak evidence to suggest a reduction in hospital admissions for breathing difficulties in this group (p=0.15). There was also weak evidence for a reduction in wheezing in the first year in the vitamin group (p=0.14) but this difference was not seen in the second year, and there were no differences in the diagnosis of asthma or eczema between the two groups (table 4). There was no evidence to suggest that the effects of vitamin supplementation on infant wheezing (ever) were modified by breast feeding, current smoking or maternal educational attainment (data not shown).

Fifty-four women given vitamin supplementation and 45 in the placebo group took part in the healthcare utilisation study (65 and 53 infants respectively, figure 1). Mothers who took part in the health utilisation study were older, with lower BMI, less likely to be white, more likely to have a college education and less likely to be a current smoker than those who did not participate (see table 2 in online supplement). Apart from a lower gestational age at recruitment, there were no differences with regard to maternal characteristics between the randomised intervention groups among women who completed the healthcare utilisation study (table 5). There were no statistically significant differences between the two groups with regard to neonatal outcome, although there was a tendency for a greater proportion of the vitamin supplementation group to have been born preterm (p=0.191, table 6). Overall, the numbers of A&E/outpatient visits (p=0.001) and GP visits (p=0.009) were greater in the vitamin supplementation group (table 7). The vitamin supplementation group also had more visits to the GP for

**Table 3** Family/household exposures and other risk factors for chronic respiratory morbidity by randomisation status

|                            | Vitamins (n=386) | Placebo (n=366) | OR (99% CI)         |
|----------------------------|------------------|-----------------|---------------------|
| Family history             |                  |                 |                     |
| Asthma                     | 31.1%            | 35.8%           | 0.81 (0.52 to 1.27) |
| Eczema                     | 38.1%            | 37.4%           | 1.03 (0.67 to 1.59) |
| Hay fever                  | 44.8%            | 46.2%           | 0.95 (0.62 to 1.46) |
| Breast feeding             | 72.5%            | 71.9%           | 1.04 (0.64 to 1.68) |
| Household smoking          | 16.3%            | 16.4%           | 0.99 (0.55 to 1.76) |
| Child contact with smokers | 13.0%            | 13.9%           | 0.90 (0.48 to 1.70) |
| Housing: owned/mortgage    | 79.0%            | 81.4%           | 0.86 (0.51 to 1.43) |

**Table 4** Infant outcomes by maternal randomisation status

|                                    | Vitamins<br>(n=386) | Placebo<br>(n=366) | Comparison<br>(99% CI) | p Value |
|------------------------------------|---------------------|--------------------|------------------------|---------|
| Chest symptoms in first 12 months  |                     |                    |                        |         |
| Asthma                             | 6.0%                | 6.3%               | 0.94 (0.42 to 2.11)    | 0.855   |
| Eczema                             | 25.4%               | 23.5%              | 1.10 (0.70 to 1.74)    | 0.586   |
| Cough                              |                     |                    |                        |         |
| At all                             | 70.5%               | 74.9%              | 0.81 (0.50 to 1.29)    | 0.245   |
| More than once/week                | 10.6%               | 11.5%              | 0.90 (0.47 to 1.74)    | 0.679   |
| Wheeze                             |                     |                    |                        |         |
| At all                             | 23.3%               | 28.1%              | 0.78 (0.50 to 1.21)    | 0.144   |
| More than once/week                | 3.9%                | 4.6%               | 0.81 (0.32 to 2.06)    | 0.568   |
| Chest symptoms in second 12 months |                     |                    |                        |         |
| At all                             | 86.8%               | 85.8%              | 1.08 (0.59 to 1.97)    | 0.752   |
| More than once/week                | 13.2%               | 10.1%              | 1.33 (0.71 to 2.51)    | 0.246   |
| Wheeze                             |                     |                    |                        |         |
| At all                             | 25.9%               | 26.5%              | 0.97 (0.62 to 1.52)    | 0.852   |
| More than once/week                | 2.6%                | 3.0%               | 0.83 (0.26 to 2.59)    | 0.669   |
| Medications for chest problems     |                     |                    |                        |         |
| Medicines (ever)                   | 38.6%               | 45.4%              | 0.75 (0.50 to 1.13)    | 0.068   |
| Medicines (in last 12 months)      | 29.5%               | 36.6%              | 0.72 (0.47 to 1.09)    | 0.041   |
| Bronchodilators                    | 13.7%               | 13.1%              | 1.06 (0.60 to 1.88)    | 0.789   |
| Antibiotics                        | 26.4%               | 32.0%              | 0.75 (0.49 to 1.16)    | 0.092   |
| Preventors                         | 5.4%                | 7.4%               | 0.72 (0.33 to 1.59)    | 0.288   |
| Palivizumab                        | 1.6%                | 0.8%               | 1.80 (0.29 to 11.2)    | 0.407   |
| Hospital admissions                |                     |                    |                        |         |
| For breathing difficulties         | 8.5%                | 11.7%              | 0.70 (0.37 to 1.33)    | 0.153   |
| n                                  | 33                  | 43                 |                        |         |
| Number of days (if any)            | 6.2 (12.2)          | 4.3 (6.4)          | 1.9 (-1.9 to 13.9)     | 0.416   |
| Other admissions                   | 14.2%               | 15.6%              | 0.91 (0.52 to 1.6)     | 0.665   |
| n                                  | 55                  | 57                 |                        |         |
| Number of days (if any)            | 10.2 (26.2)         | 5.0 (10.3)         | 5.3 (-1.6 to 23.9)     | 0.158   |
| Admissions to ICU                  | 3.6%                | 1.9%               | 2.0 (0.52 to 7.4)      | 0.195   |
| n                                  | 14                  | 7                  |                        |         |
| Number of days (if any)            | 24.1 (24.0)         | 13.1 (24.7)        | 10.9 (-51.4 to 32.4)   | 0.379   |

