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

Cindy T. McEvoy, MD, MCR[1], Lyndsey E. Shorey-Kendrick, PhD[2], Kristin Milner, BA[1], Diane Schilling, RRT[1], Christina Tiller, RRT[3], Brittany Vuylsteke, MPH,[1] Ashley Scherman, RN, PhD[1], Keith Jackson, RRT[4], David M. Haas, MD, MS[5], Julia Harris, BS[1], Robert Schuff, MS[6,7], Byung S.Park, PhD[8], Annette Vu, MPH[6], Dale F. Kraemer, PhD[6], Julie Mitchell, BS[7], Jill Metz, PhD[6], David Gonzales, PhD[9], Carol Bunten, MD[10], Eliot R. Spindel, MD, PhD[2], Robert S. Tepper, MD, PhD[3], Cynthia D. Morris, PhD, MPH[6,7]

Author Affiliations:
[1]Department of Pediatrics, Oregon Health & Science University, Portland, OR, USA
[2]Division of Neuroscience, Oregon National Primate Research Center, Beaverton, OR, USA
[3]Department of Pediatrics, Indiana University School of Medicine, Indianapolis, IN, USA
[4]PeaceHealth Southwest Medical Center, Vancouver, WA, USA
[5]Department of Obstetrics and Gynecology, Indiana University School of Medicine, Indianapolis, IN, USA
[6]Department of Medical Informatics and Clinical Epidemiology, Oregon Health & Science University, Portland, OR, USA
[7]Oregon Clinical & Translational Research Institute, Oregon Health & Science University, Portland, OR, USA
[8]Oregon Health & Science University-Portland State University School of Public Health and Knight Cancer Institute, Portland, OR, USA
[9]Division of Pulmonary & Critical Care Medicine, Oregon Health & Science University, Portland, OR, USA
[10]Vancouver Clinic, Vancouver, WA, USA

Corresponding Author: Cindy T. McEvoy, MD, MCR, Oregon Health & Science University, Department of Pediatrics, 707 SW Gaines Street, CDRC-P, Portland, OR, 97239, USA. E-mail: mcevoyc@ohsu.edu; Phone: 503-494-0085; FAX: 503-494-1542

Author Contributions:

Conception and design: all authors;
Analysis and interpretation: CTM, AV, RS, BSP, DFK, BK, JM, ERS, RST,CDM
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 FEF_{75} at 3 months of age performed with the raised volume rapid thoracic compression technique (Jaeger/Viasys). FEF_{50} and FEF_{25-75} 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 FEF_{75} (200.7 vs 188.7 mL/sec [adjusted 95% CI for difference, -3.33 to 35.64]; p=0.10), FEF_{50} (436.7 vs 408.5 mL/sec [adjusted 95% CI for difference, 6.10 to 61.30]; p=0.02), and FEF_{25-75} (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_{75} was not improved after vitamin C supplementation to pregnant smokers, the predetermined secondary outcomes FEF_{50} and FEF_{25-75} were significantly improved. These results extend our previous findings and demonstrate improved airway function (FEF_{50} and FEF_{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.[1-3] Despite intensive smoking cessation initiatives, more than 50% of smokers who become pregnant continue to smoke.[4,5] This corresponds nationwide to at least 10% of American women continuing to smoke when pregnant [6] with about 450,000 exposed infants per year at an estimated annual health care cost of over 1 billion dollars.[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 (T_{PTEF}:T_E) measured within 72 hours of birth.[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.[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.[11-13] In this model, vitamin C decreased the effects of in-utero nicotine on the offspring's FEFs.[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,[15-18] and decreased FEFs in infancy are correlated with increased risk of respiratory disease.[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.[20]

METHODS

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.[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/m^2.

Study Design and Oversight

Details of the study design were previously published.[21] 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.[22] 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 ($\leq$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_{75}, 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_{50}), as well as between 25 and 75% expired volume (FEF_{25-75}) obtained from the same expiratory curve.

