Original Investigation

Vitamin C Supplementation for Pregnant Smoking Women and Pulmonary Function in Their Newborn Infants
A Randomized Clinical Trial

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IMPORTANCE Maternal smoking during pregnancy adversely affects offspring lung development, with lifelong decreases in pulmonary function and increased asthma risk. In a primate model, vitamin C blocked some of the in-utero effects of nicotine on lung development and offspring pulmonary function.

OBJECTIVE To determine if newborns of pregnant smokers randomized to receive daily vitamin C would have improved results of pulmonary function tests (PFTs) and decreased wheezing compared with those randomized to placebo.

DESIGN, SETTING, AND PARTICIPANTS Randomized, double-blind trial conducted in 3 sites in the Pacific Northwest between March 2007 and January 2011. One hundred fifty-nine newborns of randomized pregnant smokers (76 vitamin C treated and 83 placebo treated) and 76 newborns of pregnant nonsmokers were studied with newborn PFTs. Follow-up assessment including wheezing was assessed through age 1 year, and PFTs were performed at age 1 year.

INTERVENTIONS Pregnant women were randomized to receive vitamin C (500 mg/d) (n = 89) or placebo (n = 90).

MAIN OUTCOMES AND MEASURES The primary outcome was measurement of newborn pulmonary function (ratio of the time to peak tidal expiratory flow to expiratory time [TPTEF:TE] and passive respiratory compliance per kilogram [Crs/kg]) within 72 hours of age. Secondary outcomes included incidence of wheezing through age 1 year and PFT results at age 1 year. A subgroup of pregnant smokers and nonsmokers had genotyping performed.

RESULTS Newborns of women randomized to vitamin C (n = 76), compared with those randomized to placebo (n = 83), had improved pulmonary function as measured by TPTEF:TE (0.383 vs 0.345 [adjusted 95% CI for difference, 0.011-0.062]; P = .006) and Crs/kg (1.32 vs 1.20 mL/cm H_2O/kg [95% CI, 0.02-0.20]; P = .01). Offspring of women randomized to vitamin C had significantly decreased wheezing through age 1 year (15/70 [21%] vs 31/77 [40%]; relative risk, 0.56 [95% CI, 0.33-0.95]; P = .03). There were no significant differences in the 1-year PFT results between the vitamin C and placebo groups. The effect of maternal smoking on newborn lung function was associated with maternal genotype for the α5 nicotinic receptor (rs16969968) (P < .001 for interaction).

CONCLUSIONS AND RELEVANCE Supplemental vitamin C taken by pregnant smokers improved newborn PFT results and decreased wheezing through 1 year in the offspring. Vitamin C in pregnant smokers may be an inexpensive and simple approach to decrease the effects of smoking in pregnancy on newborn pulmonary function and respiratory morbidities.

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More than 50% of smokers who become pregnant continue to smoke,[1,2] corresponding to 12% of all pregnancies.[3] Smoking during pregnancy adversely affects lung development, with lifelong decreases in pulmonary function.[4,5] At birth, newborn infants born to smokers show decreased pulmonary function test (PFT) results, with decreased respiratory flows and respiratory compliance and altered tidal breathing patterns.[6-8] These changes lead to increased wheezing, hospitalization for respiratory infections, and increased incidence of childhood asthma.[9]

The ideal solution is smoking cessation. However, smoking is addictive and heavily advertised, and certain genetic polymorphisms significantly increase risk of nicotine dependence.[10,11] Teen pregnancy, low income, low education, and cohabitation with smokers are associated with smoking during pregnancy. Clinical trials of smoking cessation during pregnancy have proven ineffective.[1] Given the addictive nature of nicotine, the socioeconomics of smokers, and tobacco company advertising, smoking during pregnancy will likely continue to adversely affect millions of infants worldwide. These realities make finding ways to lessen the effects of smoking during pregnancy of vital importance.

In nonhuman primates, Spindel et al[12-14] demonstrated that nicotine crosses the placenta, then up-regulates nicotinic receptors, leading to altered lung development and PFT results at birth.[13] They also demonstrated that vitamin C decreased the effects of in-utero nicotine on primate PFTs.[15] These data provided the foundation for this randomized trial, designed to evaluate the ability of vitamin C supplementation (500 mg/d) vs placebo during pregnancy to decrease the effects of maternal smoking on newborn PFT results. The incidence of wheezing through age 1 year and infant PFT results at age 1 year were also evaluated. A group of infants born to pregnant nonsmokers undergoing prospective follow-up was also studied as a reference group for the newborn PFTs and respiratory outcomes, including wheezing through age 1 year.

Methods

Study Design and Oversight

We conducted a randomized, double-blind, placebo-controlled study of vitamin C (500 mg/d) vs placebo in pregnant smokers. A group of pregnant nonsmokers was prospectively studied as a reference group. The protocol was approved by 3 institutional review boards, and each pregnant woman provided written informed consent. The independent data and safety monitoring board met every 6 months.

