Vitamin C to pregnant smokers persistently improves infant airway function to 12 months of age: a randomised trial

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Vitamin C supplementation coupled with smoking cessation counselling for pregnant smokers may be a safe, inexpensive, and simple intervention to improve the airway function of their offspring through 12 months of age https://bit.ly/3fa8q2X

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ABSTRACT

Background: Vitamin C (500 mg·day^{-1}) supplementation for pregnant smokers has been reported to increase newborn pulmonary function and infant forced expiratory flows (FEFs) at 3 months of age. Its effect on airway function through 12 months of age has not been reported.

Objective: To assess whether vitamin C supplementation to pregnant smokers is associated with a sustained increased airway function in their infants through 12 months of age.

Methods: This is a pre-specified secondary outcome of a randomised, double-blind, placebo-controlled trial that randomised 251 pregnant smokers between 13 and 23 weeks of gestation: 125 to 500 mg·day^{-1} vitamin C and 126 to placebo. Smoking cessation counselling was provided. FEFs performed at 3 and 12 months of age were analysed by repeated-measures analysis of covariance.

Results: FEFs were performed in 222 infants at 3 months and 202 infants at 12 months of age. The infants allocated to vitamin C had significantly increased FEFs over the first year of life compared to those allocated to placebo. The overall increased flows were 40.2 mL·s^{-1} for at FEF_{75} (75% of forced vital capacity (FVC)) (adjusted 95% CI for difference 6.6–73.8; p=0.025); 58.3 mL·s^{-1} for FEF_{50} (10.9–105.8; p=0.0081); and 55.1 mL·s^{-1} for $FEF_{25–75}$ (9.7–100.5; p=0.013).

Conclusions: In offspring of pregnant smokers randomised to vitamin C *versus* placebo, vitamin C during pregnancy was associated with a small but significantly increased airway function at 3 and 12 months of age, suggesting a potential shift to a higher airway function trajectory curve. Continued follow-up is underway.

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This study is registered at ClinicalTrials.gov with identifier NCT01723696. Individual de-identified participant data, including data dictionaries, that underlie the results reported in this article will be shared. The study protocol, statistical analysis plan and analytic code will be available. The data will be available beginning 9 months and ending 36 months following article publication. It will be made available to researchers who provide a methodologically sound proposal to achieve aims in the approved proposal. Proposals should be directed to DCC@ohsu.edu.

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Introduction

Absolute values of airway function increase with lung growth and development between infancy and early adulthood when maximal values are achieved, plateau, then progressively decline throughout adulthood [1]. Longitudinal studies demonstrate that the trajectories for airway function are established early in life. Infants and children in the lower percentiles of airway function tend to have persistently lower function into adulthood [2, 3]. Individuals with decreased airway function early in life may be at increased risk for chronic obstructive pulmonary disease during adulthood as airway function declines [4].

In utero smoke exposure is a well-established risk factor for impaired fetal lung development, decreased airway function and an increased risk for wheeze and asthma in the offspring [5, 6]. Therefore, *in utero* smoke exposure is an important determinant of a lifetime of decreased airway function and increased respiratory morbidity. ⩾10% of women in the United States (US) smoke cigarettes while pregnant [7]. A recent meta-analysis [8] reported that worldwide >50% of smokers who become pregnant continue to smoke. As smoking cessation interventions during pregnancy have not been very successful, this translates into a huge worldwide economic burden with no effective interventions [8]. The growing use of e-cigarettes during pregnancy may only exacerbate this problem [9].

We previously reported in a blinded, randomised, multicentre trial that vitamin C supplementation (500 mg·day^{-1}) given to pregnant smokers unable to quit smoking caused significant increases in their offspring's newborn pulmonary function tests performed within 72 h of birth, and decreased the incidence of wheeze through 12 months of age [10]. We recently completed a second separate multicentre randomised controlled trial and reported results at 3 months of age [11]. We now report the airway function tests from this second trial through 12 months of age. We hypothesised that vitamin C supplementation to pregnant smokers would be associated with a persistent increase in the airway function in their infants through 12 months of age. Some of the results of these studies have been reported previously [11, 12].

