Oral Vitamin C (500 mg/day) to Pregnant Smokers Improves Infant Airway Function at 3 Months: A Randomized Trial

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Drafting the manuscript: CTM, BSP, DFK, AV, ERS, RST, CDM

Key words: infant pulmonary function; forced expiratory flows; smoking in pregnancy; vitamin C; wheezing

Funding: Supported by the NHLBI (R01 HL105447) with cofunding from the Office of Dietary Supplements (ODS) and by P51OD011092. Additional support from the Oregon Clinical Translational Research Institute funded by the National Center for Advancing Translational Sciences (UL1TR000128).

Running Head: Vitamin C, in-utero smoke, infant airway function
Descriptor: 14.02 Clinical Studies: General<Pediatrics
Total word count: 3499

At a Glance Commentary
What is the current scientific knowledge on this subject?
Maternal smoking during pregnancy adversely affects lung development with near lifelong decreases in pulmonary function and increased risk of wheezing, respiratory infections, and asthma. We have previously shown that adding daily supplemental vitamin C for pregnant smokers improves their infant’s newborn pulmonary function (passive respiratory compliance and time to peak tidal expiratory flow to expiratory time) measured within 72 hours of birth. We also demonstrated that the effect of maternal smoking on newborn lung function was associated with the maternal genotype for the α5 nicotinic acetylcholine receptor (rs16969968).

What does this study add to the field?
This randomized trial expands our previous study by demonstrating increased forced expiratory flows (a more specific measurement of airway function) at three months of age in the infants of pregnant smokers randomized to daily vitamin C versus placebo. The effect of maternal smoking on infant forced expiratory flows appeared to be increased with maternal risk alleles for the α5 nicotinic acetylcholine receptor genotype. Our results suggest that vitamin C supplementation in pregnant women who cannot quit smoking may be a safe, inexpensive, and simple intervention to improve the pulmonary function of their infant by blocking some of the effects of in-utero smoke on lung development.
ABSTRACT

Rationale: We reported a randomized trial demonstrating daily supplemental vitamin C to pregnant smokers significantly improved newborn pulmonary function tests. The current study tests these results in a new cohort utilizing infant pulmonary function tests.

Objective: To determine if infants of pregnant smokers randomized to daily supplemental vitamin C would have improved forced expiratory flows (FEFs) at 3 months of age compared to those randomized to placebo, and to investigate the association of the α5 nicotinic acetylcholine receptor.

Methods: Randomized, double-blind, placebo-controlled trial conducted at three centers. Two hundred fifty-one pregnant smokers were randomized at 13 to 23 weeks of gestation: 125 randomized to vitamin C (500mg/day) and 126 to placebo.

Measurements: The primary outcome was FEF75 at 3 months of age performed with the raised volume rapid thoracic compression technique (Jaeger/Viasys). FEF50 and FEF25-75 obtained from the same expiratory curves were pre-specified secondary outcomes.

Main Results: The infants of pregnant smokers randomized to vitamin C (n=113) had the following FEFs at three months of age compared to those randomized to placebo (n=109) as measured by FEF75 (200.7 vs 188.7 mL/sec [adjusted 95% CI for difference, -3.33 to 35.64]; p=0.10), FEF50 (436.7 vs 408.5 mL/sec [adjusted 95% CI for difference, 6.10 to 61.30]; p=0.02), and FEF25-75 (387.4 vs 365.8 mL/sec [adjusted 95% CI for difference, 0.92 to 55.34]; p=0.04). Infant FEFs appeared to be negatively associated with the maternal risk alleles for the α5 nicotinic acetylcholine receptor (rs16969968).
**Conclusions:** Although the primary outcome of FEF\textsubscript{75} was not improved after vitamin C supplementation to pregnant smokers, the predetermined secondary outcomes FEF\textsubscript{50} and FEF\textsubscript{25-75} were significantly improved. These results extend our previous findings and demonstrate improved airway function (FEF\textsubscript{50} and FEF\textsubscript{25-75}) at 3 months of age in infants after vitamin C supplementation to pregnant smokers.

**Trial Registration:** Clinicaltrials.gov, Identifier: NCT01723696

**Key words:**

Nicotine; forced expiratory flows; nicotinic acetylcholine receptor; developmental origins of disease
INTRODUCTION

Smoking during pregnancy remains a large public health problem and is the largest preventable cause of childhood respiratory illness.\textsuperscript{1,3} Despite intensive smoking cessation initiatives, more than 50% of smokers who become pregnant continue to smoke.\textsuperscript{4,5} This corresponds nationwide to at least 10% of American women continuing to smoke when pregnant\textsuperscript{6} with about 450,000 exposed infants per year at an estimated annual health care cost of over 1 billion dollars.\textsuperscript{7}