Study Participants

We recruited women from 3 clinical sites in the Portland, Oregon, and Vancouver, Washington, areas who were 15 years or older, current smokers (≥1 cigarette/d), singleton gestation, randomized at 22 weeks' or less gestational age by last menstrual period, and who had declined smoking cessation. Exclusion criteria were multiple gestation, major fetal anomalies, current illicit drug or alcohol abuse, continuous daily high-dose vitamin C since last menstrual period, insulin-dependent diabetes, and history of kidney stones. After providing consent, participants entered a placebo adherence period during which they were asked to take 1 placebo capsule daily for 1 to 2 weeks. Participants were randomized if they returned for follow-up and took 75% or more of the required placebo.

A group of pregnant nonsmokers 15 years or older with a singleton gestation of 22 weeks' or less gestational age was recruited as our reference group.

Study Medications

The Oregon Health & Science University (OHSU) research pharmacy prepared the study medication consisting of crushed vitamin C (500 mg) (ascorbic acid, Rugby Laboratories Inc) or ground cornstarch (placebo) in gel capsules. Participants were instructed to take 1 study capsule daily until delivery (told to swallow them whole to minimize detecting any difference in organoleptic properties) and were supplied a standard prenatal vitamin (Prenavite, Rugby Laboratories Inc) containing 60 mg of vitamin C. The nonsmokers were not randomized to study medication but were supplied the same standard prenatal vitamin.

Randomization

Randomization was stratified according to gestational age at randomization (≤16 and >16 weeks) and clinical site. The OHSU research pharmacy dispensed study capsules. The investigators, clinicians, and patients were unaware of treatment allocation through age 1 year and analyses of all primary and secondary outcomes.

Interval Visits

Assessment of Medication Adherence

Women were asked to return study medication at each visit and after delivery for pill count. Medication adherence was calculated by dividing the number of capsules taken by the total number prescribed in a given period. Brief smoking cessation counseling (≤10 minutes) based on American College of Obstetricians and Gynecologists guidelines was provided at consent, randomization, and each visit.[16] A handout outlining the benefits of smoking cessation was distributed prior to consent. Fasting plasma ascorbic acid levels were collected at 28 to 30 weeks' gestational age.

Interval Prenatal Visits and Delivery

Study visits occurred with prenatal visits, which were approximately every 4 weeks from randomization until 34 weeks' gestation and then every 2 weeks until delivery. A detailed smoking history using a modified validated respiratory questionnaire[17] was collected at each visit. Three urine samples for cotinine levels were collected at 24, 30, and 34 weeks' gestation. Maternal blood was drawn for genetic analysis. Interim medical events were assessed. At delivery, research staff obtained maternal and neonatal outcome data.

End Points

Primary Outcome

The primary outcome was the measurement of newborn PFTs (within 72 hours of age), specifically the measurement of the ratio of time to peak tidal expiratory flow to expiratory time (TPTEF:TE) and passive respiratory system compliance per kilogram (Crs/kg). We also measured PFTs in newborns born to the nonsmokers as a reference group.

Secondary Outcomes

Secondary outcomes included the clinical respiratory outcomes of the infants through 1 year, including the occurrence of wheezing and use of medication for wheezing.[17] Pulmonary function tests were also performed in the infants born to the randomized smokers at age 1 year.

The incidence of wheezing was also determined in infants born to the nonsmokers.

Measurements

Newborn Pulmonary Function Tests

Newborns were studied in quiet sleep, supine position, and breathing through a face mask using a standardized operating procedure and standardized acceptance criteria.[18-21]

No sedation was used. Measurements were made at all sites with an infant PFT cart (Master Screen BabyBody, Jaeger/Viasys); the order of testing was tidal breathing flow-volume loops and passive respiratory system mechanics. Additional equipment (SensorMedics 2600, SensorMedics Inc) was only available at OHSU and was used to measure functional residual capacity after the prior measurements were completed. Two trained experienced respiratory therapists performed all testing, which was reviewed by the principal investigator. A minimum of 50 flow-volume loops with inspiratory and expiratory volumes within 15% were collected to calculate tidal volumes and TPTEF:TE ratios.[18,21,22]

Passive respiratory system compliance was obtained with the single-breath occlusion technique and the mean calculated from at least 10 acceptable breaths.[19,21] Passive respiratory compliance was normalized for body weight. Acceptance criteria included stable end-expiratory baseline; plateau pressure lasting more than 100 ms and varying by 0.125 cm H_2O or less; acceptable linear flow-volume curve by visual inspection; and 10 or more breaths accepted with a coefficient of variation less than 20%.[23,24]

Functional residual capacity (FRC) was measured by the nitrogen washout technique using 100% oxygen as the washout gas as previously described and applying established acceptance criteria.[21]

Wheezing

Clinical research personnel unaware of treatment assignment administered the respiratory questionnaire[17] (pediatric version) to the infant's primary caretaker when the infant was approximately 12 months of age. Presence or absence of wheezing, medication for wheezing, maternal smoking, and exposure to second-hand smoke were assessed.