Results shown are mean (SD) or percentages as appropriate. Comparisons are differences in means or ORs with 99% CIs, adjusted for effect of multiple pregnancy and for clustering by mother. For number of days in hospital, CIs are by bias-corrected bootstrap with 10 000 replications.

respiratory-related disorders ( $p=0.047$ , table 7). The overall cost of care was greater for outpatient attendances ( $p=0.008$ ), GP visits ( $p=0.01$ ) and prescriptions ( $p=0.009$ ) in the vitamin

**Table 5** Characteristics of women by randomisation status who contributed to the health utilisation study

|  | Vitamin<br>(n=54) | Placebo<br>(n=45) | Comparison<br>(99% CI) |
|--|-------------------|-------------------|------------------------|
| Mean (SD) gestational age at recruitment (weeks)     | 17.8 (2.5)        | 18.7 (2.3)        | -0.93 (-2.19 to 0.34)  |
| Mean (SD) BMI ( $\text{kg}/\text{m}^2$ )             | 26.75 (7.24)      | 27.41 (5.76)      | -0.66 (-4.10 to 2.78)  |
| Ethnicity (non-white)                                | 39%               | 33%               | 1.27 (0.44 to 3.70)    |
| Primiparous  | 48%               | 38%               | 1.53 (0.54 to 4.34)    |
| Multiple pregnancy (twins)                           | 20%               | 18%               | 1.18 (0.33 to 4.23)    |
| Maternal age (years)                                 | 32.7 (5.0)        | 33.34 (4.7)       | -0.6 (-3.18 to 1.95)   |
| College/university education                         | 61%               | 56%               | 1.26 (0.44 to 3.57)    |
| Smoking status                                       |                   |                   |                        |
| Never  | 33 (61%)          | 25 (56%)          | Reference              |
| Current smoker                                       | 3 (6%)            | 4 (9%)            | 0.57 (0.07 to 4.56)    |
| Stopped before present pregnancy                     | 15 (28%)          | 11 (24%)          | 1.03 (0.30 to 3.53)    |
| Stopped during pregnancy                             | 3 (6%)            | 5 (11%)           | 0.45 (0.06 to 3.36)    |
| Compliance with trial medication (took at least 80%) | 41 (76%)          | 31 (71%)          | 1.32 (0.42 to 4.20)    |

Data shown are mean (SD) or percentages as appropriate. Comparisons are differences in means or ORs as appropriate with 99% CIs. BMI, body mass index.