We previously reported a randomized controlled trial demonstrating that giving supplemental vitamin C (500 mg per day) to pregnant women unable to quit smoking, significantly improved their offspring’s newborn pulmonary function tests (PFTs) of passive respiratory compliance and time to peak tidal expiratory flow to expiratory time (TPTEF:TE) measured within 72 hours of birth.\textsuperscript{8} We also demonstrated that the effect of maternal smoking on newborn lung function was associated with the maternal genotype for the α5 nicotinic acetylcholine receptor (nAChR) (rs16969968). This genotype is also associated with increased risk of lung cancer, chronic obstructive pulmonary disease (COPD) and increased nicotine dependence.\textsuperscript{9,10} This trial was based on important foundation data from a non-human pregnant primate model which demonstrated that nicotine crosses the placenta, up-regulates nicotinic receptors and alters lung development in the offspring with decreased forced expiratory flows (FEFs) at birth.\textsuperscript{11-13} In this model, vitamin C decreased the effects of in-utero nicotine on the offspring’s FEFs.\textsuperscript{14}

Although our initial study showed significantly improved PFTs in the newborns of smokers allocated to vitamin C, we did not measure FEFs due to the instability of
newborn lung volumes in the first weeks of life and the required sedation to perform
testing. However, FEFs are a more direct assessment of airway function, previous
studies have demonstrated decreased FEFs in infants exposed to maternal smoking
during pregnancy,\textsuperscript{15-18} and decreased FEFs in infancy are correlated with increased risk
of respiratory disease.\textsuperscript{15,16,19} Therefore, the primary objective of this study was to
compare the FEFs at three months of age in infants of pregnant smokers randomized to
vitamin C (500 mg/day) versus placebo. We hypothesized that the supplemental vitamin
C would improve the infant’s FEFs when compared to placebo. Some of the results of
these studies were previously reported in the form of an abstract.\textsuperscript{20}

\textbf{METHODS}

\textbf{Participants}

Pregnant smokers were recruited from three clinical sites: Oregon Health &
Science University (OHSU), Portland, Oregon; Indiana University, Indianapolis, Indiana;
PeaceHealth Southwest Washington Medical Center, Vancouver, Washington. This
study cohort was comprised of different patients than those recruited in our initial trial.\textsuperscript{8}
The study protocol was approved by the three Institutional Review Boards. All women
provided written informed consent.

Inclusion criteria at randomization were: women $\geq$15 years old with a singleton
gestation between $13^{0/7}$ and $22^{6/7}$ weeks based on clinical information and confirmed by
ultrasound, current cigarette smoker ($\geq$ 1 cigarette in last week), English-speaking and
receiving prenatal care at surrounding clinics to the three sites. Exclusion criteria at
randomization included: multiple gestation, fetal anomalies, current illicit drug use,
current alcohol abuse, daily vitamin C supplementation $>3$ days/week (not including
prenatal vitamin) since last menstrual period, refusal to abstain from vitamin supplements except those from the study, history of a kidney stone, insulin dependent diabetes, complex maternal medical conditions, participation in conflicting research projects, unable to demonstrate a stable method of communication, pregnancy by in-vitro fertilization, plan to terminate pregnancy, body mass index >50 kg/m².

Study Design and Oversight

Details of the study design were previously published. We conducted a randomized, double-blind, placebo-controlled study of vitamin C (500 mg/day) versus placebo in pregnant smokers.

Study staff screened women at prenatal clinics between December, 2012 and June, 2015 in the catchment area of the three clinical sites to identify eligible women who continued to smoke cigarettes. Staff trained in smoking cessation provided participants with brief cessation counseling consistent with the US Public Health Service Clinical Practice Guideline and provided a pregnancy-specific smoking cessation pamphlet. Smoking status and counseling were documented in the study record. Upon consent, women had an ultrasound for gestational age confirmation and entered a medication adherence trial to take 1 placebo capsule/day for 7 to 21 days. If a subject returned within 21 days with the medication bottle and took at least 75% of the required placebo, she proceeded to randomization.

The vitamin C and placebo medications were manufactured in organoleptically identical tablets (Magno-Humphries Laboratories Inc, Tigard, Oregon) and dispensed through the OHSU research pharmacy. Each vitamin C tablet contained 500 mg of ascorbic acid powder; the placebo tablet contained microcrystalline cellulose and 100
mg of citric acid to mimic the taste of vitamin C. The tablets were identical in appearance, size and shape and dispensed in 100 tablet quantities during the treatment period. Participants were instructed to take 1 study capsule daily, at a consistent time, until delivery. After randomization, women were given a standard prenatal vitamin (Prenavite, Rugby Laboratories, Duluth, Georgia, 100 count) that included 60 mg of vitamin C.

Randomization was performed at the Data Coordinating Center (DCC) using a permuted block randomization stratified by gestational age at time of randomization (≤18 versus >18 weeks gestation) and clinical site. The OHSU research pharmacy prepared consecutively labelled medicine bottles to dispense, using the randomization schedule. The study medication was labeled with the study identification and a consecutive study code for the patient. The OHSU research pharmacy dispensed the study capsules to all sites. The investigators, clinicians, and patients were unaware of treatment allocation through the three month PFT and remain blinded. (CONSORT diagram, Figure 1).