Methods

Population and study protocol

Surviving infants born to pregnant smokers enrolled in the Vitamin C to Decrease the Effects of Smoking in Pregnancy on Infant Lung Function (VCSIP) study conducted in the US between 2012 and 2016 were eligible for the measurement of airway function/forced expiratory flows (FEFs) at 3 and 12 months of age. This study protocol has been described previously [13]. Briefly, this double-blind, multicentre, randomised, placebo-controlled trial enrolled smokers (⩾1 cigarette in the past week) pregnant between 13 and 23 weeks with a singleton gestation. They were randomised to vitamin C (500 mg·day^{-1}) *versus* placebo after successful completion of a medication adherence period that required 75% adherence and return for an appointment between 7 and 21 days later. Study medication was prepared in organoleptically identical capsules (Magno-Humphries Laboratories, Tigard, OR, USA) and distributed through the clinical sites' research pharmacies. Brief smoking-cessation counselling was provided to all participants beginning at screening and at every visit thereafter for the duration of the study. Randomisation was blocked in rotations of two and four subjects, and stratified by gestational age (⩽18 *versus* >18 weeks) and site (Oregon Health & Science University (OHSU), Portland, OR, USA; PeaceHealth Southwest Washington Medical Center (SWW), Vancouver, WA, USA; Indiana University (IU), Indianapolis, IN, USA). The OHSU data coordinating centre performed randomisation.

After randomisation, all women met with study staff at each pre-natal visit to assess medication use by pill count, smoking status and any health change. Staff trained in smoking cessation provided a pregnancy-specific smoking cessation pamphlet and provided brief cessation counselling consistent with the US Public Health Service Clinical Practice Guideline [13]. Serial biomarkers of fasting maternal blood for ascorbic acid (a measure of medication compliance) [11] and urine for cotinine levels were collected at randomisation and mid and late gestation.

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The trial was approved by each hospital's institutional review board and monitored by a National Institutes of Health-appointed data safety monitoring board. Written informed consent was obtained from pregnant smokers before enrolment.

Follow-up, secondary outcomes: study procedures and outcomes

We have previously reported the primary outcome of the trial, showing that FEF_{75} (the measurement of FEF at 75% of the expired volume) at 3 months of age in the infants born to pregnant smokers randomised to vitamin C *versus* placebo tended to be higher in the vitamin C group, although it did not meet statistical significance [11]. However, the pre-defined secondary outcomes of FEF_{50} (FEF at 50% of expired volume), and $FEF_{25–75}$ (FEF between 25% and 75% of expired volume) obtained from the same expiratory curves as the FEF_{75} were significantly increased in the vitamin C *versus* placebo treated infants. We are now reporting on sustained changes in FEFs between the vitamin C and placebo groups in the first year of life measured at 3 and 12 months of age and the incidence of wheeze through 12 months of age. These measurements were completed between 2013 and 2016.

Infant airway function tests at 3 and 12 months

Infant FEFs were performed at each site using identical equipment (Jaeger/Viasys Master Screen BabyBody; Yorba Linda, CA, USA) after chloral hydrate sedation following a standardised protocol [14, 15]. Testing occurred ⩾3 weeks after a respiratory illness. The 3-month test results were included if performed within the pre-determined infant age of 10–26 weeks; the 12-month test results were included if performed within 43–65 weeks. All tests were reviewed for acceptability, reproducibility and completeness.

FEFs were obtained from forced expiratory flow volume curves using the raised volume rapid thoracic compression technique following the American Thoracic Society/European Respiratory Society criteria for performance and acceptance [14]. Briefly, the lung was inflated with a pressure of 30 cm H_2O to the airway with a face mask. An inflatable jacket initiated thoracic compression at this raised volume and was maintained until residual volume was reached. Forced expiratory manoeuvres were repeated with increased pressure until flow limitation was obtained. Once flow limitation was established, the manoeuvre was repeated over a 10–15 cmH_2O range in jacket pressure until three technically acceptable curves were obtained with $FEF_{25–75}$ and forced vital capacity (FVC) within 10%. The best trial was chosen, determined as the most reliable with smooth forced expiration without evidence of early inspiration, marked flow transients or glottic closure [14, 15].

Respiratory questionnaires/incidence of wheeze

To compare the incidence of wheeze, a respiratory questionnaire modified from the International Study of Asthma and Allergies in Childhood questionnaire [16–18] was administered at least quarterly to the infant's caretaker. Presence or absence of wheeze, medications for wheeze, post-partum maternal smoking and other exposures were assessed. Composite wheeze was defined as a positive response to any of the following: parental report of wheeze, healthcare provider diagnosis of wheeze or any bronchodilator or steroids use. As defined *a priori*, only patients with at least one respiratory questionnaire completed at ⩾4 months of age were included in the clinical outcomes analysis.

Statistical analysis

Data are summarised as means and 95% confidence intervals for continuous variables and numbers and percentages for categorical variables. These analyses are all pre-planned secondary analyses and the power calculation was created for the primary analysis.