Pulmonary Function Tests at Age 1 Year

At age 1 year the infants of randomized smokers were sedated with chloral hydrate (50 mg/kg) given orally and the same PFTs as described above were performed. Infants of nonsmokers were not studied at age 1 year because of the lack of institutional review board approval to administer sedation to these infants.

Biomarkers and Genotyping

Urine cotinine levels were obtained using a chemiluminescence immunoassay on an Immulite autoanalyzer (Siemens Health Care Diagnostics).[25] Plasma ascorbic acid measurements were performed at the Linus Pauling Institute using high-performance liquid chromatography with coulometric electrochemical detection.[26]

Mothers were genotyped for 2 single-nucleotide polymorphisms (SNPs) in the α3, α5, β4 nicotinic acetylcholine receptor (nAChR) gene locus and for 3 glutathione transferase polymorphisms. This included rs16969968, which is the α5 nAChR structural polymorphism that has the strongest link to lung disease,[27] and rs588765, which is associated with increased levels of the α5 nAChR.[27] Blood was stored in EDTA tubes at −20°C. Glutathione S-transferase mu 1 (GSTM1) and T1 null allele status were genotyped by reverse-transcriptase polymerase chain reaction; the α5 nAChR SNPs and the GSTP1 SNP, rs1695, were genotyped by real-time polymerase chain reaction using predesigned reagents from Applied Biosystems.

Statistical Analysis and Sample Size

Our targeted sample size of 76 participants at delivery per treatment group was determined to detect with 80% power, at a significance level of .05, increases of 20% in mean TPTEF:TE and 25% in mean Crs in the vitamin C group compared with placebo. These calculations included allowance for 10% nonadherence to vitamin C supplementation (and 10% smoking cessation in the placebo group) and for 15% cohort loss between randomization and delivery (including PFTs not meeting acceptance criteria).

The statistical analyses were based on intention to treat. For comparison of newborn PFT outcomes between the randomized groups, linear regression analyses were used, adjusting for the stratification variable gestational age at randomization (≤16 vs >16 weeks) as well as birth weight and gestational age (as established prenatally via last menstrual period) younger than 37 weeks, known important determinants of newborn pulmonary function.[22]

For comparison of wheezing and wheezing medications between treatment groups at age 1 year, binomial regression analyses with log link were used, adjusting for gestational age at randomization, birth weight, and gestational age younger than 37 weeks. Because wheezing within the first year of life is not uncommon, we used the log link in the binomial regression analyses rather than logistic regression to obtain relative risk estimates.

For comparison of 1-year PFT outcomes between randomized groups, linear regression analyses were used, adjusting for the gestational age at randomization and postnatal age and length at PFT, known important determinants of infant PFTs.[22]

Figure 1. CONSORT Diagram for Randomized Smokers
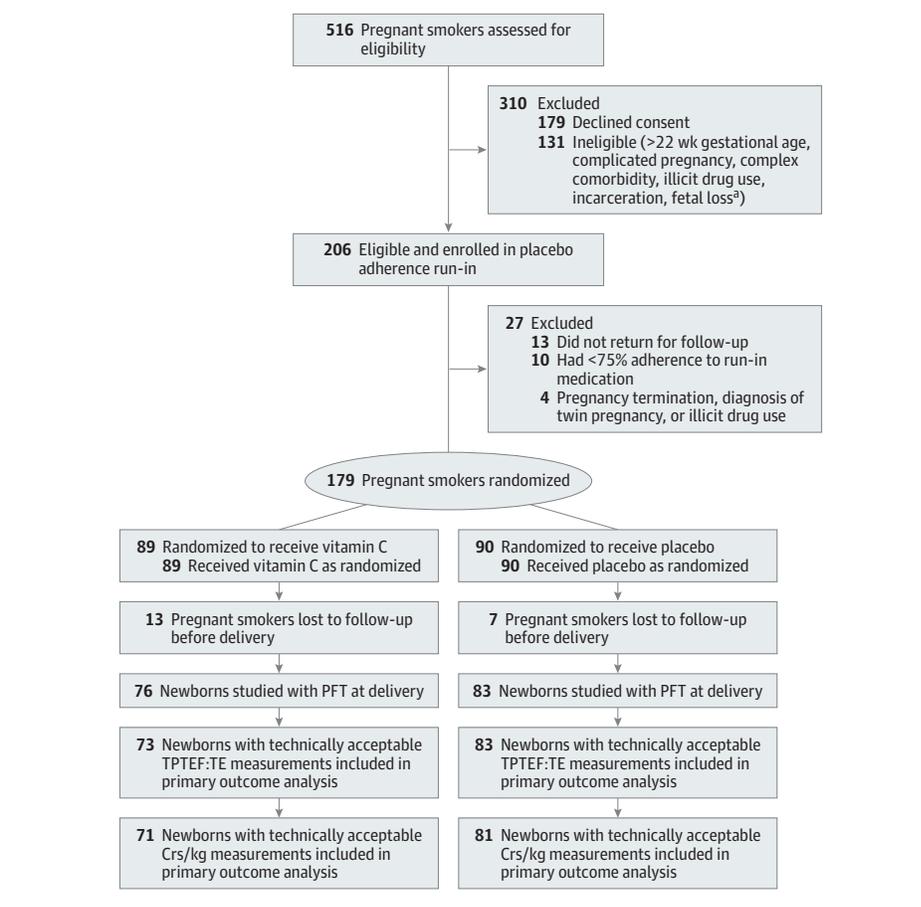
Enrollment, randomization, and follow-up of randomized smokers and their offspring through the newborn pulmonary function tests (PFTs).