Study staff met with the randomized women at each prenatal visit to collect: interval smoking histories via a standardized respiratory questionnaire,23 brief health questionnaires, medication use, and complications with review of electronic medical records (EMR). They also provided brief smoking cessation counseling. Adherence with dispensed medication (vitamin C or placebo) was assessed at each visit with pill count by study staff and at return of each medication bottle. Fasting maternal blood samples for ascorbic acid levels and maternal urine samples were obtained at randomization, and at 26 and 32 weeks of gestation.
Study End Points

The primary outcome was the comparison of infant FEF<sub>75</sub>, the measurement of FEF at 75% of the expired volume, at 3 months of age obtained using the raised volume rapid thoracic compression (RVRTC) technique in offspring of pregnant smokers randomized to vitamin C versus placebo. Pre-specified secondary outcomes were the FEF at 50% expired volume (FEF<sub>50</sub>), as well as between 25 and 75% expired volume (FEF<sub>25-75</sub>) obtained from the same expiratory curve.

Forced expiratory volumes including forced vital capacity (FVC) and forced expired volume in the initial 0.5 sec (FEV<sub>0.5</sub>) were obtained. Based on findings in our prior study, in a secondary analysis we evaluated the potential association of the α5 nAChR polymorphism rs16969968 (which is the α5 nAChR structural polymorphism that has the strongest link to lung disease and increased smoking),<sup>9</sup> in the effect of in-utero smoke on infant pulmonary function.

Measurements

Infant Pulmonary Function Tests at 3 Months of Age

Infants were studied in the infant PFT laboratories at Doernbecher Children’s Hospital (Oregon), the James Whitcomb Riley Hospital for Children (Indiana), or at Peace Health/ Southwest Medical Center (Washington) after sedation with oral chloral hydrate at 50-100 mg/kg. Each site used the same PFT equipment (Jaeger/Viasys Master Screen BabyBody; Yorba Linda, California). Operational procedures were rigorously followed across sites as outlined in the study’s manual of operations.<sup>18, 24</sup> Cross training and certification of PFT laboratories assured the same testing techniques
and acceptance criteria were applied across sites. Four experienced respiratory therapists performed all testing. All tests were reviewed by a trained, licensed respiratory therapist and reviewed for acceptability, reproducibility, and completeness. All testing was done at least three weeks after a respiratory illness and analyzed testing occurred within the predefined infant age of 10 to 26 weeks.

FEFs were obtained from forced expiratory flow volume curves using the RVRTC technique with testing performed following the American Thoracic Society/European Respiratory Society criteria for performance and acceptance. Briefly, the lung was inflated by applying a pressure of 30 cm H2O to the airway with a face mask. An inflatable jacket was used to initiate thoracic compression at this raised volume and was maintained until residual volume was reached. Forced expiratory maneuvers were repeated with increased pressure until flow limitation was obtained. Once flow limitation was established, the maneuver was repeated over a 10 to 15 cm H2O range in jacket pressure until three technically acceptable curves were obtained with FEF25-75, and FVC within 10%. The best trial was chosen that was determined to be the most reliable with smooth forced expiration without evidence of early inspiration, marked flow transients, or glottic closure.

**Biomarkers and Genotyping**

Plasma ascorbic acid measurements were performed at the Linus Pauling Institute using high performance liquid chromatography with coulometric electrochemical detection. Urine cotinine levels were measured with a widely used ELISA (Enzyme Linked Immunosorbant Assay) kit following the vendor’s protocol (Calbiotech, Spring Valley, California). DNA was prepared from EDTA whole blood
tubes using the QIAcube and QIAamp DNA Blood Mini QIAcube Kit (Qiagen, Hilden, Germany). Mothers were genotyped for rs16969968 which is the nAChR polymorphism most clearly linked to increased smoking, difficulty quitting and lung disease, by real time PCR using predesigned qPCR SNP genotyping reagents (C__26000428_20) from Thermo Fisher (Applied Biosystems, Foster City, CA). Mothers were also genotyped for GSTM1 deletion (Hs02575461_cn), GSTT1 deletion (Hs00010004_cn) and GSTP1 polymorphism (C__3237198_20). All maternal and baby DNA samples from blood were genotyped in duplicate and any samples which did not produce the same result in both replicates was repeated. As part of genotyping QC, maternal and baby genotypes were also compared for any Mendelian violations.

Safety Monitoring

All subjects were monitored for adverse events according to standard definition by interviewing the subject, EMR review, and physical exam (including vital signs monitoring during sedation). The NIH appointed Data and Safety Monitoring Board (DSMB) reviewed maternal, neonatal, and infant adverse events every 3 months.