A linear, mixed-model (with random intercepts), repeated measures analysis of covariance (ANCOVA) was performed for $FEF_{75,}$ FEF_{50}, $FEF_{25–75}$, FVC, forced expiratory volume in 0.5 s ($FEV_{0.5}$) and $FEV_{0.5}$/FVC ratio. This model included a full-factorial analysis of the design variables for the study: stratification for gestational age at randomisation and clinical site, visit (3- and 12-month test) and the randomisation group (vitamin C or placebo). In addition, the model included three covariates (sex, white/nonwhite and standardised infant length at testing), as well as their interactions. These covariates were specified *a priori* (in the protocol) as these covariates are known predictors of FEFs and potentially different among the sites. As length at test visit is collinear with visit, lengths were standardised to a z-score [14, 15]. Least squares (or adjusted means) and associated 95% confidence intervals were estimated for significant terms in the ANCOVA model.

z-scores for airway function tests were derived using equations from Lum *et al.* [19].

Logistic regression modelling was used for analysis of wheeze outcomes adjusting for treatment arm, clinical site and gestational age at randomisation (and all two-factor interactions of these three factors) and the covariates of infant sex and maternal race. Sample sizes did not permit additional interactions. Additional covariates were considered by adding to the initial design model one at a time without interactions and evaluated with respect to model fit and odds ratio for group.

All p-values are two-sided with significance set at $p<0.05$. Statistical analyses were conducted using SAS for Windows version 9.4 (SAS Institute, Cary, NC, USA).

Results

Characteristics of patients

We randomised 252 pregnant smokers; however, one subject was excluded due to a protocol violation, so 251 were randomised to receive vitamin C or placebo and their infants were followed through 12 months of age (figure 1). Of the 243 infants at delivery, 241 survived to 12 months of age with one infant death per group (both presumed sudden infant death syndrome). 225 (93%) infants attempted FEFs at 3 months of age and 222 (99%) were technically acceptable; 213 (88%) attempted FEFs at 12 months of age with 202 (95%) technically acceptable. Supplementary table S1a and b contains information on characteristics of patients with complete *versus* incomplete FEF data. 98% (n=237) of surviving infants had at least one respiratory questionnaire completed at ⩾4 months of age. The median number of respiratory questionnaires completed was nine.