[a] One hundred thirty-one participants were recorded as ineligible, but the specific frequency for each listed reason is unknown. Not shown is a reference group of 76 nonsmokers who underwent prospective follow-up in pregnancy similar to randomized smokers. Their offspring were studied with newborn PFTs. A few newborns did not have a successful measurement of either the ratio of time to peak tidal expiratory flow to expiratory time (TPTEF:TE) or passive respiratory compliance per kilogram (Crs/kg) because of inability to meet the testing criteria as outlined by the American Thoracic Society and European Respiratory Society (see Methods for details of testing acceptance criteria). Exact numbers of successful measurements for TPTEF:TE and Crs/kg are noted in the final boxes for each treatment group.

Comparisons of additional outcomes used the same regression methods with transformations applied as needed to meet distributional assumptions.

For secondary analyses that included genotype as a predictor, we also included an interaction between genotype and randomization group and allowed for different standard deviations within each genotype group. This interaction allowed us to assess whether the effect of vitamin C differed based on genotype.

We imputed missing newborn PFT and wheeze data in patients lost to follow-up using multiple imputation methods. The imputation models were based on either linear or logistic regression using the 2 primary predictors of treatment group and gestational age, as well as the baseline demographic variables. We also incorporated mid-gestation ascorbic acid levels and medication adherence measures in secondary analyses. We present summary information without formal comparison for the PFT and clinical respiratory data for the infants of the nonsmokers, because they were not part of the randomized study.

All *P* values are 2-sided, and significance was set at $P < .05$. Statistical analyses were conducted using SAS 9.3 (SAS Institute Inc) and Stata 11.2 (StataCorp). An independent data and safety monitoring board monitored the trial and reviewed in-

terim results. There was an interim analysis of the primary outcome when 33% of the total sample size had newborn PFTs performed.

Results

Participants

Participants were recruited between March 2007 and January 2011; the last infant was born in July 2011. Five hundred sixteen pregnant smokers were screened, with 310 excluded before consent; 27 were excluded after the placebo adherence run-in period (**Figure 1**). The remaining 179 women were randomized to vitamin C supplementation (500 mg/d) or placebo. Thirteen women in the vitamin C group and 7 in the placebo group (11% overall) were lost to follow-up, resulting in 76 newborns of vitamin C–treated smokers and 83 newborns of placebo-treated smokers having PFTs at delivery. Seventy-six pregnant nonsmokers received prospective follow-up similar to that received by the randomized smokers, and their newborn infants had PFTs at delivery, serving as a reference group. Respiratory histories through 1 year (secondary outcome) were obtained for 147 infants (vitamin C, 70; placebo, 77) of the 159 infants born to the randomized smokers who had PFT testing

at delivery, for a 92% follow-up rate (92.1% in the vitamin C group and 92.8% in the placebo group). Seventy of 76 infants of nonsmokers were followed up through 1 year. Pulmonary function tests were performed at age 1 year (secondary outcome) for 106 infants (vitamin C, 49; placebo, 57) of the 159 infants who had newborn PFTs, for a 67% follow-up (64.5% in the vitamin C group and 68.7% in the placebo group). However, respiratory compliance at 1 year was obtained in 49 vs 53 patients, respectively, for a 64% follow-up rate.

Baseline maternal characteristics of randomized smokers are shown in **Table 1**. At study entry, 41% of women in the vitamin C group and 36% in the placebo group reported smoking 10 cigarettes/d or more. There was no significant difference in urine cotinine levels.

The average medication adherence was 81% in the vitamin C group and 82% in the placebo group (**Table 2**). The vitamin C–treated smokers had a higher average plasma ascorbic acid level than the placebo-treated smokers (58.9 vs 39.8 μmol/L [95% CI, 11.23-26.95]; $P < .001$), which was comparable to the level of 57.8 μmol/L measured in nonsmokers. There was no significant difference in the demographic characteristics between the infants delivered to randomized smokers at a median gestational age of 39 weeks (Table 2) and having comparable medical treatment.

Primary Outcome (TPTEF:TE Ratio and Crs/kg)
The newborn infants born to the smokers randomized to receive vitamin C demonstrated a significantly increased TPTEF:TE ratio (0.383 vs 0.345 [adjusted 95% CI for difference, 0.011-0.062]; $P = .006$) and a significantly increased Crs/kg (1.32 vs 1.20 mL/cm H_2O/kg [95% CI, 0.02-0.20]; $P = .01$) and total Crs compared with those randomized to placebo (**Table 3**). The newborns randomized to vitamin C had TPTEF:TE ratios and Crs values approaching the levels measured in the infants delivered to nonsmokers (studied as a reference group). Approximately 50% of patients had FRC measurements performed, and there was no significant difference in FRC between the newborn infants delivered to randomized smokers. The average respiratory rate (at PFT) of newborns delivered to the randomized smokers was 57 breaths per minute in both groups.