Statistical Analysis and Power

Our targeted sample size of 218 infants with successful FEFs at 3 months of age was determined to detect with 90% power, at a significance level of 0.05, increases of 15% in mean FEF$_{75}$ in the vitamin C group compared to the placebo group. This was based on comparing the two means on the log scale using 0.28 as the estimate for the standard deviation of FEF$_{75}$ based on data in 155 healthy infants of smoking and nonsmoking women and included allowance for 4% of the patients to be non-compliant (took <50% of their medications) as shown in our initial trial.
Comparisons of the two treatment groups with respect to maternal characteristics assessed after randomization, delivery outcomes, and non-PFT infant characteristics at 3 months were made using the t-test for numeric variables and the Pearson chi-square test for categorical variables. Summary statistics of z scores for PFT parameters were also calculated using the approach of Lum et al. 2016.\textsuperscript{28} The statistical analyses of PFTs were based on intention to treat. We analyzed FEF\textsubscript{75}, FEF\textsubscript{50}, and FEF\textsubscript{25-75} in infants born to mothers randomized to vitamin C versus placebo, using analysis of covariance (ANCOVA) general linear models. Included in these models were treatment arm, clinical site, and gestational age at randomization (and all interactions of these three factors) and the covariates of infant sex, maternal race, and infant length at three months. Analyses for FVC, FEV\textsubscript{0.5}, and FEV\textsubscript{0.5}/FVC were done using the same ANCOVA analyses. In secondary analyses, a factor for the α5 nAChR (rs16969968) genotype (with levels for major allele homozygous, heterozygous, and minor allele homozygous) and the interaction of genotype and treatment arm were added to the above ANCOVA models for FEF\textsubscript{75}, FEF\textsubscript{50}, FEF\textsubscript{25-75}.

All p-values are 2-sided, with significance set at p<0.05. Statistical analyses were conducted using SAS® 9.4 (SAS Institute Inc.). The DSMB reviewed results for the primary outcome when 50% of the total sample size of three month PFTs were completed.

RESULTS

Characteristics of the Trial Participants

We screened 1225 pregnant women who continued to smoke in pregnancy; 892 were excluded prior to consent, and 81 were excluded after the medication adherence
period (Figure 1, CONSORT diagram). We randomized 252 women, however one subject was subsequently identified to have met critical exclusion criteria and therefore we analyzed 125 who received vitamin C (500 mg/day) and 126 who received placebo (Figure 1). Randomization balanced relevant baseline covariates including age, race and ethnicity, parity and gestational age (Table 1). At study entry, the median number of cigarettes smoked per day in each group was 7, and urine cotinine was comparable in each group. Plasma ascorbic acid levels were similar at baseline between both groups (Table 1); at mid and late gestation, the vitamin C group had significantly higher ascorbic acid levels than the placebo group (Table 2). The urine cotinine levels were comparable between groups at randomization, as well as at mid and late gestation. Of the 243 infants at delivery, there was no significant effect of the intervention on delivery mode, birth weight, gestational age, or the incidence of prematurity (Table 2).

**Pulmonary Function Tests: FEFs at 3 Months of Age**

For the analysis of the primary outcome of the 3 month FEF\textsubscript{75}, 225 infants had PFTs attempted (92.6% of infants available at delivery), and 222 infants (113 in the vitamin C and 109 in the placebo treated group) had successful FEFs performed within the predetermined window of 10 through 26 weeks of infant age. After randomization, 12 infants in the vitamin C and 17 in the placebo group did not complete the 3 month PFTs due to fetal loss, infant death, consent withdrawal, loss to follow-up or unsuccessful sedation (Figure 1). At the time of the attempted three month PFTs, infants in the vitamin C and placebo treatment groups each had a mean of 15.3 weeks and length of 60.2 centimeters (Table 2).
The infants born to the smokers randomized to vitamin C had an increased FEF\textsubscript{75} (200.7 vs 188.7 mL/sec [adjusted 95% CI for difference -3.33 to 35.64]; p=0.10) at 3 months of age, and a significantly increased FEF\textsubscript{50} (436.7 vs 408.5 mL/sec [adjusted 95% CI, 6.10 to 61.30]; p=0.02) and FEF\textsubscript{25-75} (387.4 vs 365.8 mL/sec [adjusted 95% CI, 0.92 to 55.34]; p= 0.04) compared to those randomized to placebo (Table 3A). Regarding forced expiratory volumes, there was a significant difference in FEV\textsubscript{0.5} between the groups but not for FVC or FEV\textsubscript{0.5}/FVC (Table 3A).

**Maternal Genotype**

The addition of maternal genotype (n=217) for the α5 nAChR (rs16969968) into the model of treatment and the three month FEFs, increased the effect size between the treatment arms for each FEF, however it also increased the width of the 95% confidence intervals (Table 4). There was no significant interaction between maternal genotype and treatment group. There was a significant association of the α5 nAChR genotype in the treatment model of vitamin C versus placebo for FEF\textsubscript{50}, with infants of mothers who had two copies of the risk allele for the α5 nAChR having the lowest FEF\textsubscript{50} in both the vitamin C treated and placebo subjects. Interestingly, the α5 nAChR genotype appeared to have a dose response effect on FEF\textsubscript{50} according to the number of functional alleles present with vitamin C treatment improving FEF\textsubscript{50} compared to placebo at each level.

**Adverse Events**

Adverse events were monitored. No serious adverse events related to the intervention were reported.
DISCUSSION

In this randomized, double-blinded multicenter clinical trial, infants delivered to pregnant smokers randomized to supplemental vitamin C (500 mg/day) did not have a significant improvement in the primary outcome of FEF75, but had a significant improvement in FEF50 and FEF25-75 which are flows obtained from the same expiratory curve. This confirms and expands our initial study8 of improved newborn PFTs with similar findings but in a larger population with increased diversity through the measurement of FEFs, a more specific measurement of airway function. We also confirm our previous findings of the importance of the α5 nAChR in the effect of in-utero smoke on pulmonary function.