Forced expiratory volumes including forced vital capacity (FVC) and forced expired volume in the initial 0.5 sec ($FEV_{0.5}$) 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),[9] 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.[18, 24] 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.[24] Briefly, the lung was inflated by applying a pressure of 30 cm H_2O 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 H_2O range in jacket pressure until three technically acceptable curves were obtained with FEF_{25-75}, 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.[18, 24]

Biomarkers and Genotyping

Plasma ascorbic acid measurements were performed at the Linus Pauling Institute using high performance liquid chromatography with coulometric electrochemical detection.[25] Urine cotinine levels[26] 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,[9] 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[27] and included allowance for 4% of the patients to be non-compliant (took <50% of their medications) as shown in our initial trial.[8]

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.[28] The statistical analyses of PFTs were based on intention to treat. We analyzed FEF_{75}, FEF_{50}, and FEF_{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, $FEV_{0.5}$, and $FEV_{0.5}$/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_{75}, FEF_{50}, FEF_{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_{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_{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_{50} (436.7 vs 408.5 mL/sec [adjusted 95%CI, 6.10 to 61.30]; p=0.02) and FEF_{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 $FEV_{0.5}$ between the groups but not for FVC or $FEV_{0.5}$/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 $FEF_{50,}$ with infants of mothers who had two copies of the risk allele for the α5 nAChR having the lowest FEF_{50} in both the vitamin C treated and placebo subjects. Interestingly, the α5 nAChR genotype appeared to have a dose response effect on FEF_{50} according to the number of functional alleles present with vitamin C treatment improving FEF_{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 FEF_{75}, but had a significant improvement in FEF_{50} and FEF_{25-75} which are flows obtained from the same expiratory curve. This confirms and expands our initial study[8] 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}:T_E 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 $FEV_{0.5}$ to quantify infant airway function. Among healthy infants without previous wheezing, we found that FEF_{75}, FEF_{50}, and FEF_{25-75}, but not $FEV_{0.5}$, were lower in infants whose mothers reported smoking during pregnancy.[27] Similarly, FEF_{75}, FEF_{50}, and FEF_{25-75}, but not $FEV_{0.5}$ were better at detecting low airway function among infants with a history of respiratory symptoms, but

asymptomatic at the PFT.[32] Therefore, for the current study, we chose FEF_{75} as our primary outcome to assess infant airway function in the randomized groups, however we could have just as easily chosen FEF_{50} and FEF_{25-75} as the primary outcome since they are obtained from the same expiratory curve. The summary statistics of the z scores[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 α5 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.[9] In our initial study,[8] we demonstrated that newborns of mothers who had two copies of the α5 nAChR genotype risk allele had the lowest measurements of T_{PTEF}:T_E and our current study shows a similar decrease in FEF_{50} in relation to the number of functional α5 nAChR alleles, with vitamin C improving the FEF_{50} at each level. This gene environment effect may be mediated by increased smoking, increased smoking intensity or by a direct effect on α5 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.[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.[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),[34] consistent with the increased oxidative load associated with smoking.[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[34] and demonstrate the potential impact of nutritional interventions to improve respiratory health.[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.[38-41] 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.[5] 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.[42] 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.[42,43]

Although our primary outcome of FEF_{75} at 3 months of age was not improved after vitamin C supplementation to pregnant smokers, the related measures FEF_{50} and FEF_{25-75} 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.

Reference List

1. US Department of Health and Human Services. The Health Consequences of Involuntary Exposure to Tobacco Smoke: A Report of the Surgeon General—Executive Summary. Atlanta, GA: Centers for Disease Control and Prevention; 2006.

2. Hayatbakhsh MR, Sadasivam S, Mamun AA, Najman JM, O'callaghan MJ. Maternal smoking during and after pregnancy and lung function in early adulthood: A prospective study. Thorax 2009;64:810-814.

3. Best D. From the American Academy of Pediatrics: Technical report--Secondhand and prenatal tobacco smoke exposure. Pediatrics 2009;124:e1017-e1044.

4. Filion KB, Abenhaim HA, Mottillo S, Joseph L, Gervais A, O'Loughlin J, Paradis G, Pihl R, Pilote L, Rinfret S, Tremblay M, Eisenberg MJ. The effect of smoking cessation counselling in pregnant women: a meta-analysis of randomised controlled trials. BJOG 2011;118:1422-1428.

5. Schneider S, Huy C, Schutz J, Diehl K. Smoking cessation during pregnancy: a systematic literature review. Drug Alcohol Rev 2010;29:81-90.