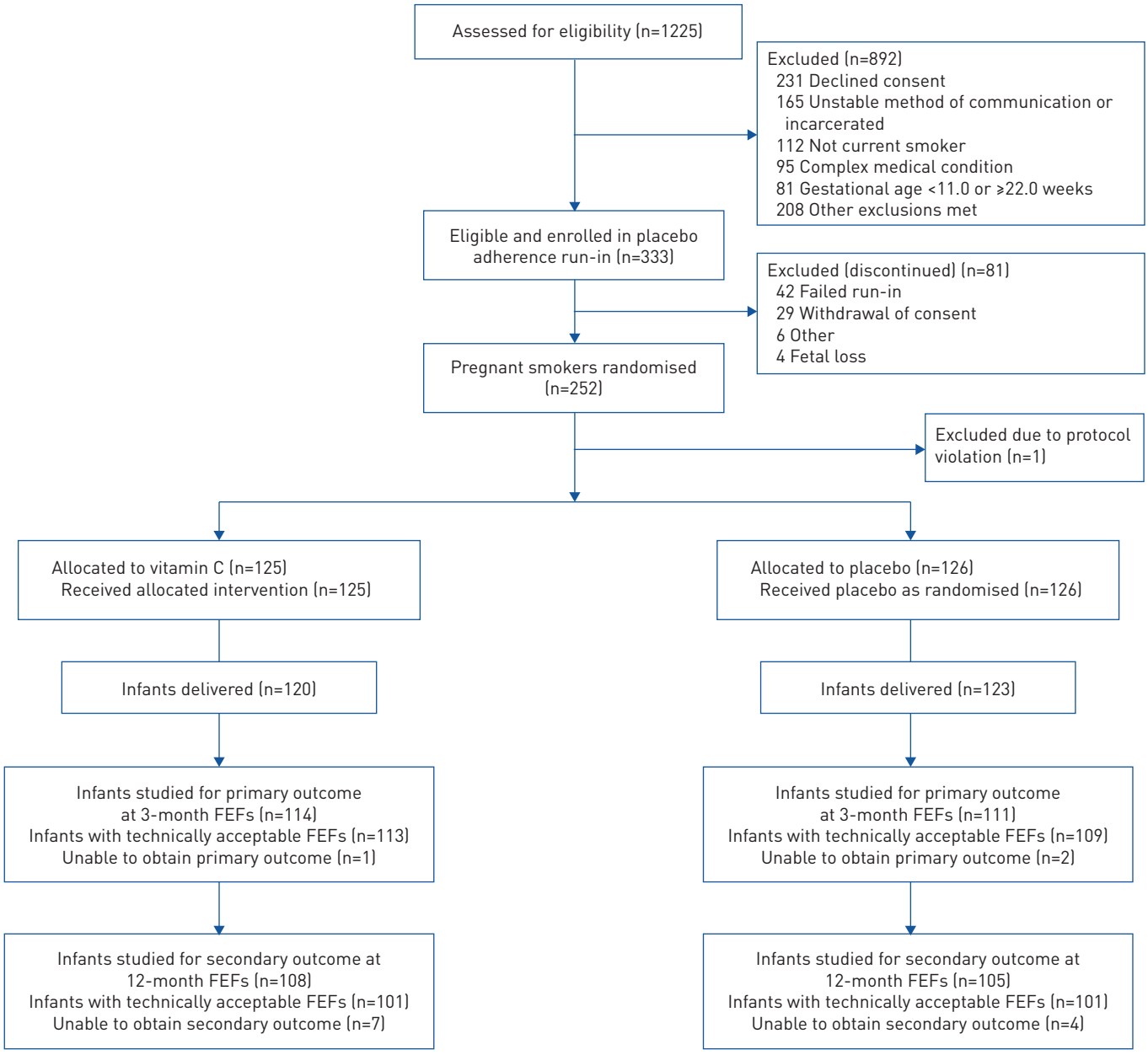

FIGURE 1 Consolidated Standards of Reporting Trials diagram for randomised smokers. Enrolment, randomisation and follow-up of randomised smokers and their infants through the 3- and 12-month measurements of airway function/forced expiratory flows (FEFs). In addition, 237 of the infants had respiratory questionnaires completed as per protocol.

The baseline characteristics of the mothers of the infants with completed respiratory clinical outcome data were similar between the vitamin C- and placebo-treated groups (table 1) including indicators of smoking status, fasting randomisation ascorbic acid level and other factors that influence offspring respiratory outcomes such as maternal asthma and body mass index (BMI) [20]. The median number of cigarettes smoked per day was seven in both groups at randomisation; six in the placebo *versus* five in the vitamin C group at mid-gestation; and five in each group at late gestation. Women allocated to vitamin C treatment *versus* placebo had significantly higher fasting ascorbic acid levels at mid (60.8 *versus* 41.6 μmol·L^{-1}; p<0.001) and late gestation (54.6 *versus* 39.6 μmol·L^{-1}; p<0.001).

The delivery characteristics of the infants were similar between randomised groups. Post-natal factors affecting infant respiratory health were comparable between groups including post-natal maternal smoking, incidence of breastfeeding and daycare attendance (table 1).

Airway function outcomes

The infants in the two treatment groups were not different in age, length, length z-scores or respiratory rate at the 3- and 12-month tests (supplementary table S2). The adjusted means for measurements of all of the airway function parameters at the 3- and 12-month evaluations are summarised in table 2. All measurements increased between 3 and 12 months of age. For all measurements except FVC and $FEV_{0.5}$/FVC

TABLE 1 Population characteristics of infants with completed respiratory clinical outcomes and their mothers[#]

	Vitamin C	Placebo	p-value
Subjects	118	119	
Baseline maternal characteristics at randomisation			
Age years	26.6±5.3	26.4±5.9	
White	93/118 (78.8)	95/119 (79.8)	
Gravida	3.0 (2.0–4.0)	3.0 (2.0–5.0)	
Body mass index kg·m^{-2}	27.2 (24.4–31.5)	29.3 (24.5–34.7)	
Married	21/118 (17.8)	31/119 (26.1)	
History of asthma	39/118 (33.1)	37/119 (31.1)	
Some college education	44/118 (37.3)	52/119 (43.7)	
Private health insurance	13/118 (11.0)	18/119 (15.1)	
Gestational age weeks	18.5±3.0	18.2±2.8	
Maternal cigarettes per day during pregnancy	7.0 (4.0–10.0)	7.5 (4.0–10.0)	
Urine cotinine ng·mL^{-1}	5031.1 (1788.7–7264.0)	5334.2 (1933.0–8291.0)	
Plasma ascorbic acid μmol·L^{-1}	49.2±19.1	48.7±19.3	
Birth characteristics of infants who completed respiratory clinical outcomes			
Birthweight g	3127±517	3067±557	0.39
Birthweight z-scores	−1.0±0.7	−1.1±0.9	0.12
Gestational age weeks	38.7±1.8	38.6±1.7	0.64
Caesarean section	40/118 (33.9)	34/118 (28.8)	0.31
Female	57/118 (48.3)	60/119 (50.4)	0.74
Pre-term birth, <37 weeks gestation	14/118 (11.9)	11/119 (9.2)	0.51
Intrauterine growth restriction	1/118 (0.8)	4/119 (3.4)	0.18
Post-natal characteristics of infants with completed respiratory clinical outcomes			
Maternal cigarettes per day at 3-month RQ	8.0 (5.0–10.0)	9.5 (5.0–10.0)	0.34
Maternal cigarettes per day at 12-month RQ	8.0 (4.0–10.0)	8.0 (3.0–10.0)	0.82
Breastfed at 3-month RQ %	42/117 (35.9)	40/116 (34.5)	0.82
Breastfed at 12-month RQ %	14/116 (12.1)	20/119 (16.8)	0.30
Daycare at 3-month RQ %	6/117 (5.1)	5/116 (4.3)	0.77
Daycare at 12-month RQ %	15/116 (12.9)	16/119 (13.4)	0.91
Pets in home in first year %	79/118 (66.9)	79/119 (66.4)	0.93
Diagnosis of eczema by physician %	25/118 (21.2)	18/119 (15.1)	0.23
Diagnosis of food allergy %	12/118 (10.2)	12/119 (10.1)	0.98