Secondary Outcomes
Clinical respiratory follow-up was obtained for 92% of the offspring (Table 3). The incidence of wheezing through 1 year in infants delivered to the smokers randomized to vitamin C vs placebo was 15/70 (21%) vs 31/77 (40%) (adjusted relative risk, 0.56 [95% CI, 0.33-0.95]; $P = .03$). The incidence of wheezing in the reference group of infants of nonsmokers was 19/70 (27%) (Table 3). Fewer patients in the vitamin C group required medications for wheezing (94% of treated patients received inhaled albuterol, 6% received inhaled albuterol and corticosteroids) in the first year of life compared with those randomized

Table 1. Baseline Maternal Characteristics

Characteristic	Treated Smokers, No. (%)[a]	
	Vitamin C (n = 76)	Placebo (n = 83)
Maternal age, mean (SD), y	26.6 (6.2)	25.5 (5.5)
White race	58 (76)	66 (80)
Private insurance	10 (13)	10 (12)
Parity ≥2	21 (28)	15 (18)
Some college education	16 (21)	17 (20)
Maternal asthma	19 (25)	18 (22)
History of substance abuse	13 (17)	16 (19)
Randomization ≤16 wk	18 (24)	18 (22)
≥10 cigarettes/d	31 (41)	30 (36)
Urine cotinine level, median (IQR), ng/mL[b]	4715 (1560-7209)	3543 (2202-5914)

Abbreviation: IQR, interquartile range.

[a] $P > .05$ for all between-group comparisons.

[b] Based on 1 to 3 occasions for each participant (64 placebo treated and 60 vitamin C treated).

Table 2. Pregnancy and Infant Characteristics of Randomized Patients

Characteristic	Treated Smokers, No./Total (%)		
	Vitamin C (n = 76)	Placebo (n = 83)	P Value
Plasma ascorbic acid at 28-30 wk, mean (SD), μmol/L[a]	58.9 (26.8)	39.8 (20.5)	<.001
Medication adherence (% medication capsules taken), mean (SD)	81 (17)	82 (18)	.67
Preeclampsia	3/75 (3.9)	2/83 (2.4)	.58
Vaginal delivery	52/76 (68)	60/83 (72)	.62
Female	35/76 (46)	43/83 (52)	.41
Gestational age, median (range), wk	39.0 (29.6-41.3)	39.1 (31.2-42.0)	.27[b]
Delivered			
<37 wk	10/76 (13)	6/83 (7)	.23
<32 wk	1/76 (1.3)	1/83 (1.2)	.97
Birth weight, mean (SD) [median], g	3163 (694) [3279]	3311 (475) [3350]	.12
Birth weight z score, mean (SD)	−0.26 (1.10)	−0.12 (0.80)	.35
Intrauterine growth restriction[c]	10/76 (13.2)	7/83 (8.4)	.34
Length, mean (SD), cm	49.8 (3.6)	50.3 (2.3)	.35
Head circumference, mean (SD), cm[d]	34.0 (2.0)	34.2 (1.7)	.69

[a] Values for ascorbic acid levels obtained in 76 placebo-treated smokers and 67 vitamin C–treated smokers.

[b] Based on a log transformation of gestational age because of skewness.

[c] Birth weight less than 10th percentile for gestational age.

[d] Values for head circumference obtained in 80 placebo-treated and 72 vitamin C–treated smokers.