6. Tong VT, Dietz PM, Morrow B, D'Angelo DV, Farr SL, Rockhill KM, England LJ. Trends in smoking before, during, and after pregnancy--Pregnancy Risk

Assessment Monitoring System, United States, 40 sites, 2000-2010. MMWR Surveill Summ 2013;62:1-19.

7. Stoddard JJ, Gray B. Maternal smoking and medical expenditures for childhood respiratory illness. Am J Public Health 1997;87:205-209.

8. McEvoy CT, Schilling D, Clay N, Jackson K, Go MD, Spitale P, Bunten C, Leiva M, Gonzales D, Hollister-Smith J, Durand M, Frei B, Buist AS, Peters D, Morris CD, Spindel ER. Vitamin C supplementation for pregnant smoking women and pulmonary function in their newborn infants: a randomized clinical trial. JAMA 2014;311:2074-2082.

9. Bierut LJ. Convergence of genetic findings for nicotine dependence and smoking related diseases with chromosome 15q24-25. Trends Pharmacol Sci 2010;31:46-51.

10. Chen LS, Baker T, Hung RJ, Horton A, Culverhouse R, Hartz S, Saccone N, Cheng I, Deng B, Han Y, Hansen HM, Horsman J, Kim C, Rosenberger A, Aben KK, Andrew AS, Chang SC, Saum KU, Dienemann H, Hatsukami DK, Johnson EO, Pande M, Wrensch MR, McLaughlin J, Skaug V, van der Heijden EH, Wampfler J, Wenzlaff A, Woll P, Zienolddiny S, Bickeboller H, Brenner H, Duell EJ, Haugen A, Bruske I, Kiemeney LA, Lazarus P, Le ML, Liu G, Mayordomo J, Risch A, Schwartz AG, Teare MD, Wu X, Wiencke JK, Yang P, Zhang ZF, Spitz MR, Amos CI, Bierut LJ. Genetic Risk Can Be Decreased: Quitting Smoking

Decreases and Delays Lung Cancer for Smokers With High and Low CHRNA5 Risk Genotypes - A Meta-Analysis. EBioMedicine 2016;11:219-226.

11. Sekhon HS, Jia Y, Raab R, Kuryatov A, Pankow JF, Whitsett JA, Lindstrom J, Spindel ER. Prenatal nicotine increases pulmonary alpha7 nicotinic receptor expression and alters fetal lung development in monkeys. J Clin Invest 1999;103:637-647.

12. Sekhon HS, Keller JA, Benowitz NL, Spindel ER. Prenatal nicotine exposure alters pulmonary function in newborn rhesus monkeys. Am J Respir Crit Care Med 2001;164:989-994.

13. Sekhon HS, Keller JA, Proskocil BJ, Martin EL, Spindel ER. Maternal nicotine exposure upregulates collagen gene expression in fetal monkey lung. Association with alpha7 nicotinic acetylcholine receptors. Am J Respir Cell Mol Biol 2002;26:31-41.

14. Proskocil BJ, Sekhon HS, Clark JA, Lupo SL, Jia Y, Hull WM, Whitsett JA, Starcher BC, Spindel ER. Vitamin C Prevents the Effects of Prenatal Nicotine on Pulmonary Function in Newborn Monkeys. Am J Respir Crit Care Med 2005;171:1032-1039.

15. Tager IB, Hanrahan JP, Tosteson TD, Castile RG, Brown RW, Weiss ST, Speizer FE. Lung function, pre- and post-natal smoke exposure, and wheezing in the first year of life. Am Rev Respir Dis 1993;147:811-817.

16. Martinez FD, Morgan WJ, Wright AL, Holberg CJ, Taussig LM. Diminished lung function as a predisposing factor for wheezing respiratory illness in infants. N Engl J Med 1988;319:1112-1117.

17. Dezateux C, Stocks J, Dundas I, Fletcher ME. Impaired Airway Function and Wheezing in Infancy. The influence of maternal smoking and a genetic predisposition to asthma. Am J Respir Crit Care Med 1999;159:403-410.

18. Jones MH, Davis SD, Grant D, Christoph K, Kisling J, Tepper RS. Forced expiratory maneuvers in very young children. Assessment of flow limitation. Am J Respir Crit Care Med 1999;159:791-795.