**Table 6** Neonatal outcome measures by maternal randomisation status of those contributing to health utilisation study

|   | Vitamins<br>(n=65) | Placebo<br>(n=53) | Comparison<br>(99% CI) | p Value |
|---|--------------------|-------------------|------------------------|---------|
| Mean (SD) birth weight (g)                          | 2828 (827)         | 3026 (723)        | -170 (-518 to 178)     | 0.203   |
| IUGR  |                    |                   |                        |         |
| <5th birth weight centile                           | 28%                | 28%               | 0.88 (0.28 to 2.7)     | 0.775   |
| <10th birth weight centile                          | 37%                | 30%               | 1.33 (0.43 to 4.1)     | 0.515   |
| Mean (SD) gestational age at birth (weeks)          | 37.4 (2.9)         | 38.2 (2.8)        | -0.7 (-2.1 to 0.6)     | 0.161   |
| Preterm birth <37 weeks (singletons and twins only) | 37%                | 23%               | 2.11 (0.48 to 9.2)     | 0.191   |
| Preterm birth <34 weeks                             | 14%                | 6%                | 2.63 (0.36 to 19.2)    | 0.211   |
| Admission to NNU/SCBU                               | 20%                | 11%               | 1.94 (0.47 to 8.08)    | 0.229   |
| APGAR <7 at 1 min                                   | 11%                | 4%                | 3.26 (0.39 to 27.45)   | 0.153   |
| APGAR <7 at 5 min                                   | 1.5%               | 0.0%              |                        |         |
| Surfactant  | 5%                 | 4%                | 1.31 (0.11 to 15.03)   | 0.774   |
| Mechanical ventilation                              | 6%                 | 2%                | 3.69 (0.19 to 71)      | 0.255   |

Data shown are mean (SD) or percentages as appropriate. Comparisons are differences in means or ORs as appropriate with 99% CIs. IUGR, intrauterine growth restriction; NNU, neonatal unit; SCBU, special care baby unit.

supplementation group (table 8). The cost of care related to respiratory-related conditions was also greater in the vitamin supplementation group for outpatient attendances ( $p=0.043$ ) and GP visits ( $p=0.047$ ) and prescriptions ( $p=0.056$ , table 8).

## DISCUSSION

In this randomised placebo controlled trial of women at risk of pre-eclampsia, we found no strong evidence for a beneficial effect of prenatal supplementation with vitamins C and E on respiratory symptoms or other outcomes in the first 2 years of life, except for a lower use of medication for chest problems in the second year. In fact, this intervention was associated with increased healthcare utilisation and cost of care for their infants. These results are consistent with adverse short-term neonatal outcomes in the VIP trial<sup>16</sup> as supplementation was associated with significantly lower birth weight, higher rates of cord blood acidosis and lower 5-min Apgar scores.<sup>16</sup> In a recent study, however, using an identical protocol in 1500 high-risk women in developing countries, no difference was demonstrated in neonatal outcomes between the supplemented and placebo groups.<sup>21</sup> The increased healthcare utilisation and cost of care tended to be greater for non-respiratory rather than for

**Table 7** Infant healthcare utilisation by maternal randomisation status

|                     | Vitamins<br>(n=65) | Placebo<br>(n=53) | Comparison<br>(99% CI) | p Value |
|---------------------|--------------------|-------------------|------------------------|---------|
| Any cause           |                    |                   |                        |         |
| Hospital admission  | 17%                | 19%               | 0.88 (0.26 to 2.96)    | 0.779   |
| ICU                 | 0%                 | 0%                |                        |         |
| A&E/outpatient ever | 86%                | 72%               | 2.46 (0.74 to 8.18)    | 0.054   |
| A&E/outpatient (n)  | 4.5 (5.8)          | 1.9 (2.3)         | 2.6 (0.8 to 5.1)       | 0.001   |
| GP visits ever      | 65 (100%)          | 53 (100%)         |                        |         |
| GP visits (n)       | 11.6 (8.0)         | 8.4 (4.9)         | 3.2 (0.2 to 6.9)       | 0.009   |
| Respiratory-related |                    |                   |                        |         |
| Hospital admission  | 8%                 | 11%               | 0.65 (0.12 to 3.43)    | 0.508   |
| Total hospital days | 0.3 (1.5)          | 0.5 (1.7)         | -0.1 (-1.1 to 0.6)     | 0.671   |
| Any A&E/outpatient  | 28%                | 25%               | 1.18 (0.36 to 3.86)    | 0.722   |
| A&E outpatient (n)  | 0.6 (1.4)          | 0.4 (0.8)         | 0.2 (-0.3 to 0.9)      | 0.390   |
| GP visits (ever)    | 78%                | 77%               | 1.07 (0.32 to 3.61)    | 0.892   |
| GP visits (n)       | 3.4 (3.6)          | 2.3 (2.0)         | 1.1 (-0.2 to 2.8)      | 0.047   |

