VCSIP2:CCC-Lead Application

Vitamin C to Decrease the Effects of Smoking in Pregnancy on Infant Lung Function (VCSIP):

Follow-up of a Randomized Trial

Funded by the National Heart, Lung & Blood Institute

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1.1 Protocol Abstract

Despite strong anti-smoking efforts, at least 12% of American women do not quit smoking when pregnant, resulting in >450,000 smoke-exposed infants born yearly.[1] Maternal smoking during pregnancy adversely affects lung development as seen by lifelong decreases in pulmonary function and increased risk of asthma.[2;3] with annual heath care costs exceeding 1 billion dollars per year.[4] In a randomized controlled trial (RCT) published in *JAMA,*[5] we have provided evidence that vitamin C supplementation (500 mg daily during pregnancy) ameliorates the effects of maternal smoking during pregnancy on offspring lung function and decreased the incidence of wheeze by 48% through 1 year of age. We are currently completing a second RCT of vitamin C supplementation in pregnant smokers (NCT01723696 ["Vitamin C to Decrease Effects of Smoking in Pregnancy on Infant Lung Function"]) which has more robust measures of pulmonary outcomes through 12 months of age in the offspring. This minimal risk follow-up protocol will study the offspring from the current RCT. This will allow us to study the duration of the protection vitamin C provides in the face of in-utero smoke, the relationship between PFTs and the development of recurrent wheeze and/or asthma.

2. Objectives/ Scientific Aims

2.1 Specific Aim 1 (Primary Outcome): The primary aim of this study is to demonstrate improved pulmonary function at 5 years of age in the offspring of pregnant smokers randomized to vitamin C (500 mg/day) versus placebo. We hypothesize that vitamin C supplementation in pregnancy will block the adverse effects of maternal smoking on offspring pulmonary function measured at 5 years of age by spirometry. Impulse oscillometry (IOS) will also be used at 3-5 years of age to assess pulmonary function.

2.2 Specific Aim 2 (Secondary Outcome): The secondary aim of this study is to demonstrate a decreased incidence of wheeze at 5 years of age in offspring of pregnant smokers randomized to vitamin C (500 mg/day) versus placebo. We hypothesize that vitamin C supplementation during pregnancy will decrease the incidence of wheeze at 5 years of age in offspring of smokers. Respiratory health will be assessed by quarterly validated respiratory questionnaires and clinician report.

2.3 Purpose of the Study Protocol

This protocol serves as a road map for the execution of the study "**VCSIP2:CCC-Lead Application** "Vitamin C to Decrease the Effects of Smoking in Pregnancy on Infant Lung Function (VCSIP): Follow-up of a Randomized Trial". It will be reviewed by the Study Clinical Center (CCC) the Study Data Center (DCC), and Safety Monitoring Board (DSMB) appointed by the NHLBI. Any significant changes to the protocol during the study require the approval of the CCC, DCC, the NHLBI, and the DSMB. We are requesting that the Oregon Health & Science University (OHSU) IRB provides oversight of this minimal risk protocol to the IRBs at Indiana University (IU) and the PeaceHealth System IRB of PHSW. This will be done via signed authorization forms between OHSU and the respective IRBs.

A manual of operating procedures (MOP) will supplement the protocol and includes detailed information on the execution of the study and all procedures used.

3.Background

3.1 Introduction and Significance

Smoking during pregnancy remains a large public health issue and **more than 50% of smokers who become pregnant continue to smoke** despite the Surgeon General's warning of the associated health problems for over 40 years.[6;7] Newborns of smokers have decreased pulmonary function when measured after delivery and before exposure to postnatal smoke, confirming the importance of in utero exposure. 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.[8-11] Therefore, it is critical to develop early life strategies to maximize lung growth and development. Cohort studies have found that the increase in these adverse respiratory outcomes related to maternal smoking during pregnancy track into childhood and adulthood.[3;12]

3.2 Background Studies

3.2.1.Epidemiology of Smoking in Pregnancy and Offspring Pulmonary Function Tests (PFTs).

Smoking during pregnancy is the largest preventable cause of low birth weight, prematurity and perinatal mortality.[13;14] Maternal smoking during pregnancy is also the largest preventable cause of childhood respiratory illness, and children whose mothers smoked during pregnancy show lifetime decreases in pulmonary function and increased respiratory illnesses and asthma.[2;3;15] Maternal smoking is estimated to cause 10% of direct medical expenditures in the first year of life[16] and Stoddard and Grey[4] estimated that approximately 20% of expenditures for childhood respiratory illness are caused by maternal smoking amounting to over $1 billion annually in current health care dollars.

Multiple studies have shown an increased lower respiratory illness in infants born of mothers who smoke.[8;9;17;18] There is also a direct link between the clearly documented decreases in indices of offspring pulmonary function caused by maternal smoking during pregnancy and increased rates of respiratory illness.[19-26] A prospective study of offspring of pregnant smokers with a 21 year follow up has now documented persistent decreases in forced expiratory flows to 21 years of age in males.[3] In turn, decreased pulmonary function in infants correlates with increased rates of respiratory illness.[27-34] Critically, results from the multiple cohorts have now definitively shown that children with lower pulmonary function continue to track at lower percentiles of pulmonary function throughout life, putting them at **increased risk for developing COPD as adults**.[34-39]

Given the above, the obvious question is: why can't pregnant women stop smoking to avoid the associated morbidities in their offspring. The reality is that smoking is a unique morbidity in that it is addictive, heavily advertised [40-42] and as discussed above, certain genotypes significantly increase the likelihood of nicotine addiction and failure to quit. [43-45] Teen pregnancy, low income, low education, and living with a smoker are important factors increasing the odds of smoking during pregnancy.[46-48] Therefore, infants are victimized through no fault of their own by the addiction of their mothers caused in part by socioeconomic class, advertising, and genetics. Given nicotine's addictive nature, the low socio-economics of this population, and the constant advertising by tobacco companies, smoking during pregnancy will continue to adversely affect millions of babies worldwide. CDC statistics show that decreases in smoking rates have plateaued[49] and the rates have increased in recent years for teenagers.[25] The statistic that delineates the magnitude of the problem, is that multiple studies have shown, that upon learning of pregnancy, approximately 50% of smokers will immediately quit; the other 50% will continue to smoke no matter the intervention. This unfortunate reality makes finding ways to lessen the impact of smoking during pregnancy of vital importance.

3.2.2 Maternal Vitamin C Supplementation Prevents the Effects of Prenatal Nicotine Exposure on Newborn Rhesus Monkey PFTs.

There is strong support for the continuing success and follow-up study of the VCSIP ECHO cohort. Data from preclinical work in primates from Dr. Spindel's laboratory, demonstrated that the major effects of in utero smoke are caused by nicotine crossing the placenta to interact with nicotinic receptors in developing lung airways and vasculature.[50] This leads to altered patterns of lung growth / development and hence altered pulmonary function at birth.[51] Spindel then showed that supplemental vitamin C blocked the effects of in utero nicotine exposure on newborn monkey lung function (Figure 1).[52] This is consistent with studies showing that a primary mediator of smoking-induced oxidant injury is nicotine[53-56] and the multiple studies that show better pulmonary function correlating with increased vitamin C intake[57;58]. The results (Figure 1) are almost identical to the effects of maternal smoking during pregnancy on infant PFTs. These data provided the basis for VCSIP1 and VCSIP2.