Data are presented as n, mean±SD, n (%) or median (interquartile range), unless otherwise stated. RQ: respiratory questionnaire. [#]: infants who had at least one respiratory questionnaire completed at ⩾4 months of age.

TABLE 2 Airway function tests in infants at 3- and 12-month visits

	Vitamin C at 3 months	Vitamin C at 12 months	Overall values in vitamin C-treated infants	Placebo at 3 months	Placebo at 12 months	Overall values in placebo-treated infants	Adjusted p-value for overall difference between vitamin C *versus* placebo groups[#]
Subjects	113	101		109	101		
FEF_{75} mL·s^{-1}	213±16	368±17	290±15	184±10	317±10	250±8	0.025
FEF_{50} mL·s^{-1}	446±23	679±26	562±21	401±15	607±15	504±12	0.0081
$FEF_{25–75}$ mL·s^{-1}	397±22	629±24	513±20	357±14	559±14	458±11	0.013
$FEV_{0.5}$[¶] mL	191±7	320±8	256±7	174±5	305±5	239±4	0.014
FVC mL	229±10	401±11	315±10	212±7	392±7	302±5	0.13
$FEV_{0.5}$/FVC[¶]	0.845±0.015	0.812±0.016	0.829±0.014	0.828±0.009	0.782±0.009	0.805±0.007	0.11

Data are presented as n or adjusted mean±SEM, unless otherwise stated. The infants in the two treatment groups were not different in age, length, or length z-scores at the 3- or 12-month tests. FEF_{75}: forced expiratory flow at 75% of the expired volume; FEF_{50}: FEF at 50% of the expired volume; $FEF_{25–75}$: FEF between 25% and 75% of the expired volume; $FEV_{0.5}$: forced expiratory volume in 0.5 s; FVC: forced vital capacity. [#]: represents significantly improved FEF_{75}, FEF_{50}, $FEF_{25–75}$ and $FEV_{0.5}$ for vitamin C *versus* placebo through 12 months of age. Adjusted p-values were estimated using mixed-model repeated measures analysis of covariance models that include treatment group, visit, other design variables (site and gestation age at randomisation) and potential covariates (sex, white/non-white and standardised length at testing). See text for details. While there were significant interactions involving treatment group for each airway function measure other than FEF_{75}, there was no evidence that these interactions modified the overall difference between the treatment groups. That is, there were no significant differences between the treatment groups for any combination of the other variables in the interaction terms. For example, if the interaction between gestational age (GA) and treatment group were significant, the difference between the two treatment groups was not significant for either the children with earlier GA or the children with later GA; [¶]: one infant in the vitamin C group at the 3-month visit did not have an $FEV_{0.5}$ value (or corresponding ratio), reducing the sample sizes in these tests by one.

ratio, offspring of women allocated to vitamin C compared to placebo had on average significantly higher flows through the first year of life (figure 2). There were no significant interactions for treatment group by study visit (3- and 12-month), suggesting effectively parallel differences between the treatment groups. These adjusted least-squared means were all significantly different from zero, indicating that infants in the vitamin C group had improved FEFs over the 3- and 12-month visits: FEF_{75} 40.2 (adjusted 95% CI 6.6–73.8 mL·s^{-1}), FEF_{50} 58.3 (10.9–105.8) mL·s^{-1}, $FEF_{25–75}$ 55.1 (9.7–100.5) mL·s^{-1} and $FEV_{0.5}$ 16 (1.0–31.6) mL. The effective percentage increases with vitamin C compared to placebo were 16.1% for FEF_{75}, 11.6% for FEF_{50}, 12% for $FEF_{25–75}$ and 6.8% for $FEV_{0.5}$. There were significant higher order interactions that included both treatment group and visit for all airway function parameters except FEF_{75}. However, there were no differences between treatment groups for any combination of the other design variables, supporting the use of the overall mean differences between the treatment groups above. Analysis summaries are provided in more detail in supplementary table S3a and b. Our statistical analysis plan contained a pre-defined analysis of FEFs at 3 months (previously reported [11]) and at 12 months, in addition to the repeated measures analysis. The standalone analysis of FEFs at 12 months also showed a significantly increased FEF_{75}, FEF_{50}, and $FEF_{25–75}$ at 12 months with maternal vitamin C treatment (supplementary table S4).