Data shown are mean (SD) or percentages as appropriate. Comparisons are differences in means or ORs as appropriate with 99% CIs and p values for mean differences bootstrapped (10 000 replications), allowing for clustering by mother.

**Table 8** Cost of infant care by maternal randomisation status

|                                  | Vitamins<br>(n = 65) | Placebo<br>(n = 53) | Difference<br>(99% CI) | p Value |
|----------------------------------|----------------------|---------------------|------------------------|---------|
| <b>Any cause</b>                 |                      |                     |                        |         |
| All hospital admissions          | £242 (672)           | £171 (381)          | £71 (–138 to 384)      | 0.475   |
| Outpatients                      | £390 (624)           | £165 (285)          | £226 (27 to 488)       | 0.009   |
| A&E                              | £76 (156)            | £54 (106)           | £22 (–33 to 103)       | 0.363   |
| Prescriptions                    | £43 (64)             | £21 (26)            | £22 (3 to 50)          | 0.007   |
| GP visits                        | £209 (143)           | £151 (88)           | £57 (3 to 123)         | 0.011   |
| Total cost (any cause)           | £1309 (1931)         | £785 (1185)         | £524 (–132 to 1352)    | 0.066   |
| <b>Respiratory-related</b>       |                      |                     |                        |         |
| All admissions                   | £74 (303)            | £98 (293)           | £–23 (–177 to 119)     | 0.519   |
| Outpatients                      | £31 (98)             | £7 (32)             | £25 (–2 to 73)         | 0.046   |
| A&E                              | £23 (72)             | £24 (54)            | £–1 (–27 to 39)        | 0.908   |
| GP visits                        | £61 (66)             | £41 (36)            | £20 (–5 to 49)         | 0.049   |
| Prescriptions                    | £8 (12)              | £4 (7)              | £3 (–0 to 9)           | 0.056   |
| Total cost (respiratory-related) | £271 (758)           | £290 (644)          | £–19 (–328 to 374)     | 0.859   |

Mean (SD) costs are given in £ sterling.

Mean differences between groups are given with bootstrapped 99% CIs and p values (10 000 replications), allowing for clustering by mother.

respiratory-related disorders. The vitamin group tended to be born more preterm and thus a possible explanation for the increased healthcare utilisation is non-respiratory complications of premature birth.

Our findings do not support previous observational findings from two birth cohort studies that suggested that a higher maternal intake of vitamin E was associated with less wheezing in the offspring in the first 2 years of life,<sup>12 13</sup> and suggest that the earlier observations may have been confounded. They also challenge the antioxidant hypothesis with respect to asthma inception. Indeed, it has been suggested that dietary antioxidants may increase the risk of asthma and allergic disease.<sup>22</sup> Furthermore, a recent study from a large UK birth cohort found no evidence that greater adherence in pregnancy to a 'health-conscious' dietary pattern, which is likely to be antioxidant-rich, was associated with a lower prevalence of wheezing or asthma in the offspring.<sup>23</sup> In the current study there was weak evidence to suggest possible benefits of supplementation on wheezing in the first year of life only, and a larger study would be needed to confirm or refute this. The lack of benefit for wheezing in the second year, however, would suggest that prenatal supplementation is unlikely to reduce the prevalence of asthma when this diagnosis can be reliably made later in childhood.