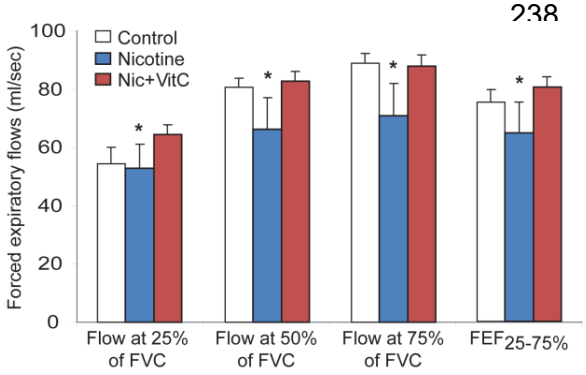


Figure 1. *Forced expiratory flows in animals treated as shown (Mean ± SEM). *p<.05 for overall comparison of Nicotine-treated group to control and nicotine + vitamin C-treated groups by MANOVA. $FEF_{25\%-75\%}$ = the average flow between 25% and 75% of forced expired volume. N= 20* [52]

3.2.3 Maternal Vitamin C Supplementation Improved Newborn Infant PFTs and Decreased Wheeze through One Year of Age

The likely success of our current study is strongly supported by our exciting data from VCSIP1 which was published in JAMA. In this study,[5] 159 pregnant smokers were randomized to vitamin C (500 mg/day) versus placebo. The offspring born to pregnant smokers randomized to vitamin C had significant improvements in newborn PFTs with significant increases in the time to peak tidal expiratory flow to expiratory time (TPTEF:TE) and respiratory compliance per kilogram (Crs/kg).

Clinical respiratory follow-up was obtained on 92% of the patients and infants delivered to pregnant smokers who received supplemental vitamin C importantly had a **48% decrease in the incidence of wheeze** through 1 year of age (Table 1). Fewer patients in the vitamin C group required medications in the first year of life for wheezing versus those randomized to placebo.

Table 1. Respiratory Outcomes in Randomized Infants through One Year of Age

	Newborns of non-smokers (n=76)	Newborns of placebo-treated smokers (n=83)	Newborns of vitamin C-treated smokers (n=76)	P value for treatment groups*	95% CI for difference in means in treated groups
Incidence of wheeze (%)	19 (27%)	31 (40%)	15 (21%)	0.019	0.53 (0.32, 0.90)
Medication for wheezing (%)	7 (10%)	17 (22%)	9 (13%)	0.14	0.58 (0.28, 1.20)

*Adjusted p value for vitamin C versus placebo based on binomial regression adjusting for randomization.

The above published data from the VCSIP1 trial[5] showed that vitamin C supplementation reversed the adverse effects of maternal smoking on infant pulmonary function and also suggests that vitamin C will

prevent the smoking-induced increase in offspring wheezing. Thus our preliminary data supports a likely positive result for continuing to study our VCSIP1 and VCSIP2 cohorts.

3.2.4 Genotype Modifies Infant Susceptibility to Effects of Smoking.

The importance of our study is supported by the multiple implications for our findings. Data published within our JAMA article[5] showed a significant interaction between the genotype for rs16969968 (the α5 nicotinic acetylcholine receptor (nAChR) structural polymorphism that has the strongest link to lung disease[59]) and altered offspring pulmonary function (Figure 2). These results add to the growing literature linking the effects of smoking on fetal development directly to nicotinic receptors which also raises concerns about the effects of e-cigarette usage during pregnancy. We found a significant interaction (p=0.0006) between genotype and treatment group, indicating that the effect of vitamin C treatment depended on genotype. Although the sample size for smoking mothers homozygous for the risk allele was small, infants from these mothers showed the biggest decrease in TPTEF:TE (p<0.0001) and this decrease was reversed by vitamin C supplementation. This finding emphasizes the power of a centralized approach to SNP analysis of prenatal and birth cohorts that is likely to come from the ECHO project.

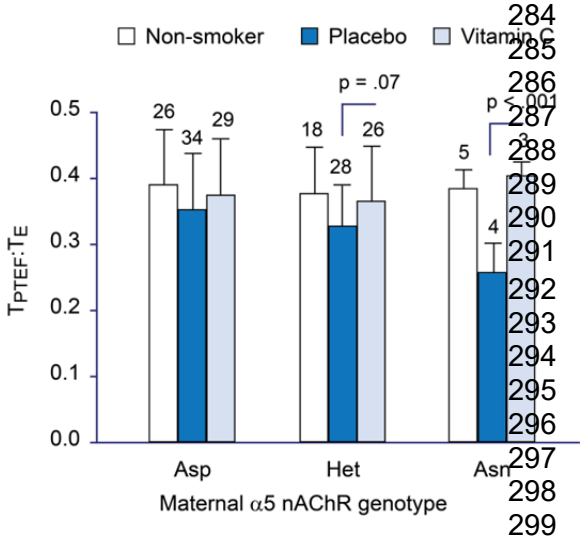


Figure 2. *Maternal α5 nAChR genotype (rs16969968) modulates the effect of maternal smoking during pregnancy on offspring pulmonary function. Infants whose mothers were homozygous for the risk allele in which amino acid 398 of the α5 nAChR is changed from Asp to Asn showed the largest decrease in TPTEF:TE comparing placebo to vitamin C treatment. Asp = mothers homozygous for non risk allele (Asp/Asp), Het = heterozygotes (Asp/Asn), Asn = mothers homozygous for risk allele (Asn/Asn).*

3.3 Rationale for the Follow-up of the Current Cohort

There is growing data that an individual continues to track along the same pulmonary function percentile that is established early in life. Given that in-utero smoke exposure adversely affects the pulmonary function of the offspring, the long-term health of children whose mothers smoke in pregnancy will likely be compromised with increased wheeze, asthma, and respiratory illness. In our earlier work, we demonstrated that vitamin C protects lung development as measured by pulmonary function tests early in life, and may reduce the incidence of early wheeze. This proposed study is innovative as we will determine if the trajectory of lung development as measured through pulmonary function testing and the clinical outcome of children as measured by wheezing and respiratory illness burden is improved in children at 5 years old whose mother's received a vitamin C supplement during pregnancy. **If successful, vitamin C supplementation to pregnant smokers will be a safe, inexpensive and simple measure that will have great public health significance in improving respiratory health in the US as well as worldwide.**

While all mothers should be counseled for smoking cessation in pregnancy, the reality is that some mothers are unable or unwilling to quit smoking and their children may be affected throughout their lives. If this study is successful, this simple approach may alter the respiratory health of these children, and potentially their later health as adults. In addition, this study is innovative as we will expand our unique biorepository of maternal, placental, and offspring blood, tissue and genetic samples that is coupled with prospective data collected on lifestyle, demographics, maternal history, and childhood

health and exposures. This will allow investigators to answer important scientific questions about the mechanisms by which exposure to cigarette smoke and vitamin C supplementation has life-long effects on respiratory function. The diligent follow-up of mother-child pairs in this study will create a highly important resource for studying the developmental origins of lung health and disease.