Clinical respiratory outcomes

Clinical respiratory questionnaire follow-up was obtained in 98% of the infants who survived from delivery to 12 months of age. There were no differences at baseline between the intervention groups in maternal or infant characteristics or postnatal factors known to influence wheeze (table 1). The overall incidence of composite wheeze was 51 (43.2%) out of 118 in the vitamin C group and 63 (53.9%) out of 119 in the placebo group (unadjusted p=0.13).

Treatment group allocation was significant (p=0.03) in the overall multivariable analysis, as were study site (p=0.03) and sex (p=0.03). However, there were significant interactions between treatment group and study site on the incidence of wheeze (p=0.03), as well as between treatment group and gestational age at randomisation (p=0.004) (table 3).

At SWW, the odds ratio of wheeze was 0.16 (95% CI 0.06–0.44) in infants of pregnant smokers taking vitamin C starting at ⩽18 weeks' gestation *versus* placebo. Similarly, at OHSU the odds ratio was 0.095 (95% CI 0.02–0.51). Yet at IU, the odds ratio of wheeze was 3.30 (95% CI 1.22–8.93) when vitamin C was begun at >18 weeks gestational age.

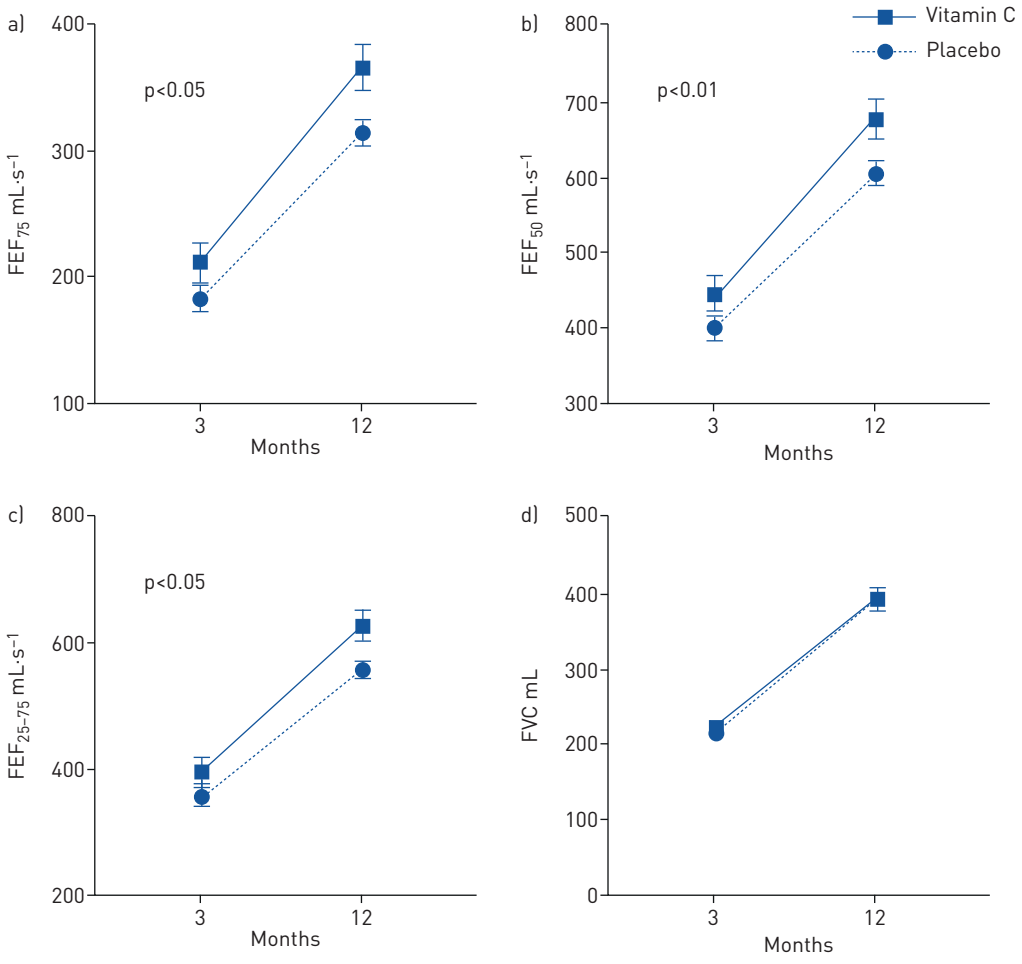

FIGURE 2 Effect of vitamin C supplementation during pregnancy on infant airway function tests at 3 and 12 months of age. Plots of unadjusted means (expressed as mean±SEM) for a) forced expiratory flow at 75% of the expired volume (FEF_{75}), b) FEF at 50% of the expired volume (FEF_{50}), c) FEF at between 25% and 75% of the expired volume (FEF_{25-75}) and d) forced vital capacity (FVC). FEF_{75}, FEF_{50} and FEF_{25-75} were significantly improved in the vitamin C *versus* placebo group through 12 months of age by repeated measures analysis of covariance. There were no significant interactions for treatment group by study visit (3- and 12-month), suggesting effectively parallel differences between the treatment groups. See also table 2 legend and text.