Table 3. Respiratory Outcomes in Offspring

Outcome	Nonsmokers[a]	Treated Smokers Vitamin C	Placebo	Adjusted[b] Mean Difference (95% CI) (Vitamin C − Placebo)	P Value[c]
Mean (SD)					
Newborns, Pulmonary function testing	n = 76	n = 76	n = 83	**Mean Difference (95% CI) (Vitamin C − Placebo)**	**P Value[c]**
Respiratory rate, bpm[d]	53 (12)	57 (12)	57 (12)	−0.27 (−4.14 to 3.60)	.89
TPTEF:TE[d]	0.399 (0.077)	0.383 (0.084)	0.345 (0.078)	0.036 (0.011 to 0.062)	.006
Crs/kg, mL/cm H_2O/kg[e]	1.36 (0.30)	1.32 (0.30)	1.20 (0.24)	0.11 (0.02 to 0.20)	.01
Total Crs, mL/cm H_2O[e]	4.46 (1.10)	4.16 (1.13)	3.92 (0.81)	0.32 (0.02 to 0.62)	.04
FRC/kg, mL/kg[f]	24.5 (5.2)	25.1 (3.9)	23.9 (4.9)	0.96 (−1.21 to 3.14)	.38
Total FRC, mL[f]	81.1 (20.8)	79.9 (20.2)	77.3 (15.6)	3.45 (−3.49 to 10.38)	.32
Infants, clinical respiratory outcomes through age 1 y	n = 70	n = 70	n = 77	**Relative Risk (95% CI)**	**P Value[c]**
At least 1 episode of wheezing, No. (%)	19 (27)	15 (21)	31 (40)	0.56 (0.33 to 0.95)	.03
Medication for wheezing, No. (%)	7 (10)	9 (13)	17 (22)	0.56 (0.27 to 1.18)	.13
Infants, pulmonary function testing at age 1 y		n = 49	n = 57	**Mean Difference (95% CI) (Vitamin C − Placebo)**	**P Value[c]**
Respiratory rate, bpm[g]	NA[h]	30 (4)	30 (5)	0.80 (−1.0 to 2.6)	.37
TPTEF:TE[g]	NA[h]	0.260 (0.061)	0.247 (0.061)	0.009 (−0.015 to 0.034)	.44
Crs/kg, mL/cm H_2O/kg[i]	NA[h]	1.40 (0.36)	1.26 (0.27)	0.13 (0.00 to 0.25)	.05
Total Crs, mL/cm H_2O[i]	NA[h]	13.9 (3.7)	12.5 (2.8)	0.62 (−0.53 to 1.77)	.29
Respiratory resistance, cm H_2O/mL/s[j]	NA[h]	0.032 (0.011)	0.035 (0.009)	−0.002 (−0.006 to 0.002)	.29

Abbreviations: Crs, passive respiratory compliance; FRC, functional residual capacity; NA, not available; PFT, pulmonary function test; TPTEF:TE, ratio of time to peak tidal expiratory flow to expiratory time.

[a] Nonsmokers are reference group only and not included in formal statistical analysis.

[b] For PFT values of newborns and clinical outcomes among infants aged 1 year: P values and 95% confidence intervals adjusted for gestational age at randomization (>16 weeks vs ≤16 weeks), birth weight, and gestational age <37 weeks. For PFT values among infants aged 1 year: P values and 95% confidence intervals adjusted for gestational age at randomization (>16 weeks vs ≤16 weeks) and length and postnatal age at PFT.

[c] For comparison of randomized groups (vitamin C vs placebo).

[d] Values obtained in 74 nonsmokers, 83 placebo-treated patients, and 73 vitamin C–treated patients.

[e] Values obtained in 73 nonsmokers, 81 placebo-treated patients, and 71 vitamin C–treated patients.

[f] Values obtained in 49 nonsmokers, 33 placebo-treated patients, and 32 vitamin C–treated patients.

[g] Values obtained in 57 placebo-treated patients and 48 vitamin C–treated patients.

[h] Unable to perform PFTs at 1 year for infants of nonsmokers because of inability to perform sedation as per the institutional review boards.

[i] Values obtained in 53 placebo-treated patients and 49 vitamin C–treated patients.

[j] Values obtained in 53 placebo-treated patients and 48 vitamin C–treated patients.

to placebo, although this was not significant (*P* = .13). Pulmonary function tests at age 1 year were obtained for 67% of the offspring studied at delivery. Respiratory compliance was higher in infants born to the smokers randomized to vitamin C but did not reach statistical significance. There was no significant difference in other PFT parameters measured at 1 year (Table 3). Twenty-seven vitamin C–treated and 30 placebo-treated infants had measurements of FRC, and there were no significant differences. The results of the multiple imputation methods for the primary and secondary clinical outcomes were negligibly different from the main results (eTable in Supplement).
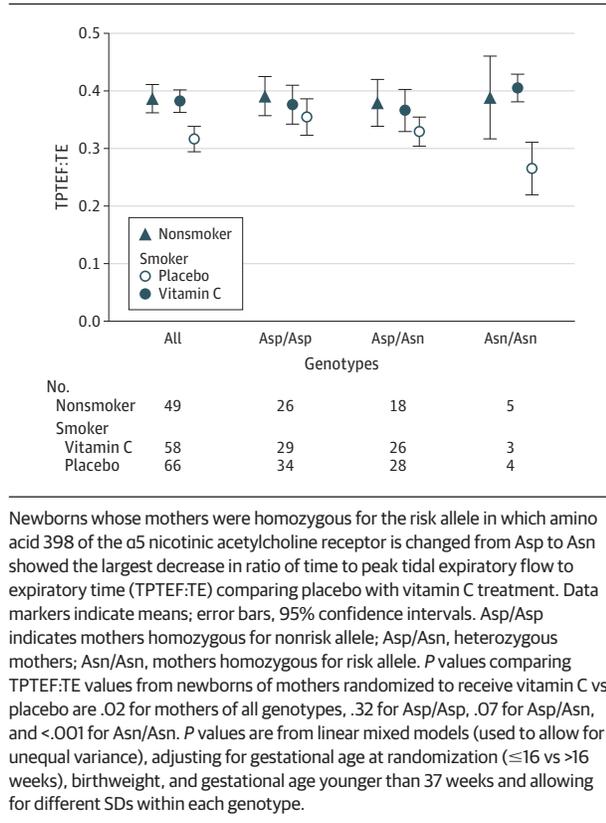
A subgroup of mothers (n = 173) were genotyped for 2 nAChR and 3 glutathione transferase polymorphisms associated with smoking and lung disease. This aspect of the study was added after the study was already under way, so only a subgroup of mothers were tested. Only the maternal genotype for rs16969968 significantly affected offspring pulmonary function. We found a significant interaction (*P* < .001) between maternal genotype and treatment group, indicating that