4.Study Design

4.1 Study Design Summary for the Follow-up of the Current Cohort

This study is the prospective follow-up of offspring from the current RCT of pregnant smokers randomized to supplemental vitamin C (500 mg/day) versus placebo to follow the respiratory outcomes of the offspring at 5 years of age. The ongoing study is only funded to follow patients to 12 months of age although we have kept updated contact information on those more than 12 months old. The primary outcome for this proposal is pulmonary function tests at 5 years and the secondary outcome is recurrent wheeze at 5 years of age. Children will continue to be followed for up to 5 years, regardless of age at time of study entry. We have excellent recruitment and retention rates and project that 232 children will enter this continuation and assuming a 10% cohort loss to 5 years, there will be 208 children (104 per arm) for the analysis. **Of note, we have met the NIH timeline and milestone accrual plan throughout the study and have kept dropouts to a minimum, pointing to the success of our efforts used to retain study subjects.**

4.2 Summary of Procedures of Current Vitamin C Study

4.2.1 General Background

Recruitment of the pregnant smokers and their offspring is finished for the current RCT, but we summarize it here as context for the continuation of the study. The primary aim of the study was to demonstrate improved pulmonary function tests (PFTs) at 3 months of age. The secondary aims were to demonstrate improved PFTs and decreased wheeze at 12 months of age.(Figure 3). See IRB for full protocol.

4.2.2. Eligibility Criteria

To qualify for the study at randomization, pregnant cigarette smokers had to be ≥15years old, with a singleton gestation, gestational age between $13^0/_7$ and $22^6/_7$ weeks, receiving care at clinics near the study sites, unable to quit smoking, English speaking. Important exclusion criteria: multiple gestation, fetal anomalies, illicit drug use, unstable communication, failure of medication adherence period.

4.2.3. Screening, Recruitment, and Enrollment

There was a single research coordinator at each site for all patient interactions. Pregnant smokers were recruited by referrals from providers, self-referral from advertisements, and hospital medical record queries. Eligible patients were approached at their prenatal visit about participation in the study, and the consent was reviewed in detail. S**moking cessation and education of the adverse effects of smoking on the fetus occurred at consent and each prenatal visit.**

4.2.4 Randomization and Study through Delivery

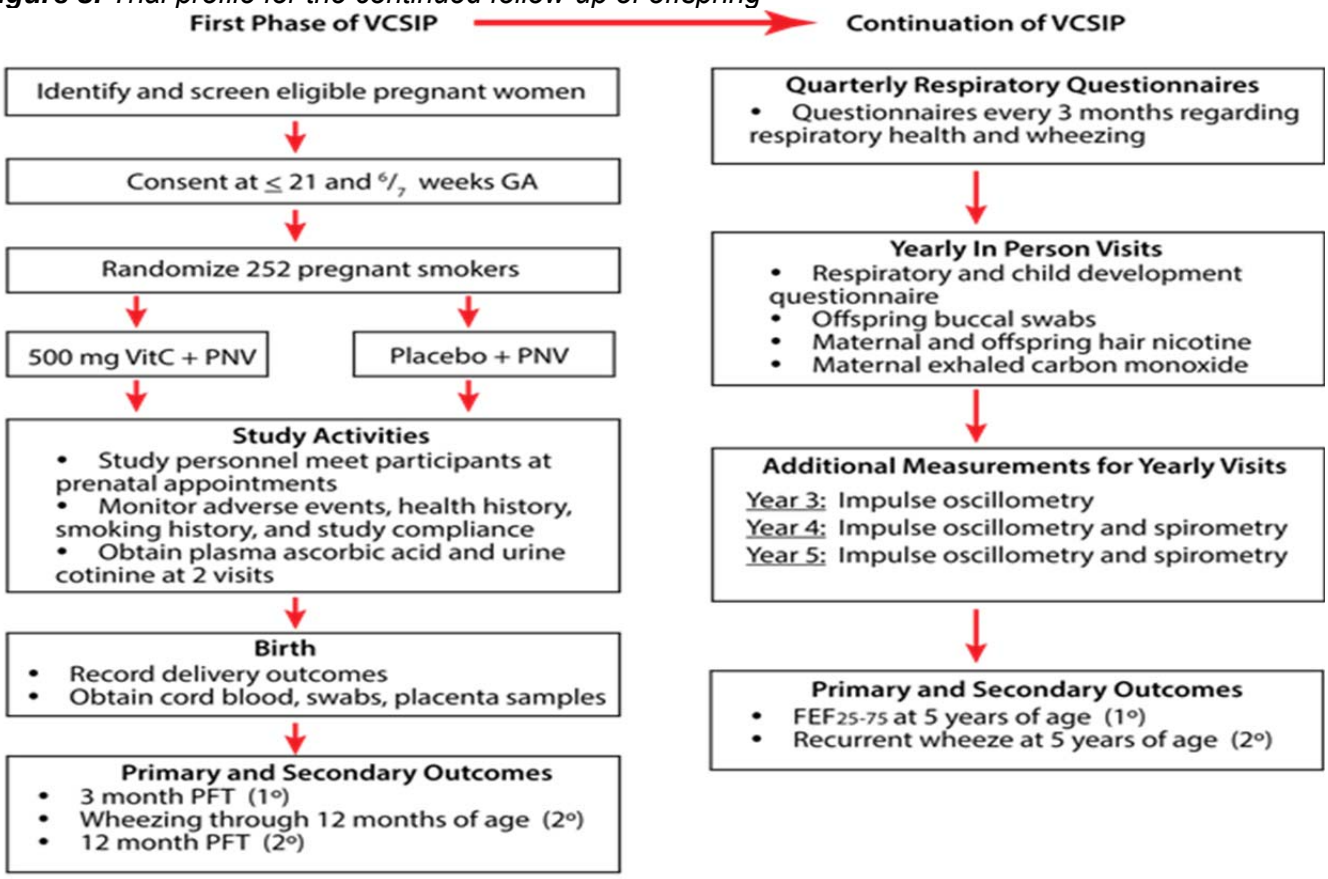
Randomization was done through the study's data coordinating center (DCC) and the OHSU research pharmacy. The vitamin C and placebo medications were compounded in identical capsules at an outside site and distributed through the respective research pharmacies of OHSU and Indiana University. **Blinding has been maintained for the patients, all clinical study personnel, and primary care providers for both studies at all sites**. Maternal fasting ascorbic acid levels, urine and hair samples and exhaled carbon monoxide were collected prior to randomization and at two other prenatal visits. Patients were met at monthly prenatal visit and pill counts, smoking, and medical history

were documented. Blood, placenta samples, buccal swabs, maternal and newborn hair, and delivery history were collected at delivery.

4.2.5.Follow-up from Delivery through 12 Months

A standardized respiratory questionnaire for the offspring was done at monthly for the current study and PFTs at 3 and 12 months of age. At the PFTs, maternal and infant hair for nicotine, infant buccal swabs, and maternal exhaled carbon monoxide were collected. The same research coordinator maintained consistent patient contact. At the 12 month PFTs, the potential of follow-up through 5 years of age was discussed in person.