Our study has strengths and limitations. A major strength is the randomised trial design which reduces the likelihood of bias and confounding which are difficult to exclude in observational studies. To maximise the numbers of children from whom information would be available, an easy to complete questionnaire was used which had been previously used in a large study.<sup>24</sup> Respiratory morbidity can also be determined by recording the child's healthcare utilisation and cost of care by careful examination of GP and hospital records.<sup>17</sup> Such a detailed study is labour intensive, so we collected this information on a subset, but a large enough sample to confidently determine differences that were clinically important. Of the 2410 women who took part in the VIP study,<sup>16</sup> only 752 took part in the respiratory questionnaire study and 118 in the healthcare utilisation study. Nevertheless, the sample sizes are substantial and gave sufficient power to determine if there were clinically relevant differences in important outcomes. In addition, the main focus of this paper is in the comparison between the two randomised groups and the two groups examined in this study

remain closely comparable (table 1). In particular, there were no significant differences in the proportions of women in the two groups who were also taking multivitamins and/or folate supplements. After completion of the VIP trial the women were invited to ask about their allocation and approximately 30% did so (see online supplement). It was impossible for mothers to differentiate between the active and placebo treatment, so this would not have influenced them with regard to asking about their allocation. A limitation of our study is that the mothers studied were at high risk of pre-eclampsia and thus our findings may not be generalisable. We cannot exclude the possibility that lower dose antenatal supplementation with vitamins C and E in women not at risk of pre-eclampsia might have beneficial effects on respiratory outcomes in the offspring. The dosages chosen in the VIP trial, however, were based on pregnancy versus non-pregnancy data, suggesting they were likely to have a biological effect. The mothers included in this follow-up study were of higher socioeconomic status and were less likely to smoke than those who did not participate. They are therefore likely to have had a higher intake of fruit and vegetables containing antioxidants and lower levels of oxidative stress, and consequently potentially beneficial effects of antioxidant supplementation on respiratory outcomes may have been diluted. However, when we examined possible interactions with maternal smoking and socioeconomic status we found no evidence to suggest that antioxidant supplementation in pregnancy protected against infant wheezing, even among mothers who smoked and were of lower socioeconomic status.

In conclusion, high-dose vitamins C and E (1000 mg vitamin C, 400 IU RRR  $\alpha$ -tocopherol) supplementation daily from the second trimester of pregnancy did not improve respiratory outcome in the offspring. Furthermore, our results suggest that such high-dose prenatal supplementation is disadvantageous, at least in women at high risk of pre-eclampsia, as it is associated with increased healthcare utilisation and cost of care for infants. These deleterious consequences should be considered in the context of recent evidence from randomised trials of higher adult mortality associated with vitamin supplementation.<sup>25</sup>

**Acknowledgements** We are grateful to Mrs Deirdre Gibbons for secretarial assistance, the research midwives Sally Neville, Jenny Carter and Clare Raiman who collected the healthcare utilisation data and Annette Briley who supervised them and facilitated establishment of the database.

**Funding** This study was supported by Asthma UK. SOS was an Asthma UK Senior Research Fellow. Support was also given by the Wellcome Trust and Tommy's, the baby charity, to the Vitamins In Pre-eclampsia trial.