The odds ratios for the other three combinations of site and gestational age were not statistically significant. Adding maternal asthma and BMI as potential covariates did not explain the differences in treatment effect between centres. Male sex and maternal asthma were associated with increased risk of wheeze. When the analysis was repeated using healthcare provider diagnosis of wheeze or use of asthma medication, the results were similar.

Adverse events

No serious adverse events were related to the intervention.

Discussion

This is the first randomised controlled trial to demonstrate a persistent increase in infant airway function after an *in utero* intervention targeted to block a specific environmental insult known to adversely affect subsequent childhood respiratory health. In this study of singleton infants of pregnant smokers randomised at <23 weeks of gestation to receive vitamin C supplementation (500 mg·day^{-1}) or placebo for the remainder of the pregnancy, vitamin C produced a persistently significant increase of the offspring's airway function at 3 and 12 months of age. The results of this study, as well as our previous clinical trial in newborns [10] cumulatively demonstrate that vitamin C improves lung function at birth and these improvements are maintained throughout the first year of life.

TABLE 3 Incidence of wheeze by recruitment site and gestational age stratification

	Vitamin C	Placebo	OR (95% CI)[#]
Subjects	118	119	
PeaceHealth Southwest Washington Medical Center			
Gestational age ⩽18 weeks	6/24 (25)	22/28 (78.6)	0.16 (0.06–0.44)
Gestational age >18 weeks	15/30 (50)	12/27 (44.4)	0.82 (0.32–2.10)
Oregon Health and Science University			
Gestational age ⩽18 weeks	1/9 (11.1)	6/12 (50)	0.095 (0.02–0.51)
Gestational age >18 weeks	2/11 (18.2)	3/8 (37.5)	0.50 (0.10–2.60)
Indiana University			
Gestational age ⩽18 weeks	8/16 (50)	7/17 (41.2)	0.62 (0.20–1.92)
Gestational age >18 weeks	19/28 (67.9)	13/27 (48.2)	3.30 (1.23–8.93)

Data are presented as n or n (%), unless otherwise stated. [#]: logistic regression modelling was used for analysis of wheeze outcomes adjusting for treatment arm, clinical site and gestational age at randomisation (and all two-factor interactions of these three factors) and the covariates of infant sex and maternal race.

Data from longitudinal birth cohorts demonstrate that an individual's airway function is established very early in infancy and subsequently tracks over time [3, 21], although there may be some potential for improvement between infancy and childhood [22]. This study is the first to provide evidence that these parameters can be reset with targeted pre-natal interventions yielding changes in the first year of life, and potentially shifting airway function to a higher percentile on the trajectory curve [1]. In the current study, FEFs in the vitamin C-treated group were increased by 11.6–16.1% compared to placebo, which is consistent with differences in the newborn pulmonary function tests assessed in our first trial [10]. Additionally, it is consistent with percentage decreases that we [23] and others [24, 25] have reported in infants born to smokers *versus* nonsmokers, which was the basis for the power calculations of sample size for the differences in the 3-month FEFs in the current study [23]. The summary statistics of the z-scores [19] of the FEFs of the vitamin C- and placebo-treated infants indicate that their flows fall within normal limits and are consistent with the statistical analyses of the raw values (table 4).

The persistence in the difference in FEFs for the vitamin C *versus* placebo did not change over time; specifically, there was not a significant interaction of treatment and time indicating that the protective effect of vitamin C on FEFs appeared constant throughout the first 12 months of life. This is consistent with our pre-clinical nonhuman primate data demonstrating that nicotine crosses the placenta and upregulates nicotinic acetylcholine receptors. While the exact mechanism of action is unknown, nicotine treatment in this model was associated with collagen deposition around the airways and decreased FEFs in the offspring [26–28]. Vitamin C supplementation during nicotine exposure in pregnancy blocked these structural changes [29], which could explain the persistently higher FEFs through 12 months of age observed in the current study of human infants. In addition, the vitamin C treatment may be acting through antioxidant mechanisms to prevent nicotine's ability to alter airway geometry in favour of

TABLE 4 Summary table of z-scores for airway function tests at 3 and 12 months of age