Figure 3. *Trial profile for the continued follow-up of offspring*



5. Study Population of Current Vitamin C Study

5.1 Number of Subjects

The ongoing RCT is being conducted in the Pacific Northwest and in Indianapolis, Indiana. Pregnant smokers were recruited at obstetric clinics delivering at OHSU, PeaceHealth Southwest Washington Medical Center in Vancouver, Washington or Indiana University in Indianapolis, Indiana. Recruitment and randomization began in December, 2012 and ended in May, 2015 and exceeded the original NIH accrual plan and timeline. 333 pregnant smokers were consented and 252 randomized. Cohort retention from delivery through the 12 month follow-up has been excellent. We have excellent recruitment and retention rates and project that 232 children of smokers (65% of patients will be in the Pacific Northwest and 35% in Indianapolis) will enter into the follow-up phase.

5.1.2 Retention Strategies.

The clinical centers will maintain their respective cohorts of mothers and their children through application of novel and evidenced based retention techniques. Techniques include regular multimodal contact such as phone calls, text messages, and emails, social media private messaging, birthday cards and pre-arranged check-ins at mother or child's appointments. In instances where contact proves difficult, alternative retention strategies are: searching databases for updated contact information; calling at different times of the day and on weekends; and contacting close friends and/or relatives who were listed by the participant as back-up contacts or the participant's work if permission was obtained during consent. Additionally, we successfully branded our study with three logos placed on items such as magnets with our contact information, tote-bags for the mothers, onesies for the infants, and lanyards for hospital and clinic staff (see appendix). These items have proven priceless in enhancing rapport with participants and recruitment site staff by increasing excitement around the study and reminding participants and clinicians of their commitment. There is a website http://vcsip.org and a Facebook page https://www.facebook.com/Vcsip.ohsu with publicly available information as well as protected pages for investigator and participant communication. There will also be a yearly retreat to enhance communication, problem solving for cohort retention, and capturing outcomes data.

5.2 Inclusion and Exclusion Criteria for Follow-up

5.2.1 Inclusion criteria for prospective follow-up:

1. Women and their offspring randomized to vitamin C versus placebo during pregnancy as well as pregnant nonsmokers and their offspring enrolled as the reference group in the current RCT.

5.2.2 Exclusion criteria for prospective follow-up:

1. Patients specifically withdrawing consent.

5.3. Vulnerable Populations

This is a follow-up of children born to pregnant smokers randomized to supplemental vitamin C versus placebo in the current RCT. Continued follow-up of these children is critical to determine if maternal vitamin C supplementation during pregnancy leads to prolonged improvements on offspring's pulmonary function tests (PFTs) and respiratory health. This will allow us to study the duration of the protection vitamin C provides in the face of in-utero smoke, the relationship between PFTs and the development of recurrent wheeze and/or asthma.

5.4. Settings

The research settings for this follow-up will be the same sites that randomized pregnant smokers and enrolled pregnant nonsmokers i.e. (metropolitan Portland, Oregon (PNW) including OHSU and PeaceHealth Southwest Medical Center (PHSW) and Riley Children's Hospital in Indianapolis, Indiana). Both sites demonstrate track records of successful clinical research in pregnancy and infant/ childhood PFTs, and offer ethnic diversity. The non invasive procedures as outlined in Table 2 will be performed by designated research personnel hired by the respective sites and performed at HIPPA compliant locations at the respective hospitals or other locations to decrease patient burden. The data center will continue to be housed at OHSU under the direction of Dr. Morris as was the case in the current vitamin C study and is being funded through a linked data coordinating application. This follow-up will continue the strong ongoing collaborations between Drs. McEvoy, Morris, and Spindel at OHSU, and Tepper (at Indiana University School of Medicine). Dr. McEvoy is internationally recognized for her clinical trials [60] and the VCSIP-ECHO will use her successful research infrastructure as used in VCSIP1 and VCSIP2.[60-62] She will be the PI at OHSU and PHSW. Dr. Tepper, a pediatric pulmonologist, will be the PI at Indiana University. He is widely known for his expertise in PFTs[63;64] with extensive experience and

publications evaluating pre-school and school aged children, both ages which will be studied in VCSIP-ECHO. He has also followed a cohort of infants with eczema longitudinally from the age of 1 with spirometric measurements pre and post bronchodilator at 4 years of age,[65] and methacholine bronchial challenge at 5 years of age[66].

Each PI will be responsible for the conduct of the research at their institution. OHSU will submit all required documents for approval to the OHSU IRB and is requesting that the Indiana University IRB and PeaceHealth IRB waive oversight of this follow-up study to the OHSU IRB.

OHSU will be the coordinating center of this multi-center trial.

5.5. Recruitment Methods

This is a follow-up study of women and their children randomized in the current RCT. We have IRB approval to recontact these patients in the future follow-up. Subjects may be contacted to set up an appointments via phone calls, text messages, emails, or social media private messaging. Please see attached telephone script if contact is by phone.

Time and travel reimbursements will be $25 for each questionnaire and $75 for visits that includes sample collections and/or PFTs. This will be reimbursed after the questionnaires or visits are completed and appropriate receipts signed and inventoried.

5.6. Consent Process

Written informed consent will be obtained from each subject's parent/legal guardian appropriately prior to any study procedures taking place. Individuals authorized to obtain written consent are the principal investigator (PI), co-investigators, and assigned staff specifically designated by the PI to work on this project. The informed consent process will occur in the participating hospitals, or a HIPPA compliant location of convenience to the family to maximize cohort retention. If consent is obtained by phone, a hard copy of the consent will be mailed to the patient with a self-addressed envelope, reviewed over the phone with the research staff, signed by the patient, returned to the research office and signed by the research staff and detailed in the patient's chart. If a respiratory questionnaire (RQ) is done at the same time as a phone consent, no information will be recorded until the signed consent is obtained by the research staff.

The information provided in the consent and assent will cover the elements in the CFR Part 50.25 and be approved by the OHSU Institutional Review Board (IRB) which will provide IRB oversight on behalf of Indiana University and PeaceHealth IRBs. This includes the observational nature and objective of the trial; the procedures and treatments involved and their attendant risks, discomforts, and benefits; and the potential alternative therapies, alternative to not participate and right to withdraw without penalty, all of which will be explained to the parents in detail. All of the parent/legal guardian's questions will be answered before signing the consent form. If the parent/legal guardian wishes to take the consent form home to consult with other family members or health care providers, or to allow more time for consideration, they will be allowed to do so. A copy of the signed consent form will be given to the parent/legal guardian.

The informed consent process will be an ongoing active process of sharing information between the investigator and the parent/legal guardian(s). If a protocol change requires a change to the consent form, parents/legal guardian(s) will be notified in a timely manner and the new informed consent form will be signed.

Permission may be obtained from legally authorized representatives, described as an individual who is authorized under applicable state or local law to consent on behalf of a child to general medical care when general medical care includes participation in research. We may contact the patients after completion of this study to conduct a follow-up of this study or to conduct other studies. We would obtain separate consents for those studies.

Patient participation may be ended at any time for medical reasons, if the researcher feels that it is in your child's best interest, or because the NHLBI finds it necessary to limit or terminate the study.