19. Martinez FD. The origins of asthma and chronic obstructive pulmonary disease in early life. Proc Am Thorac Soc 2009;6:272-277.

20. McEvoy CT, Milner KF, Schiling DG, Scherman A, TIller A, Buylsteke B, Jackson K, Haas D, Bunten C, Harris J, Vu A, Schuff R, Kraemer D, Mitchell J, Metz J, Shorey-Kendrick L, Spindel ER, Tepper RS, Morris CD. Improved forced expiratory flows in infants of pregnant smokers randomized to daily vitamin C versus placebo. Am J Respir Crit Care Med 2018:A4192.

21. McEvoy CT, Milner KF, Scherman AJ, Schilling DG, Tiller CJ, Vuylsteke B, Shorey-Kendrick LE, Spindel ER, Schuff R, Mitchell J, Peters D, Metz J, Haas D, Jackson K, Tepper RS, Morris CD. Vitamin C to decrease the effects of smoking in pregnancy on infant lung function (VCSIP): Rationale, design, and methods of a randomized, controlled trial of vitamin C supplementation in pregnancy for the

primary prevention of effects of in utero tobacco smoke exposure on infant lung function and respiratory health. Contemp Clin Trials 2017;58:66-77.

22. Fiore MC, Jaen CR, Baker TB et al. Treating Tobacco Use and Dependence: 2008 Update. Clinical Practice Guideline. Rockville, MD: U.S. Department of Health and Human Services. Public Health Service. May 2008.

23. Ferris BG. Epidemiology Standardization Project (American Thoracic Society). Am Rev Respir Dis 1978;118:1-120.

24. ATS/ERS statement: raised volume forced expirations in infants: guidelines for current practice. Am J Respir Crit Care Med 2005;172:1463-1471.

25. Frei B. Efficacy of dietary antioxidants to prevent oxidative damage and inhibit chronic disease. J Nutr 2004;134:3196S-3198S.

26. Benowitz NL, Hukkanen J, Jacob P, III. Nicotine chemistry, metabolism, kinetics and biomarkers. Handb Exp Pharmacol 2009:29-60.

27. Jones M, Castile R, Davis S, Kisling J, Filbrun D, Flucke R, Goldstein A, Emsley C, Ambrosius W, Tepper RS. Forced expiratory flows and volumes in infants. Normative data and lung growth. Am J Respir Crit Care Med 2000;161:353-359.

28. Lum S, Bountziouka V, Wade A, Ah-Fong H, Kirby J, Moreno-Galdo A, deMir I, Sardon-Prado O, Corcuera-Elosegui P, Mattes, J, Borrego LM, Davies G, Stocks J. New reference ranges for interpreting forced expiratory manoeuvers in infants and

implications for clinical interpretation: a multicenter collaboration. Thorax 2016; 71: 276-283.

29. Hanrahan JP, Tager IB, Segal MR, Tosteson TD, Castile RG, Van Vunakis H, Weiss ST, Speizer FE. The effect of maternal smoking during pregnancy on early infant lung function. Am Rev Respir Dis 1992;145:1129-1135.

30. Hoo AF, Henschen M, Dezateux C, Costeloe K, Stocks J. Respiratory function among preterm infants whose mothers smoked during pregnancy. Am J Respir Crit Care Med 1998;158:700-705.

31. Tager IB, Weiss ST, Munoz A, Rosner B, Speizer FE. Longitudinal study of the effects of maternal smoking on pulmonary function in children. N Engl J Med 1983;309:699-703.

32. Jones MH, Howard J, Davis S, Kisling J, Tepper RS. Sensitivity of spirometric measurements to detect airway obstruction in infants. Am J Respir Crit Care Med 2003; 167:1283-1286.

33. Moshammer H, Hoek G, Luttmann-Gibson H, Neuberger MA, Antova T, Gehring U, Hruba F, Pattenden S, Rudnai P, Slachtova H, Zlotkowska R, Fletcher T. Parental Smoking and Lung Function in Children: an International Study. Am J Respir Crit Care Med 2006; 173: 1255-1263.