the effects of maternal smoking and vitamin C treatment may have been influenced by genotype. Although the sample size for smoking mothers homozygous for the risk allele was small, newborns from these mothers showed the biggest decrease in TPTEF:TE (*P* < .001), and this decrease was not present in those receiving vitamin C supplementation. Offspring of mothers heterozygous for the risk allele showed no significant interaction (*P* = .07) (**Figure 2**). The frequency of the risk allele in our study population was 28%, which is consistent with our population of primarily European origin.[27] Further analysis of the 1-year respiratory outcomes incorporating the effect of maternal α5 nAChR genotype demonstrated that infants of pregnant smokers homozygous or heterozygous for the rs16969968 risk allele randomized to vitamin C had a decreased incidence of wheezing through 1 year compared with those randomized to placebo (14% vs 48%; *P* = .02).

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

Figure 2. Effect of Maternal Smoking During Pregnancy on Newborn Pulmonary Function as Modulated by Maternal α5 Genotype (rs16969968)

No.	All	Asp/Asp	Asp/Asn	Asn/Asn
Nonsmoker	49	26	18	5
Smoker				
Vitamin C	58	29	26	3
Placebo	66	34	28	4

Newborns whose mothers were homozygous for the risk allele in which amino acid 398 of the α5 nicotinic acetylcholine receptor is changed from Asp to Asn showed the largest decrease in ratio of time to peak tidal expiratory flow to expiratory time (TPTEF:TE) comparing placebo with vitamin C treatment. Data markers indicate means; error bars, 95% confidence intervals. Asp/Asp indicates mothers homozygous for nonrisk allele; Asp/Asn, heterozygous mothers; Asn/Asn, mothers homozygous for risk allele. P values comparing TPTEF:TE values from newborns of mothers randomized to receive vitamin C vs placebo are .02 for mothers of all genotypes, .32 for Asp/Asp, .07 for Asp/Asn, and <.001 for Asn/Asn. P values are from linear mixed models (used to allow for unequal variance), adjusting for gestational age at randomization (≤16 vs >16 weeks), birthweight, and gestational age younger than 37 weeks and allowing for different SDs within each genotype.

Discussion

Infants born to pregnant smokers randomized to supplemental vitamin C (500 mg/d) during pregnancy had significant increases in newborn PFT results (TPTEF:TE and Crs/kg) and a significantly decreased incidence of wheezing through 1 year when compared with infants born to pregnant smokers randomized to placebo. We chose the primary outcome of TPTEF:TE and Crs/kg based on studies demonstrating alterations in these parameters of pulmonary function in newborn infants of pregnant smokers,[6,8] data from nicotine-exposed primates showing that maternal vitamin C supplementation improved offspring pulmonary function,[15] and our experience with newborn pulmonary function testing.[19,21,23] The daily dose of 500 mg of vitamin C was based on data indicating that this dose would likely saturate vitamin C receptors and maximize plasma concentrations.[28] We demonstrated that pregnant smokers allocated to vitamin C had a significantly higher fasting plasma ascorbic acid level measured at 28 to 30 weeks' gestation compared with those allocated to placebo, providing a supportive biomarker for this randomized trial.

The PFT measurements of TPTEF:TE and respiratory compliance are known to be decreased in offspring of pregnant smokers.[6,8] The TPTEF:TE is a measurement performed during noninvasive, nonsedated newborn PFTs; a decreased

TPTEF:TE measured soon after birth is strongly associated with important respiratory clinical outcomes in later childhood.[29] Decreased TPTEF:TE may reflect expiratory airflow limitation and precedes and predicts subsequent wheezing.[29] This is consistent with the increase in small-diameter airways caused by prenatal nicotine exposure that we have previously reported.[12] Martinez et al[29] reported increased recurrent wheezing at ages 1 and 3 years in infants who had reduced TPTEF:TE in the initial weeks of life. Premature infants born to pregnant smokers have significant decreases in TPTEF:TE.[6] In animal models, in-utero nicotine increased collagen expression around airways[12,14] and altered airway geometry.[30] Either could contribute to the altered TPTEF:TE measured in this study. Supplemental vitamin C may act by blocking formation of reactive oxygen species, which stimulate abnormal patterns of airway cell proliferation, resulting in narrowed airways and abnormal airway geometry, but this requires further study. Decreased respiratory compliance in offspring of smokers may reflect the increased airway collagen and decreased elastin in the lung caused by in-utero nicotine[12]; supplemental vitamin C may ameliorate these connective tissue abnormalities by blocking formation of reactive oxygen species, but this also requires further study.