6. Study Procedures and Methods

6.1 Overview

The study personnel will continue to administer a similar standardized respiratory questionnaire (updated for age) asked during the first 12 months of the current study. RQs will be obtained up to four times per year. The coordinators will schedule yearly in person visits at which time study procedures will be carried out including the RQ and additional appropriate "Ages and Stages", childhood health questionnaires, infant buccal swabs, maternal and child hair nicotine samples, and maternal exhaled carbon monoxide (eCO) reading. Pulmonary function testing will begin when the child is at least 3 years of age. If the child Is 3 years of age at enrollment, the PFT may be done at the time of the consent or scheduled as a separate appointment. Pre and post bronchodilator spirometry testing will be done yearly in children ≥ 4 years of age to identify under recognized airway hyperreactivity. These early attempts will allow the child to practice and feel comfortable with the surroundings and testing. Blood collection for genetics and epigenetics will be done once at age 5-6. A summary of the data collection in the proposed study is shown in the Table 2 below.

Table 2. Summary of Questionnaires or Procedures

Age in years	1	2	3	4	5***
Quarterly Respiratory questions	X	X	X	X	X
Impulse Oscillometry			X	X	X
Spirometry				X	X
Weight, height*,	X	X	X	X	X
Head, chest, abdominal circumference*	X	X	X	X	X
Buccal swab	X	X	X	X	X
Hair nicotine-mom and child	X	X	X	X	X
Exhaled carbon monoxide-Mom	X	X	X	X	X
Bronchodilator response				X	X
Blood for epigenetics/genetics**					X

* Measurements will be taken at each in-person visit
** This will be up to 3 teaspoons of blood
*** Child will continue to be followed in study for up to 5 years total regardless of age

6.2 Respiratory Questionnaires

To compare the incidence of recurrent wheeze[67-69] between the children of randomized smokers, we will use data obtained four times per year from the same standardized respiratory questionnaire (RQ) being used in the ongoing study [70-73](appendix) which will be updated for patient's age, and from clinician reports. The use of this RQ was requested by current study's NIH appointed DSMB and has been applied in similar NIH trials. The RQs may be done in person or over the phone. Parents will be asked about new episodes of wheeze since the last questionnaire, medication prescriptions with specifically outlined respiratory medications, other illnesses including physician diagnosed croup,

bronchitis, bronchiolitis, or pneumonia, symptoms of or physician diagnosed eczema, emergency room visits, second hand smoke exposure and hospitalizations. If an infant has wheezing associated with an illness lasting several days, this will be counted as one episode. The patient's electronic medical record may also be reviewed when available to confirm diagnosis, medications, etc. or when we are unable to perform a respiratory questionnaire. A small time reimbursement will be given to those patients who complete the questionnaires. Once per year we will ask additional questions about diet, behaviors, home environment, and potential sources of stress.

6.3 Child Anthropometrics and Blood Pressure

Height, weight, head, chest, and abdominal circumference, and blood pressure of the child will be taken at each in person visit.

6.4 Maternal and Child Hair Nicotine Testing

Small samples of maternal and child hair will be taken at each yearly in person visit as a quantification of second hand smoke exposure. Hair nicotine is determined by a modification of the methods of Hegstad et al [74] and Pichini et al[75] by tandem mass spectrometry (LC-MS/MS) using deuterated internal standards. Hair samples may also be analyzed for other substances such as cortisol that is an important determinant of lung development and function.

6.5 Maternal Exhaled Carbon Monoxide Testing

Non-invasive measurements of exhaled carbon monoxide (CO) will be performed by the child's mother at each yearly in person visit as a quick, non invasive estimate of acute second hand smoke exposure (Smokelyzer, Bedfont Scientific, London, UK). [76] The highest level of the parts per million (ppm) of carbon monoxide and the percent of carboxyhemoglobin (COHb) are then recorded.

6.6 Child Buccal Swabs

Buccal swabs will be collected on the child yearly to investigate DNA methylation patterns that may mediate subsequent respiratory disease after prenatal exposure to nicotine.[77] Buccal swabs will be done using Epicentre Catch-All wrapped soft foam swabs. Swabs will be done by rolling the swab on the inside of the infant's cheek, approximately 10-20 times on each side, making certain to roll the brush over the entire cheek. After collection the swab will be placed in the collection tube and stored at –20°C. To increase yield of cells a separate swab will be used for each cheek surface and each cheek will be swabbed separately twice.

6.7 Continued Smoking Cessation Counseling

Smoking cessation counseling will continue at the yearly visits under the guidance of co-investigator Dr. David Gonzales[78;79] of the OHSU Smoking Cessation Center. The best thing for the children and families would be the elimination of exposure to the toxicants in tobacco smoke. The annual visits will provide an opportunity for extra "teachable moments" for stopping smoking. Study participants will be provided brief smoking cessation counseling and educational materials. Staff will discuss dangers of first and secondhand smoke at annual visits and the implications of the exhaled carbon monoxide measurement as a proxy for toxicant exposure.

6.8 Blood for Epigenetic Changes

One blood sample will be drawn at age 5-6. Blood will be collected in 3 mL EDTA tubes and store at -80º C and analyzed for global and gene specific methylation changes.

6.9 Pulmonary Function Tests on Child

All pulmonary function tests will be done by trained personnel from the infant/ child pulmonary function laboratories at OHSU, Riley Hospital for Children in Indianapolis, Indiana, and PHSW. The equipment being used is portable so this testing can be done either in one of these laboratories or in a HIPPA compliant location to ease the burden of the study on the patient. No testing will be done within 3 weeks of a respiratory illness and a test may be repeated if the quality was not acceptable. Of note, the PFT laboratory at Riley Children's hospital published the first normative data for spirometric measurements in young children between 3 and 6 years of age.[80] Cross training and certification will occur between the PFT laboratories prior to the start of the study to assure the same testing techniques and acceptance criteria are applied across sites. In addition, similar to the ongoing study, there will be weekly discussions between the sites regarding testing techniques to problem solve issues that arise for performance and acceptance criteria. For the spirometric measurements, acceptance criteria as modified per Eigen/Tepper et al [80;81] will be followed with each data set consisting of at least 3 reproducible attempts (see protocol in appendix). Measurements will be reported as absolute values as well as corresponding z scores.[82] As an additional outcome, we will also evaluate both pre and post bronchodilator (albuterol) spirometry yearly in children ≥ 4years of age. An increase in FEF_{25-75} with a bronchodilator indicates increased airway tone and heightened airway responsiveness. Also, if the difference between the two groups (vitamin C vs. placebo) is present pre-bronchodilator, but not post bronchodilator, this suggests that the lower baseline spirometry is related to increased airway tone and not fixed structural difference.