34. Schleicher RL, Carroll MD, Ford ES, Lacher DA. Serum vitamin C and the prevalence of vitamin C deficiency in the United States: 2003-2004 National

Health and Nutrition Examination Survey (NHANES). Am J Clin Nutr 2009;90:1252-1263.

35. Kirkham PA, Barnes PJ. Oxidative stress in COPD. Chest 2013;144:266-273.

36. Litonjua AA, Carey VJ, Laranjo N, Harshfield BJ, O'Connor GT, Sandel M, Strunk RC, Bacharier LB, Zeiger RS, Schatz M, Hollis BW, Weiss ST. Effect of prenatal supplementation with vitamin D on asthma or recurrent wheezing in offspring by age 3 years: the VDAART Randomized Clinical Trial. JAMA 2016;315:362-370.

37. Manuck TA, Levy PT, Gyamfi-Bannerman C, Jobe AH, Blaisdell CJ. Prenatal and perinatal determinants of lung health and disease in early life: A National Heart, Lung, and Blood Institute Workshop Report. JAMA Pediatr. 2016; 170: e154577.

38. Martinez FJ, Han MK, Allinson JP, Barr RG, Boucher RC, Calverley PMA, Celli BR, Christenson SA, Crystal RG, Fageras M, Freeman CM, Groenke L, Hoffman EA, Kesimer M, Kostikas K, Paine R III, Rafii S, Rennard SI, Segal LN, Shaykhiev R. Stevenson C, Tal-Singer R, Vestbo J, Woodruff PG, Curtis JL, Wedzicha JA. At the Root: Defining and Halting Progression of Early Chronic Obstructive Pulmonary Disease. Am.J.Respir.Crit Care Med, 2018;197:1540-1551.

39. Taylor B, Wadsworth J. Maternal smoking during pregnancy and lower respiratory tract illness in early life. Archives of Disease in Childhood 1987;62:786-791.

40. Burke H, Leonardi-Bee J, Hashim A, Pine-Abata H, Chen Y, Cook DG, Britton JR, McKeever TM. Prenatal and passive smoke exposure and incidence of asthma and wheeze: systematic review and meta-analysis. Pediatrics 2012;129:735-744.

41. Neuman A, Hohmann C, Orsini N, Pershagen G, Eller E, Kjaer HF, Gehring U, Granell R, Henderson J, Heinrich J, Lau S, Nieuwenhuijsen M, Sunyer J, Tischer C, Torrent M, Wahn U, Wijga AH, Wickman M, Keil T, Bergstrom A. Maternal smoking in pregnancy and asthma in preschool children: a pooled analysis of eight birth cohorts. Am J Respir Crit Care Med 2012;186:1037-1043.

42. Stocks J, Hislop A, Sonnappa S. Early lung development: lifelong effect on respiratory health and disease. Lancet Respir Med 2013;1:728-742.

43. Ten Have-Opbroek AA. The development of the lungin mammals: an analysis of concepts and findings. American Journal of Anatomy 1981; 162: 201-219

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

	Vitamin C treated smokers (n=125)	Placebo treated smokers (n=126)
Age, mean (SD), years	26.6 (5.2)	26.4 (5.9)
Caucasian, n (%)	97 (77.6)	100 (79.4)
Gravida, median (IQR)	3.0 (2.0-4.0)	3.0 (2.0-5.0)
Gestational age, mean (SD), weeks	18.4 (3.0)	18.2 (2.8)
BMI, mean (SD), kg/m^2	28.6 (6.5)	30.0 (7.3)
Education status, n (%)		
<High school	20 (16.0)	31 (24.6)
High school or equivalent	59 (47.2)	40 (31.7)
Some college	43 (34.4)	49 (38.9)
Bachelor's degree	3 (2.4)	6 (4.8)
Marital status, n (%)		
Married	22 (17.6)	31 (24.6)
Single	39 (31.2)	49 (38.9)
Divorced	8 (6.4)	7 (5.6)
Significant other	56 (44.8)	39 (31.0)
Health insurance, n (%)		
Government assistance	112 (89.6)	106 (84.1)
Private	13 (10.4)	18 (14.3)
None or self-pay	0 (0)	2 (1.6)
Cigarettes/day, median (IQR)	7.0 (4.0-10.0)	7.5 (4.0-10.0)
Urine cotinine, median (IQR), ng/mL[a]	5031 (1885-7058)	5409 (1950-8662)
Randomization plasma ascorbic acid, mean (SD), µmol/L[b]	48.7 (19.1)	49.3 (21.8)
Asthma, n (%)	44 (35.2)	38 (30.2)
History of substance abuse, n (%)	20 (16.0)	16 (12.7)

Abbreviations: SD, standard deviation; IQR, interquartile range; BMI, body mass index
[a]Values for urine cotinine based on 121 vitamin C and 124 placebo treated smokers.
[b]Values for ascorbic acid based on 119 vitamin C and 123 placebo treated smokers.