We chose the primary outcome of infant FEFs to build on our strong preclinical data in nicotine exposed primates,12,14 and to expand our initial clinical study of significantly improved passive pulmonary mechanics and T_{PTEF:TE} in newborns of pregnant women randomized to vitamin C versus placebo.8 Several studies, including our own,2;27,28,29 have demonstrated that a key effect of maternal smoking during pregnancy is a decrease in infant FEFs, which is considered a sensitive measurement of infant airway function. In contrast to older children and adults, infants have a very short rate constant for forced expiration and the entire FVC occurs in < 1-second,24 therefore some investigators use FEV0.5 to quantify infant airway function. Among healthy infants without previous wheezing, we found that FEF75, FEF50, and FEF25-75, but not FEV0.5, were lower in infants whose mothers reported smoking during pregnancy.27 Similarly, FEF75, FEF50, and FEF25-75, but not FEV0.5 were better at detecting low airway function among infants with a history of respiratory symptoms, but
asymptomatic at the PFT. Therefore, for the current study, we chose FEF\textsubscript{75} as our primary outcome to assess infant airway function in the randomized groups, however we could have just as easily chosen FEF\textsubscript{50} and FEF\textsubscript{25-75} as the primary outcome since they are obtained from the same expiratory curve. The summary statistics of the z scores\textsuperscript{28} of the FEFs of the vitamin C and placebo treated infants (Table 3B) indicate their flows fall within normal limits (range of mean scores from -0.38 to 0.29 for all parameters) and follow the same general pattern/direction of the raw scores (i.e. the z scores of the placebo treated infants being lower than those of the vitamin C treated.)

In this study, we confirm our previous findings of the importance of the maternal α\textsubscript{5} nAChR genotype (rs16969968) in the effect of in-utero smoke on infant lung function. This relatively common polymorphism has been associated with an increased risk of lung cancer and nicotine addiction.\textsuperscript{9} In our initial study,\textsuperscript{8} we demonstrated that newborns of mothers who had two copies of the α\textsubscript{5} nAChR genotype risk allele had the lowest measurements of T\textsubscript{PTEF:TE} and our current study shows a similar decrease in FEF\textsubscript{50} in relation to the number of functional α\textsubscript{5} nAChR alleles, with vitamin C improving the FEF\textsubscript{50} at each level. This gene environment effect may be mediated by increased smoking, increased smoking intensity or by a direct effect on α\textsubscript{5} nAChR receptor signaling in developing lung. This also identifies a group at particular risk of the effects of smoking during pregnancy on infant lung function and emphasizes the importance of nicotine and nicotinic signaling in mediating the effects of maternal smoking during pregnancy.

Although this study was a randomized placebo-controlled trial, there are several limitations. Our study was powered to detect a 15% vitamin C treatment effect, which
we based upon our previous observation of 15% lower FEFs among healthy infants tested at 11 months of age in which the history of maternal smoking during pregnancy was gathered retrospectively and not quantified.\textsuperscript{27} We were able to demonstrate significant increases of 7-9% in FEFs with vitamin C treatment at three months of age, showing the ability of RVRTC maneuvers to detect differences of this magnitude in infant airway function. Importantly, studies have demonstrated that decreases in FEFs in children of mothers who smoked during pregnancy are in this range and even decreases of 4-6% in FEFs are associated with increased lung disease in the children.\textsuperscript{33} Our study was also analyzed on intention to treat and there were about 10% of women who quit smoking after randomization which may have diluted our results. Some portion of these may reflect the effect of smoking cessation counseling provided during the study.

Our results support the hypothesis that oxidative mechanisms are in part responsible for the effects of in-utero nicotine on lung development. At randomization, both groups of pregnant smokers had levels of fasting ascorbic acid of 49 µmol/L that were decreased compared to levels reported for nonsmoking women (58 µmol/L),\textsuperscript{34} consistent with the increased oxidative load associated with smoking.\textsuperscript{35} Pregnant women randomized to the supplemental vitamin C had significantly increased fasting ascorbic acid levels at 26 and 32 weeks of gestation when compared to baseline and when compared to those randomized to placebo. Importantly, these levels were within the range reported in nonsmokers\textsuperscript{34} and demonstrate the potential impact of nutritional interventions to improve respiratory health.\textsuperscript{36,37}
Decreased pulmonary function early in life is associated with increased respiratory illnesses early in life, and maternal smoking during pregnancy is a major contributor to these adverse respiratory outcomes. While smoking cessation remains the top priority, it is important to recognize the reality that 50% of pregnant smokers will continue to smoke despite multiple interventions. Therefore, it is critical to develop early life strategies, such as prenatal vitamin C supplementation to mitigate the effects of maternal smoking in order to maximize lung growth and development. Cohort studies have found that increases in adverse respiratory outcomes related to maternal smoking during pregnancy track into adulthood and may be related to subsequent onset of COPD. This emphasizes the importance of continuing to follow the respiratory outcomes of the cohort described here. Future trials of vitamin C supplementation in pregnant smokers could investigate whether a greater treatment effect might be achieved by earlier treatment which would cover more of the canalicular phase of lung development or more prolonged vitamin C treatment as well as treatment of the infant postnatally that would cover more of lung alveolarization.