In addition, pulmonary function will be measured with the forced oscillation technique (FOT). FOT is a method of assessing lung function in children who cannot perform spirometry or plethysmography due to age or other factors.[83-85] With FOT, the child performs tidal breathing of ambient air via a pneumotachograph while measurements are performed with different frequencies of air oscillation. This is basically the human equivalent of oscillometry widely used in animals to measure airway resistance.[86;87] A measure of resistance to the flow of air into and out of the lung is usually determined from the ratio of pressure and flow during spontaneous breathing. This methodology has been demonstrated to differentiate between preschool children with and without asthma.[83] The infant PFT laboratory in Indianapolis has experience using FOT in 4 year old children.[88] We propose this measurement starting at 3 years of age. The use of this complementary technique to spirometry will maximize our ability to determine differences in lung function between children born to pregnant smokers randomized to vitamin C versus placebo. Cross training will be done prior to testing, the same equipment will be used at both sites, and the results will be reviewed by an independent reader. The primary outcome measure of the FOT will be the resistance at 8 Hz, and measurements at 5-11-19 Hz signal will also be recorded as obtainable.

6.10.1 Quality Control of Pulmonary Function Tests

The same equipment and testing software for spirometry (Morgan Scientific, Haverhill MA) and FOT (Resmon Pro, MGC Diagnostics, Saint Paul, MN) will be used at all testing facilities. Testing will be performed following the American Thoracic Society/ European Respiratory Society (ERS) criteria.[81] All test results will be reviewed by a trained, licensed Respiratory Technician and reviewed weekly for acceptability, reproducibility, and completeness. There will be annual review training sessions on the

proper use and maintenance of the equipment and coaching of the spirometry maneuvers appropriate to children.

7. Data and Specimens

7.1 Handling of Specimens

Specimens (maternal and child hair, child blood and cheek swabs) will be coded with subject study ID and date and time of collection. Patient name, initials, birth date and other potential identifiers are known only to the investigators at each study site and not made available to other personnel. The hair samples will be stored in static free manila envelopes at room temperature, the blood will be collected in EDTA tubes and stored at -80º C and the buccal swabs will be stored at –20°C. All samples will be stored in a secure location until they are batched by the respective study coordinators and shipped to Dr. Spindel's laboratory at the Oregon National Primate Research Center (ONPRC). Sample processing will occur and residual specimens will be stored in the IRB approved repository located at the ONPRC.
As below, specimen logs will be implemented in the REDCap study database. Detailed information about processing, storage location, and chain of custody will be recorded.

7.2. Handling of Data

7.2.1 Background of Data Center

With the current study, we formed the structure of the Clinical Coordinating Center (CCC) which handled the clinical side of the study and the Data Coordinating Center (DCC) which handled the data side, thus providing the essential firewall needed to maintain blinding and allow for safety monitoring. To support the data coordination needs of the follow-up, we will continue the DCC to provide support for data collection and management, data quality, maintenance of protocol compliance, and biostatistical analysis. The biostatistics unit will be responsible for all data analyses and DSMB reports.

Most importantly Dr. Morris and the DCC will ensure continued blinding of all clinical personnel and patients to the study treatment allocation from both the VCSIP1 and VCSIP2 trials.
Maintaining the complete security of the allocation table and treatment assignment is of critical importance to this study. The allocation table is password protected and is stored off site for security.

7.2.2 Data Management and Quality Assurance.

The primary data management goal is to provide high quality and timely data to study investigators, and effective study management tools to study staff. Key features of the data management system include a powerful and flexible data dictionary; real-time access to study data; real-time query generation and resolution; real-time audit trail and comprehensive reports for effective study management. The system also eliminates delays in identifying missing data and in cleaning data; real-time reports enable more efficient workflow; allows monitoring of study progress in real-time. The DCC has a data management plan that provides information on data management activities, responsibilities, scheduled study visits, required data collection forms, and data handling conventions, including data recorded in paper and

electronic formats. The data management plan serves as a reference guide to the operational procedures governing the data management activities throughout the lifecycle of the study.

7.2.3 REDCap Data Management System

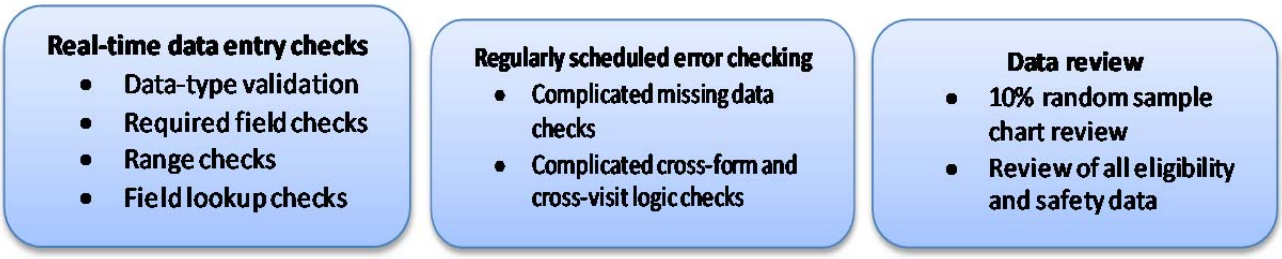
We will utilize the REDCap (Research Electronic Data Capture) system to enable high-quality data collection and robust quality assurance processes by employing features such as discrepancy adjudication; field level validation including range and format checks; and branching/skip logic. REDCap provides an integrated database while simultaneously allowing remote site staff access to only their participants' data. This approach enables real-time data quality checks, safety monitoring, and together with its participant calendaring system, enables cohort retention to be monitored closely. REDCap's robust security system allows customized security schemes for individual case report forms, data export functions, and reporting. REDCap employs algorithms that allow easy creation of de-identified datasets to assist in producing sharable datasets to meet NIH requirements for data sharing. REDCap automatically maintains an audit trail of all data changes is maintained including timestamp, user initiating change, change record, type of change, changed fields, and field values. REDCap offers an automated export mechanism to several common statistical packages (SPSS, SAS, Stata, R/S-Plus). Data exports can be customized and security can be attached to the data export process and the reporting system providing access study data while maintaining blinding and maximizing participant data confidentiality. The power of REDCap will be a strength in providing the ECHO consortium with needed data elements.

Daily backups of all center data, code, documents, and other study-related digital assets will be created by OHSU's Advanced Computing Center. Two-weeks of daily backups and two weekly-backups are immediately available in the unlikely event that a restore is needed. After one month, backups are stored offline in a secure vault maintained by OHSU.

7.2.4 Data Collection Forms, Entry, Cleaning

Data will be collected on standardized forms on which nearly all responses have been precoded. The data collection protocol for the VCSIP study will utilize paper case report forms and will be entered into REDCap by the clinical center study staff. All paper charts will be stored in locked file cabinets. There will be a 10% random sample chart review. The data entry workflow and validation scheme are illustrated in the figure below.

***Figure 4**. Multi-stage data validation*



The discrepancy management process includes a variety of important data quality tasks. These include running production edit checks, reviewing data discrepancies produced by edit checks, generating queries, resolving discrepancies, and updating any relevant data in the database. All discrepancies will be reviewed by the Data Center staff who will either resolve the discrepancies or forward queries to

each site for clarification or resolution on data clarification forms, with a full audit trail generated for each modification.

We will routinely perform quality control checks of data and implement cleaning procedures throughout the study duration. In addition, the data management team will perform the final quality control checks and clean the data to an optimal degree prior to locking the database at the end of the study. The data management team will ensure that all of the data have been entered, all discrepancies resolved, and final data updates completed before it begins the final quality control checks of data prior to data lock.