There are epidemiologic data linking vitamin C intake to improved pulmonary function in children[31] and adults.[32] Plasma levels of vitamin C are decreased in smokers, most likely because of the increased oxidative load associated with smoking,[33,34] and our data reconfirm this (Table 2). Vitamin C supplementation is safe during pregnancy,[35] and several studies demonstrate that maternal vitamin supplementation during pregnancy may improve subsequent childhood respiratory health.[36,37]

Although smoking cessation is the foremost goal, most pregnant smokers continue to smoke, supporting the need for a pharmacologic intervention. We provided smoking cessation counseling throughout the study, and 10% of patients quit smoking. Birth cohort studies demonstrate that reduced pulmonary function in offspring of smokers continues into childhood and up to age 21 years.[5] Longitudinal PFT cohorts indicate that individuals track along their predetermined PFT percentiles.[38] This emphasizes the important opportunity of in-utero intervention. Individuals who begin life with decreased PFT measures may be at increased risk for chronic obstructive pulmonary disease.[39]

We examined several genetic polymorphisms associated with increased sensitivity to smoking. Only the maternal genotype for rs16969968, which encodes a single amino acid change in the α5 nAChR and which has been associated with increased risk of lung cancer, nicotine addiction, and chronic obstructive pulmonary disease,[27] correlated with increased sensitivity of offspring to maternal smoking. Newborns whose mothers were homozygous for the risk allele showed the greatest decrease in TPTEF:TE of the randomized participants. Although the association with the polymorphism was statistically significant, the number of participants homozygous for the risk allele was relatively small, as expected in a population of European ancestry.[27] This finding is however sup-

ported by recent findings in 2 much larger studies, in which infants born to smoking mothers who had the risk genotype in the α5 gene cluster showed the largest decreases in birth weight.[40,41] These polymorphisms may affect lung development by increased smoking intensity caused by the altered α5 receptor or by a direct effect of the α5 receptor, which is expressed in bronchial epithelial cells in developing lung.[14]

Although our study was a randomized, placebo-controlled trial, we acknowledge several limitations. There was a 7.7% cohort loss through delivery in women randomized to placebo vs a 14.6% cohort loss in those randomized to vitamin C. This may have biased our outcomes. However, there was no difference in the baseline characteristics between the women who remained in the study through delivery vs those who did not. Also, the etiology for cohort loss was similar between the 2 randomized groups, primarily because of social reasons. We showed significant increases of 11% in TPTEF:TE and 10% in Crs/kg in the newborn PFTs. For reference, changes of this magnitude in forced expiratory volume in the first second of expiration (FEV_1) are used by the National Heart, Lung, and Blood Institute to define a positive response to bronchodilators,[42] and even smaller changes in FEV_1 are used to test for effective therapies for cystic fibrosis.[43] Birth cohorts have shown that changes in PFTs of this magnitude at delivery translate into important clinical benefits over time.[38] Importantly, in our study, changes in newborn PFTs were associated with a significant decrease (48%) in wheezing in patients receiving vitamin C vs placebo. Of the offspring studied at delivery, 92% had clinical respiratory follow-up (wheezing) through 1 year, whereas fewer patients (67%) had PFTs performed at 1 year because parents declined to provide consent for the necessary sedation. In addition, TPTEF:TE is a complex measure that represents an integrated response of the infant's entire respiratory system, including the neural, laryngeal, and intrathoracic components, and is strongly influenced by respiratory rate and postnatal age. Both of these lat-ter factors were comparable in the newborns of the randomized smokers in our study, and several studies have shown TPTEF:TE to be decreased in newborn infants born to smokers.[6,8] Respiratory compliance was not significantly higher in infants born to the vitamin C–treated smokers when measured at age 1 year. This may have been attributable to cohort loss and the use of passive respiratory mechanics, which is not the most sensitive technique for detecting abnormalities in the peripheral airways in infants. The lack of significant differences at 1 year may also mean that the initial differences in pulmonary function testing were transient. However, one of the key effects of maternal smoking on infant PFTs is a decrease in infant forced expiratory flows, which we did not measure for this study. We are currently performing a randomized trial of vitamin C vs placebo given to pregnant smokers that includes the measurement of infant forced expiratory flows at ages 3 and 12 months and monthly respiratory questionnaires through age 12 months. Last, it is important to note that although supplemental vitamin C may improve respiratory outcomes, no evidence is presented for beneficial effects on prematurity, intrauterine growth restriction, or behavioral effects associated with maternal smoking during pregnancy.

Conclusions

Vitamin C (500 mg/d) supplementation in pregnant smokers improved the newborn PFT results of their offspring. A significant decrease in wheezing through age 1 year was also observed. Vitamin C supplementation in pregnant smokers may be an inexpensive and simple approach (with continued smoking cessation counseling) to decrease some of the effects of smoking in pregnancy on newborn pulmonary function and ultimately infant respiratory morbidities, but further study is required.

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