**Competing interests** None.

**Ethics approval** This study was conducted with the approval of the hospital research ethics committees.

**Provenance and peer review** Not commissioned; externally peer reviewed.

## REFERENCES

- Greenough A**, Maconochie I, Yuksel B. Recurrent respiratory symptoms in the first year of life following preterm delivery. *J Perinatal Med* 1990;**18**:489–94.
- Giffin F**, Greenough A, Yuksel B. Prediction of respiratory morbidity in the third year of life in infants born prematurely. *Acta Paediatr* 1994;**83**:157–8.
- Yuksel B**, Greenough A, Maconochie I. Effective bronchodilator therapy by a simple spacer device for wheezy premature infants in the first two years of life. *Arch Dis Child* 1990;**65**:782–5.
- Yuksel B**, Greenough A. Randomised trial of inhaled steroids in preterm infants symptomatic at follow up. *Thorax* 1992;**47**:910–13.
- Emond A**, Evans JA, Howat P. The continuing morbidity and use of health services by preterm infants after discharge from hospital. *Ambulatory Child Health* 1997;**3**:121–9.
- Brooke AM**, Lambert PC, Burton PR, *et al*. The natural history of respiratory symptoms in preschool children. *Am J Respir Crit Care Med* 1995;**152**:1872–8.
- Rona R**, Gulliford MC, Chinn S. Effects of prematurity and intrauterine growth on respiratory health and lung function in childhood. *BMJ* 1993;**306**:817–20.
- Barker DJ**, Godfrey KM, Fall C, *et al*. Relation of birth weight and childhood respiratory infection to adult lung function and death from chronic obstructive airway disease. *BMJ* 1991;**303**:671–5.
- Greenough A**, Alexander J, Burgess S, *et al*. Home oxygen status on rehospitalisation and primary care requirements of chronic lung disease infants. *Arch Dis Child* 2002;**86**:40–3.
- Saugstad OD**. Bronchopulmonary dysplasia-oxidative stress and antioxidants. *Semin Neonatal* 2003;**8**:39–49.
- Islam S**, Narra V, Coté GM, *et al*. Prenatal vitamin E treatment improves lung growth in fetal rats with congenital diaphragmatic hernia. *J Pediatr Surg* 1999;**34**:172–7.
- Martindale S**, McNeill G, Devereux G, *et al*. Anti-oxidant intake in pregnancy in relation to wheeze and eczema in the first two years of life. *Am J Respir Crit Care Med* 2005;**171**:121–8.
- Litonjua AA**, Rifas-Shiman SL, Ly NP, *et al*. Maternal antioxidant intake in pregnancy and wheezing illnesses in children at 2 years of age. *Am J Clin Nutr* 2006;**84**:903–11.
- Devereux G**, Turner SW, Craig LC, *et al*. Low maternal vitamin E intake during pregnancy is associated with asthma in 5-year-old children. *Am J Respir Crit Care Med* 2006;**174**:499–507.
- Shaheen SO**. Prenatal nutrition and asthma: hope or hype? *Thorax* 2008;**63**:483–5.
- Poston L**, Briley AL, Seed PT, *et al*. Vitamins in Pre-eclampsia (VIP) Trial Consortium. Vitamin C and vitamin E in pregnant women at risk for pre-eclampsia (VIP trial): randomised placebo-controlled trial. *Lancet* 2006;**367**:1145–54.
- Greenough A**, Cox S, Alexander J, *et al*. Health care utilisation of chronic lung disease infants related to hospitalisation for respiratory syncytial virus infection. *Arch Dis Child* 2001;**85**:463–8.
- Gates S**, Brocklehurst P. How should randomised trials including multiple pregnancies be analysed? *Br J Obstet Gynaecol* 2004;**111**:213–19.
- Binder DA**. On the variances of asymptotically normal estimators from complex surveys. *Int Stat Rev* 1983;**51**:279–92.
- Barber JA**, Thompson S. Analysis of cost data in randomised trials: an application of the non-parametric bootstrap. *Stat Med* 2000;**19**:3219–36.
- Villar J**, Purwar M, Merialdi M, *et al*; WHO Vitamin C and Vitamin E trial group. World Health Organisation multicentre randomised trial of supplementation with vitamins C and E among pregnant women at high risk for pre-eclampsia in populations of low nutritional status from developing countries. *Br J Obstet Gynaecol* 2009;**116**:780–8.
- Murr C**, Schroeksnadel K, Winkler C, *et al*. Antioxidants may increase the probability of developing allergic diseases and asthma. *Med Hypotheses* 2005;**64**:973–7.
- Shaheen SO**, Northstone K, Newson RB, *et al*. Dietary patterns in pregnancy and respiratory and atopic outcomes in childhood. *Thorax* 2009;**64**:411–17.
- Greenough A**, Yuksel B, Naik S, *et al*. First trimester invasive procedures. Effects on symptom status and lung volume in very young children. *Pediatr Pulmonol* 1997;**24**:415–22.
- Bjelakovic G**, Nikolova D, Gluud LL, *et al*. Mortality in randomised trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. *JAMA* 2007;**297**:842–57.