7.2.5 Data Access and Security

Access to data in the study database will be controlled through REDCap's role-based security system based upon the principle of least access. Each site will only be able to access the data from subjects enrolled at their own site. Blinding is maintained by restricting access to a separately-maintained REDCap form to only those data management staff needed to maintain group assignment in accordance with the treatment assignment blinding procedure. Security assignment will be managed and maintained by the Data Coordinator, Julie Mitchell.

7.2.6 Manual of Operations and Training

The manual of operations will be appended with procedures for this additional follow-up period. This includes specimen handling procedures, data collection forms, all study data collection instruments, procedures for pulmonary function testing, procedures for completing and handling the collection forms, data entry screens and associated procedures, and monitoring procedures for adverse events, accrual, lab quality and timeliness. The steering committee will review and approve the manual of operations before study enrollment begins.

While at present we anticipate no or minimal change in personnel, we will plan for a training procedure in order to ensure that the protocol is followed accurately and completely. We will structure training sessions and materials around specific competency-based objectives. The training program and supporting materials will address study objectives, study design, screening and recruitment procedures, data collection procedures, the data management process, and protection of patient rights.

7.2.7 Site Monitoring.

On-site performance monitoring of a clinical site is an important component in the overall plan for quality assurance and data quality control. During this follow-up, site monitoring will be performed at the time of periodic pulmonary function testing calibration that will occur between Pacific Northwest and Indiana sites. At that visit, data coordination personnel will monitor that consent has been obtained; ensure the accuracy and completeness of data; and will review for protocol modifications.

7.3 Sharing of Results with Subjects

Any clinically significant results from the PFT spirometry of blood pressure measurements will be shared with the offspring's primary care practitioner. Results of the FOT testing and genetic information will not be shared with the patients as its clinical significance at present is unknown. The blood testing will not be done until the study is over and will only be used for research.

7.4 Data and Specimen Banking

Data and specimens may be used for future research that may include genetic research. All data and specimens will be de-identified and will be stored in an existing OHSU repository that contains data and specimens from the two vitamin C studies. See OHSU eIRB 2893 repository protocol also uploaded with this submission. Repository procedures will be followed to release specimens or data to outside investigators. There is no scheduled date on which the samples will be destroyed. The samples may be stored for research until they are "used up".

8. Data Analysis

8.1 Analysis Plan of Primary Aim

Specific Aim 1 (Primary Outcome): The primary aim of this study is to demonstrate improved pulmonary function at 5 years of age in the offspring of pregnant smokers randomized to vitamin C (500 mg/day) versus placebo. We hypothesize that vitamin C supplementation in pregnancy will block the adverse effects of maternal smoking on offspring pulmonary function measured at 5 years of age by spirometry. Impulse oscillometry (IOS) will also be used at 3-5 years of age to assess pulmonary function.

We anticipate that those children whose mothers were treated with placebo during pregnancy will have lower baseline spirometry at 5 years of age compared to those whose mothers were treated with Vitamin C. We have an excellent cohort retention and project that 208 children will be have PFTs performed at 5 years of age (Figure 7). As per the linked DCC statistical analysis, this sample sizes will give us a 90% power to show a 13% to 15% increase in the FEF_{25-75} for children born to smokers randomized to vitamin C versus placebo which would be both statistically and clinically significant as demonstrated by our published data and other outcome trials.[5;89] Our previous experience[80] assessing spirometry in this young age group suggests that we will obtain technically acceptable measurements in at least 90% of the children. We anticipate this high success rate by having experienced laboratory personnel, repeated contact with these young children and their mothers/caregivers to ensure they feel comfortable with study staff, and practicing the spirometric testing during visits at a younger age. If the test does not meet acceptance criteria we will also approach the parents about a repeat attempt in a few weeks. This has already been discussed with the parents. We will provide a time and travel stipend at the yearly visit and we have an additional reimbursement in place for patients who have moved out of the area.

8.1.3. Additional Pulmonary Function Tests

Although the primary endpoint is FEF_{25-75}, similar analyses will be repeated for other forced expiratory flow measures: FEF_{75}, FEF_{50},. Forced expiratory volumes will also be evaluated including forced vital capacity (FVC), forced expired volume in the initial 0.4 sec (FEV0.4) and 0.5 sec (FEV0.5) and the ratio of FEV0.5/FVC.

8.2 Analysis Plan of Secondary Aim of Wheezing

Specific Aim 2 (Secondary Outcome): The secondary aim of this study is to demonstrate a decreased incidence of wheeze at 5 years of age in offspring of pregnant smokers randomized to vitamin C (500 mg/day) versus placebo. We hypothesize that vitamin C supplementation during pregnancy will decrease the incidence of wheeze at 5 years of age in offspring of smokers. Respiratory health will be assessed by quarterly validated respiratory questionnaires and clinician report.

We continue to have a 92.4% completion of quarterly RQs and have the infrastructure in place to continue at this high quality rate of data collection. We anticipate that those children whose mothers were treated with placebo during pregnancy will have a higher occurrence of recurrent wheeze compared to those children whose mothers were treated with vitamin C. In our published pilot study, the occurrence of wheeze was 40% in the placebo treated group and 21% in the vitamin C treated group. Even if there is an unexpected 25% decrease in our projected sample size of 104 per treatment group, we will have 80% power to detect a 50% decrease in recurrent wheeze if the control incidence is 40% or higher, which given our current aggregate occurrence of wheeze is achievable. The power will exceed 90% for the same RR if the control incidence is 50% or higher (see DCC statistical plan for more detail). If the higher incidence of recurrent wheeze is also related to lower baseline spirometry, which disappears following a bronchodilator, it would suggest that the vitamin C treatment suppressed the development of airway hyper-responsiveness, and the potential for asthma. However, if the higher incidence of recurrent wheeze in the placebo treated group is associated with lower pre and post bronchodilator spirometry, this would suggest that vitamin C suppressed the development of fixed airways obstruction secondary to maternal smoking during pregnancy.

8.4 Missing Data.

We will make every reasonable effort to retain this cohort to preserve the integrity of the study and to minimize possible bias associated with withdrawal or loss to follow-up. The statistical analysis using mixed models as described provides valid inference if the missing data is missing at random (MAR). However, in the case that the data are not missing at random (NMAR), we will perform multiple imputation propensity score modeling and then sensitivity analysis will be performed to examine the sensitivity of the inferences to departures from the MAR assumption.

8.5 Timeline

Yearly PFTs, longitudinal data collection, analysis of epigenetic changes and interaction with the overall cohort will occur in years 1-4.5 of the study.

9. Privacy, Confidentiality, and Data Security

Upon enrollment into the initial portion of the studies, mothers and their offspring were assigned a code that was used instead of their name, medical record number or other personally identifying information. Electronic files for data analysis contain only that subject code and this code will be continued in this follow-up study. The subject codes do not contain any part of the 18 HIPAA identifiers (initials, DOB, MRN). The key associating the codes and the subjects personally identifying information are restricted to the PI and the clinical study staff. The key will be kept secure as outlined below. Data will be entered into the REDCap system by site study personnel.