	Vitamin C at 3 months	Placebo at 3 months	Vitamin C at 12 months	Placebo at 12 months
Subjects	113	109	101	101
FEF_{75} mL·s^{-1}	−0.02±1.37	−0.24±1.33	0.62±0.95	0.32±1.16
$FEF_{25–75}$ mL·s^{-1}	0.29±1.18	0.02±1.29	0.77±1.00	0.46±1.11
$FEV_{0.5}$ mL	−0.01±1.07	−0.24±1.16	0.30±0.96	0.20±1.13
FVC mL	−0.28±1.12	−0.39±1.29	−0.18±0.96	0.09±1.02
$FEV_{0.5}$/FVC	0.28±0.98	0.10±1.13	0.82±0.95	0.53±0.95

Data are presented as n or mean±SD. Means are unadjusted for other variables. z-scores were calculated after LUM *et al.* [19]. Forced expiratory flow at 50% of the expired volume (FEF_{50}) is not included, as LUM *et al.* [19] did not include a z-score equation for FEF_{50}. FEF_{75}: FEF at 75% of the expired volume; $FEF_{25–75}$: FEF between 25% and 75% of the expired volume; $FEV_{0.5}$: forced expiratory volume in 0.5 s; FVC: forced vital capacity.

smaller-diameter airways [30]. The actions of vitamin C on airway geometry, connective tissue and alveolar structure probably underlies why vitamin C treatment affects FEFs, but not overall lung volumes.

Improved airway function in infants has been associated with a lower incidence of wheeze early in life [31, 32]. Although vitamin C produced a persistent increase in FEFs at all study sites, we did not find that the incidence of wheeze was significantly lower across all study sites. Therefore, it becomes difficult to interpret the magnitude of the effect size of improved FEFs upon the clinical outcome of decreased wheeze. Composite wheeze was 43.2% for the vitamin C-treated group and 53.9% for the placebo-treated group. This difference was not statistically significant across all sites and gestational age groups. Interpretation of the clinical assessment of wheeze is complex as there were significant interactions for treatment, site and gestational age. The decrease in the incidence of wheeze in the vitamin C-treated group in the combined OHSU and SWW sites in the current study (32% incidence of wheeze for vitamin C and 57% incidence of wheeze for placebo for a 44% decrease in the incidence of wheeze) was consistent with the decrease reported in our first study [10] at these same sites (21% incidence of wheeze for vitamin C and 40% incidence of wheeze for placebo for a 48% decrease in the incidence of wheeze; p=0.03). However, in the current study, at the IU site, the incidence of wheeze in the vitamin C treated group was not lower than the placebo group. There was a higher incidence of pregnant smokers with a history of asthma (32% *versus* 11%) and a higher incidence of infants with eczema (52% *versus* 23%) allocated to the vitamin C group in Indiana; however, secondary analysis using these covariates did not adequately account for site differences.

Strengths and limitations

A major strength of our study is the assessment of FEFs, the gold standard for assessing intrathoracic airway function in cooperative children and adults [33]. Additional strengths include the small loss to follow-up, the significantly increased ascorbic acid levels in women randomised to vitamin C providing biological support for the intervention [10] and the use of standardised respiratory questionnaires [16]. The increased FEFs in the vitamin C-treated groups were consistent across all three sites regardless of the gestational age at the initiation of intervention, supporting the generalisability of our findings, although the primary outcome of the study FEF_{75} at 3 months of age was not significantly different between groups [11]. While there was a higher proportion of infants from Indiana with missing 3-month and 12-month outcome data, the strong correlation between site and nonwhite race in this trial needs additional study to determine if this effect is seen across all populations. Despite the pattern of missingness, there was no significant effect of site or race on the effect of vitamin C on FEFs in the formal statistical model.

Wheeze is a complex clinical respiratory outcome, particularly for infants, who often have upper respiratory infections. Nonatopic and atopic mechanisms can contribute to airways obstruction and wheeze [34, 35], and vitamin C supplementation may only affect some of the mechanisms. Both groups of pregnant smokers reported a comparable decrease in the median number of cigarettes smoked over the pregnancy, which may have decreased the impact of vitamin C. In addition, we were probably underpowered for wheeze, which was a secondary clinical outcome of the study. Additionally, vitamin C supplementation was not continued postnatally, which might provide additional protection for growing airways exposed to post-natal smoking. Lastly, 12 months of age is a short period of follow-up for respiratory symptoms. These infants are now in continued follow-up through 5 years of age.

Conclusions

In this analysis of pre-defined secondary outcomes of a randomised clinical trial of vitamin C supplementation ($500\ mg{\cdot}day^{-1}$) to pregnant smokers, vitamin C significantly increased the airway function in the infants through 12 months of age. While smoking cessation in pregnancy remains the number one priority and vitamin C supplementation does not justify continued smoking, in reality 50% of pregnant smokers continue to smoke despite vigorous antismoking campaigns [36, 37]. This study demonstrates that the safe, inexpensive [38] and simple intervention of vitamin C supplementation can persistently improve the airway function of infants exposed to *in utero* smoke.

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