Table 2. Pregnancy, Delivery, and Infant Characteristics

	Vitamin C Treated (n=120)	Placebo Treated (n=123)	Mean Difference (95% CI) (Vitamin C- placebo)
Plasma ascorbic acid, mean (SD), µmol/L			
Mid gestation			
Women, n	111	116	
Mean (SD), µmol/L	**60.8 (22.6)**	**41.6 (20.0)***	**19.2 (13.7 to 24.8)***
Late gestation			
Women, n	107	110	
Mean (SD), µmol/L	**54.6 (23.0)**	**39.6 (17.6)***	**15.0 (9.7 to 20.6)**
Preeclampsia/hypertension, n (%)[c]	8 (6.7)	13 (10.6)	3.9 (-10.9 to 3.1)
Mode of delivery, n (%)			
Vaginal	80 (66.7)	86 (69.9)	3.2 (-14.9 to 8.4)
Cesarean delivery	40 (33.3)	36 (29.3)	
Female, n (%)[c]	59 (49.2)	61 (49.6)	0.4 (-13.0 to 12.1)
Gestational age, weeks			
Infant, n	120	123	
Mean (SD)	38.7 (1.8)	38.6 (1.7)	0.1 (-0.3 to 0.5)
Birth weight, g			
Infant, n	120	120	
Mean (SD)	3123 (516)	3078 (552)	44.6 (-91.2 to 180.5)
Birth Length, cm			
Infant, n	113	111	
Mean (SD)	49.6 (3)	49.2 (3.2)	0.4 (-0.4 to 1.2)
Birth head circumference, cm			
Infant, n	106	104	
Mean (SD)	33.7 (1.9)	33.3 (2)	0.4 (-0.1 to 0.9)
Delivered at < 37 weeks, n (%)[c]	14 (11.7)	11 (8.9)	-2.7 (-10.4 to 4.9)
IUGR, n (%)[a, c]	2 (1.7)	4 (3.3)	1.6 (-2.3 to 5.5)
Infant characteristics at 3 month PFT			
Infant, n	**114**	**111**	
White race, n (%)[b, c]	89 (78.1)	88 (79.3)	1.2 (-9.5 to 11.9)
Female, n (%)[c]	55 (48.2)	54 (48.6)	0.4 (-16.7 to 13.5)

Age, mean (SD), weeks	15.3 (3.2)	15.3 (3.0)	0.0 (-5.6 to 5.8)
Length, mean (SD), cm	60.2 (3.2)	60.2 (2.9)	0.0 (-0.8 to 0.8)
Weight , mean (SD), kg	6.4 (1.0)	6.3 (1.0)	0.1 (-0.2 to 0.3)
Respiratory rate, mean (SD), breaths/minute	40 (7)	40 (7)	0.7 (-1.1 to 2.5)

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

[a]Birth weight less than the 10th percentile for gestational age

[b]Maternal race used as proxy for infant race

[c]Wald 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

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

Abbreviations: SD= standard deviation; FEF_{75}= forced expiratory flow at 75% of the expired volume; FEF_{50}= forced expiratory flow at 50% of the expired volume; FEF_{25-75} = forced expiratory flow between 25 and 75% of the expired volume; $FEV_{0.5}$ = forced expired volume in the initial 0.5 sec; FVC= forced vital capacity.

[a]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

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

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

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

Abbreviations: SD= standard deviation; α5 nAChR = alpha 5 nicotinic acetylcholine receptor; FEF_{75}= forced expiratory flow at 75% of the expired volume; FEF_{50}= forced expiratory flow at 50% of the expired volume; FEF_{25-75} = 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.