Although our primary outcome of FEF75 at 3 months of age was not improved after vitamin C supplementation to pregnant smokers, the related measures FEF50 and FEF25-75 obtained from the same forced expiratory curve were significantly improved. This extends our previous findings of improved newborn pulmonary function tests in infants of pregnant smokers randomized to vitamin C versus placebo. Our findings also suggest that vitamin C supplementation in pregnant women who cannot quit smoking may be a safe, inexpensive, and simple intervention to improve their offspring’s pulmonary function by
blocking some of the effects of in-utero smoke on lung development. These infants are in continued follow-up to track lung function and respiratory outcomes.
Author disclosures are available with the text of this article at www.atsjournal.org

Acknowledgement: The VCSIP research team deeply thanks the women who participated in our study. The VCSIP team also thanks and acknowledges the members of the Vitamins for Early Lung Health (VITEL) DSMB for their advice, support, and data monitoring during the trial and Dr. Manuel Durand for advice and careful manuscript review.
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Figure Legends

Figure 1. CONSORT Diagram for Randomized Smokers. Enrollment, randomization, and follow-up of randomized smokers and their infants through the 3 month pulmonary function tests (PFTs)/ forced expiratory flows (FEFs).
Table 1. Baseline Maternal Characteristics at Randomization

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<th>Placebo treated smokers (n=126)</th>
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</tr>
<tr>
<td>Single</td>
<td>39 (31.2)</td>
<td>49 (38.9)</td>
</tr>
<tr>
<td>Divorced</td>
<td>8 (6.4)</td>
<td>7 (5.6)</td>
</tr>
<tr>
<td>Significant other</td>
<td>56 (44.8)</td>
<td>39 (31.0)</td>
</tr>
<tr>
<td>Health insurance, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Government assistance</td>
<td>112 (89.6)</td>
<td>106 (84.1)</td>
</tr>
<tr>
<td>Private</td>
<td>13 (10.4)</td>
<td>18 (14.3)</td>
</tr>
<tr>
<td>None or self-pay</td>
<td>0 (0)</td>
<td>2 (1.6)</td>
</tr>
<tr>
<td>Cigarettes/day, median (IQR)</td>
<td>7.0 (4.0-10.0)</td>
<td>7.5 (4.0-10.0)</td>
</tr>
<tr>
<td>Urine cotinine, median (IQR), ng/mL&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5031 (1885-7058)</td>
<td>5409 (1950-8662)</td>
</tr>
<tr>
<td>Randomization plasma ascorbic acid, mean (SD), µmol/L&lt;sup&gt;b&lt;/sup&gt;</td>
<td>48.7 (19.1)</td>
<td>49.3 (21.8)</td>
</tr>
<tr>
<td>Asthma, n (%)</td>
<td>44 (35.2)</td>
<td>38 (30.2)</td>
</tr>
<tr>
<td>History of substance abuse, n (%)</td>
<td>20 (16.0)</td>
<td>16 (12.7)</td>
</tr>
</tbody>
</table>

Abbreviations: SD, standard deviation; IQR, interquartile range; BMI, body mass index
<sup>a</sup> Values for urine cotinine based on 121 vitamin C and 124 placebo treated smokers.
<sup>b</sup> Values for ascorbic acid based on 119 vitamin C and 123 placebo treated smokers.
Table 2. Pregnancy, Delivery, and Infant Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Vitamin C Treated (n=120)</th>
<th>Placebo Treated (n=123)</th>
<th>Mean Difference (95% CI) (Vitamin C - placebo)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma ascorbic acid, mean (SD), µmol/L</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mid gestation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women, n</td>
<td>111</td>
<td>116</td>
<td></td>
</tr>
<tr>
<td><strong>Mean (SD), µmol/L</strong></td>
<td>60.8 (22.6)</td>
<td>41.6 (20.0)*</td>
<td>19.2 (13.7 to 24.8)*</td>
</tr>
<tr>
<td><strong>Late gestation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women, n</td>
<td>107</td>
<td>110</td>
<td></td>
</tr>
<tr>
<td><strong>Mean (SD), µmol/L</strong></td>
<td>54.6 (23.0)</td>
<td>39.6 (17.6)*</td>
<td>15.0 (9.7 to 20.6)</td>
</tr>
<tr>
<td>Preeclampsia/hypertension, n (%)</td>
<td>8 (6.7)</td>
<td>13 (10.6)</td>
<td>3.9 (-10.9 to 3.1)</td>
</tr>
<tr>
<td><strong>Mode of delivery, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal</td>
<td>80 (66.7)</td>
<td>86 (69.9)</td>
<td>3.2 (-14.9 to 8.4)</td>
</tr>
<tr>
<td>Cesarean delivery</td>
<td>40 (33.3)</td>
<td>36 (29.3)</td>
<td></td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>59 (49.2)</td>
<td>61 (49.6)</td>
<td>0.4 (-13.0 to 12.1)</td>
</tr>
<tr>
<td><strong>Gestational age, weeks</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infant, n</td>
<td>120</td>
<td>123</td>
<td></td>
</tr>
<tr>
<td><strong>Mean (SD)</strong></td>
<td>38.7 (1.8)</td>
<td>38.6 (1.7)</td>
<td>0.1 (-0.3 to 0.5)</td>
</tr>
<tr>
<td><strong>Birth weight, g</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infant, n</td>
<td>120</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td><strong>Mean (SD)</strong></td>
<td>3123 (516)</td>
<td>3078 (552)</td>
<td>44.6 (-91.2 to 180.5)</td>
</tr>
<tr>
<td><strong>Birth Length, cm</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infant, n</td>
<td>113</td>
<td>111</td>
<td></td>
</tr>
<tr>
<td><strong>Mean (SD)</strong></td>
<td>49.6 (3)</td>
<td>49.2 (3.2)</td>
<td>0.4 (-0.4 to 1.2)</td>
</tr>
<tr>
<td><strong>Birth head circumference, cm</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infant, n</td>
<td>106</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td><strong>Mean (SD)</strong></td>
<td>33.7 (1.9)</td>
<td>33.3 (2)</td>
<td>0.4 (-0.1 to 0.9)</td>
</tr>
<tr>
<td>Delivered at &lt; 37 weeks, n (%)</td>
<td>14 (11.7)</td>
<td>11 (8.9)</td>
<td>-2.7 (-10.4 to 4.9)</td>
</tr>
<tr>
<td>IUGR, n (%)</td>
<td>2 (1.7)</td>
<td>4 (3.3)</td>
<td>1.6 (-2.3 to 5.5)</td>
</tr>
<tr>
<td><strong>Infant characteristics at 3 month</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infant, n</td>
<td>114</td>
<td>111</td>
<td></td>
</tr>
<tr>
<td>White race, n (%)</td>
<td>89 (78.1)</td>
<td>88 (79.3)</td>
<td>1.2 (-9.5 to 11.9)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>55 (48.2)</td>
<td>54 (48.6)</td>
<td>0.4 (-16.7 to 13.5)</td>
</tr>
<tr>
<td></td>
<td>Mean (SD) 15</td>
<td>Mean (SD) 26</td>
<td>Mean Difference (95% CI)</td>
</tr>
<tr>
<td>------------------------</td>
<td>-------------</td>
<td>-------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>Age, mean (SD), weeks</td>
<td>15.3 (3.2)</td>
<td>15.3 (3.0)</td>
<td>0.0 (-5.6 to 5.8)</td>
</tr>
<tr>
<td>Length, mean (SD), cm</td>
<td>60.2 (3.2)</td>
<td>60.2 (2.9)</td>
<td>0.0 (-0.8 to 0.8)</td>
</tr>
<tr>
<td>Weight, mean (SD), kg</td>
<td>6.4 (1.0)</td>
<td>6.3 (1.0)</td>
<td>0.1 (-0.2 to 0.3)</td>
</tr>
<tr>
<td>Respiratory rate, mean (SD), breaths/minute</td>
<td>40 (7)</td>
<td>40 (7)</td>
<td>0.7 (-1.1 to 2.5)</td>
</tr>
</tbody>
</table>

Abbreviations: SD, standard deviation; IQR, interquartile range; IUGR, intrauterine growth restriction

aBirth weight less than the 10th percentile for gestational age
bMaternal race used as proxy for infant race
cWald confidence interval

*p<0.05, ascorbic acid levels significantly different between groups at 26 and 32 weeks gestation
Table 3A. Pulmonary Function Tests in Infants at Three Months of Age

<table>
<thead>
<tr>
<th></th>
<th>Vitamin C Mean (SD) (n=113)</th>
<th>Placebo Mean (SD) (n=109)</th>
<th>Unadjusted Mean Difference (95% CI) (Vitamin C-placebo)</th>
<th>Unadjusted P value</th>
<th>Adjusted Mean Difference (95% CI) (Vitamin C-placebo)*</th>
<th>Adjusted P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEF&lt;sub&gt;75&lt;/sub&gt; (mL/sec)</td>
<td>200.7 (71.1)</td>
<td>188.7 (66.4)</td>
<td>12.1 (-6.1, 30.3)</td>
<td>0.19</td>
<td>16.2 (-3.3, 35.6)</td>
<td>0.10</td>
</tr>
<tr>
<td>FEF&lt;sub&gt;50&lt;/sub&gt; (mL/sec)</td>
<td>436.7 (101.5)</td>
<td>408.5 (94.0)</td>
<td>28.1 (2.3, 54.0)</td>
<td>0.033</td>
<td>33.7 (6.1, 61.3)</td>
<td>0.02</td>
</tr>
<tr>
<td>FEF&lt;sub&gt;25-75&lt;/sub&gt; (mL/sec)</td>
<td>387.4 (98.1)</td>
<td>365.8 (98.8)</td>
<td>21.6 (-3.8, 47.0)</td>
<td>0.096</td>
<td>28.1 (0.9, 55.3)</td>
<td>0.04</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;0.5&lt;/sub&gt; (mL)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>180.0 (35.4)</td>
<td>173.7 (32.8)</td>
<td>6.3 (-2.8, 15.4)</td>
<td>0.17</td>
<td>8.6 (0.7, 16.4)</td>
<td>0.03</td>
</tr>
<tr>
<td>FVC (mL)</td>
<td>211.2 (43.7)</td>
<td>207.3 (43.3)</td>
<td>3.8 (-7.7, 15.4)</td>
<td>0.51</td>
<td>5.7 (-3.7, 15.1)</td>
<td>0.24</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;0.5&lt;/sub&gt; / FVC&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.80 (0.10)</td>
<td>0.90 (0.10)</td>
<td>0.01 (-0.01, 0.03)</td>
<td>0.18</td>
<td>0.01 (-0.01, 0.03)</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Abbreviations: SD= standard deviation; FEF<sub>75</sub>= forced expiratory flow at 75% of the expired volume; FEF<sub>50</sub>= forced expiratory flow at 50% of the expired volume; FEF<sub>25-75</sub>= forced expiratory flow between 25 and 75% of the expired volume; FEV<sub>0.5</sub>= forced expired volume in the initial 0.5 sec; FVC= forced vital capacity.

<sup>a</sup>Value not reported for one infant

*P values and 95% confidence intervals adjusted for design factors of site, gestational age at randomization and covariates of length, race, and sex.
Table 3B. Summary Table of Z Scores for Pulmonary Function Tests in Randomized Infants

<table>
<thead>
<tr>
<th>PFT Parameter</th>
<th>Vitamin C Mean (SD) (n=113)</th>
<th>Placebo Mean (SD) (n=109)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEF75 (mL/sec)</td>
<td>-0.02 (1.37)</td>
<td>-0.24 (1.33)</td>
</tr>
<tr>
<td>FEF25-75 (mL/sec)</td>
<td>0.29 (1.18)</td>
<td>0.02 (1.29)</td>
</tr>
<tr>
<td>FEV0.5 (mL)</td>
<td>-0.01 (1.07)</td>
<td>-0.24 (1.16)</td>
</tr>
<tr>
<td>FVC (mL)</td>
<td>-0.28 (1.12)</td>
<td>-0.38 (1.29)</td>
</tr>
<tr>
<td>FEV0.5 / FVC</td>
<td>0.28 (0.98)</td>
<td>0.10 (1.13)</td>
</tr>
</tbody>
</table>

Means are unadjusted for other variables. FEF-50 is not included as no z-scores calculation was included for this parameter in Lum et al., 2016.28
Table 4. Forced Expiratory Flows in Infants at Three Months of Age Incorporating α5 nAChR Genotype

<table>
<thead>
<tr>
<th></th>
<th>Vitamin C Mean (SD) (n=112)</th>
<th>Placebo Mean (SD) (n=105)</th>
<th>Adjusted Mean Difference without α5 genotype (95% CI) (Vitamin C-placebo)*</th>
<th>Adjusted P value*</th>
<th>Adjusted Mean Difference with α5 genotype (95% CI) (Vitamin C-placebo)*</th>
<th>Adjusted P value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEF&lt;sub&gt;75&lt;/sub&gt; (mL/sec)</td>
<td>200.7 (70.0)</td>
<td>188.7 (66.3)</td>
<td>12.1 (-6.1, 30.3)</td>
<td>0.12</td>
<td>24.15 (-0.31 to 48.61)</td>
<td>0.053</td>
</tr>
<tr>
<td>FEF&lt;sub&gt;50&lt;/sub&gt; (mL/sec)</td>
<td>438.4 (100.2)</td>
<td>406.4 (94.0)</td>
<td>28.1 (2.3, 54.0)</td>
<td>0.016</td>
<td>47.00 (12.8 to 81.3)</td>
<td>0.007</td>
</tr>
<tr>
<td>FEF&lt;sub&gt;25-75&lt;/sub&gt; (mL/sec)</td>
<td>389.4 (96.3)</td>
<td>363.8 (93.9)</td>
<td>21.6 (-3.8, 47.0)</td>
<td>0.0493</td>
<td>40.00 (6.1 to 73.8)</td>
<td>0.021</td>
</tr>
</tbody>
</table>

Abbreviations: SD= standard deviation; α5 nAChR = alpha 5 nicotinic acetylcholine receptor; FEF<sub>75</sub> = forced expiratory flow at 75% of the expired volume; FEF<sub>50</sub> = forced expiratory flow at 50% of the expired volume; FEF<sub>25-75</sub> = forced expiratory flow between 25 and 75% of the expired volume.

*P values and 95% confidence intervals adjusted for design factors of site, gestational age at randomization and covariates of length, race, and sex

**P values and 95% confidence intervals adjusted for design factors of site, gestational age at randomization and covariates of length, race, sex and α5 nAChR genotype.