Standard practices will be followed to maintain the confidentiality and security of data collected in this study. Study staff will be trained with regard to these procedures. Paper files will be stored in locked filing cabinets in restricted access locations. Electronic data will be stored in a web-accessible encrypted REDCap database housed on the OHSU secure server under the direction of Dr. Morris. Access to data/specimens is restricted to study personnel.

10. Provision to Monitor Safety

This is a minimal risk protocol. The data center under Dr. Morris and in conjunction with the NIH-appointed DSMB(see uploaded list of members) which monitored the current vitamin C study will monitor safety, protocol adherence, patient retention, AEs and SAEs. We will adhere to the schedule and content of reports that the NIH DSMB requests. Any serious adverse events and protocol deviations that are determined to be unanticipated problems will be sent to the DSMB chair within 7 days of the occurrence of the event.

We will document and report all events that are considered possibly related to the study in any way and all Serious Adverse Events (SAE's) that occur in the pediatric study population. A Serious Adverse Event is any event meeting the following criteria:

- Death
- Life Threatening
- Hospitalization or prolongation of hospitalization
- Disability or permanent damage
- Congenital abnormality
- Required intervention to prevent permanent impairment or damage
- Other (important medical event)

These events will be identified by interviewing the subject's parents, completing the respiratory questionnaires, physical exam/observation (during the in person visits) and by reviewing medical records when necessary/available.

1. Plan for reporting both anticipated and unanticipated adverse events.

a. Each child is evaluated for any adverse events.

b. Any event that is possibly related to the study or meets criteria for a Serious Adverse event will be documented as such.

c. All documented events will be reported per each site's IRB policy.

e. All adverse events will be summarized annually and submitted to the IRB.

f. Any action resulting in a temporary or permanent suspension of the study (e.g. IRB actions or actions by the investigators or co-investigators) will be reported to the DCC and the appropriate NIH program official.

11. Risks and Benefits

11.1 Minimal Risks to Subjects

The risks associated with participation in this study are minimal and are related to the administration of a bronchodilator during the non sedated lung function testing, phlebotomy (one blood sample at 5-6 years of age), and any potential breach of confidentiality.

Bronchodilator

Each child will be monitored closely after the administration of the bronchodilator and during the test. This treatment can uncommonly cause a temporary increase in heart rate similar to what occurs after children do vigorous exercise or have a caffeinated drink. When this occurs the trained staff will reassure the child that the experience will be short-lived and resolve without intervention. In addition studies have shown that 50% of children receive a bronchodilator treatment in the first 5 years of life.

Blood draw

The subject may feel some pain when their blood is drawn. There is a small chance the needle will cause bleeding, a bruise, an infection, or fainting.

Personnel trained in blood draws in children will perform the blood draw using standard precautions. When possible, a topical numbing agent will be used to minimize pain to the child.

Confidentiality

There is a risk to confidentiality. This risk will be minimized by storing the subjects personal and medical information collected as part of this study in a secure location as detailed in section 8 above. Risks will be minimal as there is no intervention being done.

11.2 Potential Benefit to Subjects

The subject may or may not benefit from taking part in this follow-up study. The benefits of being in the study may be a chance that the PFTs demonstrate an unrecognized airway hyper responsiveness which would be cautionary for the subject and their parents. The subject and their family will also receive additional smoking cessation information and education on the effects of second hand smoke exposure. Participation may provide information about the long term effects of supplemental vitamin C given to pregnant smokers on the respiratory outcomes of their offspring. Still, the subject may get no direct benefit from this study.

12. Resources Available

PFTs in infants less than 5-6 years of age can be challenging but of note, Dr. Tepper and the laboratory at the collaborating center at Riley Children's hospital published the first normative data for spirometric measurements in young children between 3 and 6 years of age.[80] All sites have a track record for reproducible infant PFTs and extensive cross training will occur between sites prior to testing to ensure reproducibility.

13. Drugs and Devices

A bronchodilator, albuterol, will be used during spirometry. See package insert for more information.

A topical anesthetic, LMX4, will be used when possible during blood draws. See package insert for more information.

14. Multi-Site Coordination

Dr. McEvoy at OHSU will obtain approval of the protocol, consent, and other pertinent items at OHSU and will request that OHSU provides oversight of this minimal risk protocol to the IRBs at Indiana University (IU) and the PeaceHealth System IRB of PHSW and will request that these IRBs accept the oversight of the OHSU IRB. This will be done via signed authorization forms between OHSU and the respective IRBs. All approved items will be uploaded and maintained at the IU and PHSW IRB prior to starting and throughout the study.

Prior to beginning the study, all sites will have a site initiation by the PI. At that visit, the study protocol and procedures will be reviewed, with site sponsor, study coordinator, study respiratory staff, and other medical staff. A manual of operations will be provided and reviewed. The PI will meet separately with study coordinator to review all regulatory and study procedures. Appropriate recording of clinical data as specified by FDA Regulations for Good Clinical Practice will be reviewed with study personnel.

During subject enrollment, the PI will communicate regularly with site coordinators and through the data center to review consent and subject status and data completion. Sites will complete data on case report forms (CRFs) and then enter data into a secure REDCap database housed on the UCSF server. Data will be assessed for quality via automated and manual processes. Queries (data discrepancies) will be generated to identify potential errors in the study data and to resolve them in a timely manner. Copies of the original site CRFs will be faxed or pdf to DCC on a scheduled basis for comparison with REDCap entries. Original CRF documents are kept by the site in a secure location for their records. The study coordinator at each site (or designate) will review all CRFs for completeness, accuracy and consistency prior to entry into REDCap.

15. Community-based Participatory Research- Not applicable

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Statistical Analysis Plan: VCSIP Follow-up through 5 years of age

Airway Function Outcomes: Covariates and Stratification

The statistical analysis of the FEFs performed at 5 years of age will be based on intention to treat. We will analyze the FEFs in infants born to mothers randomized to vitamin C versus placebo, using analysis of covariance general linear models. Included in these models will be treatment arm, clinical site, and gestational age at randomization (see Table 1) and the covariates race (using mothers' race as a binary variable i.e. white/non-white), infant's sex, and length at the time of the 5 year old airway function testing.

Table 1. Randomization Strata

	Gestational age at randomization	
	≤ 18 weeks	**> 18 weeks**
OHSU	Strata 1	Strata 2
SWW	Strata 3	Strata 4
IU	Strata 5	Strata 6

Primary Outcome: FEF_{25-75} at Five Year Airway Function Testing

Forced expiratory flow at 25-75% of expired volume, (FEF_{25-75}) measured at 5 years of age with spirometry in infants born to mothers randomized to vitamin C versus placebo is the primary outcome. The statistical analysis of airway function tests will be based on intention to treat. General linear mixed model, analysis of covariance (ANCOVA) will be used to compare the FEF_{25-75} at the 5-year-old time point between treatment groups. Included in these models will be treatment arm, clinical site, and gestational age at randomization (≤18 vs >18 weeks) and the covariates of infant sex, maternal race, and infant length at testing. We will initially examine the distribution of each outcome variable using the Shapiro Wilk test and apply data transformations if indicated. If there is a significant difference among the strata at the significance level of 0.05, interaction between study sites and gestational age at randomization, the interaction term will be included in the model and differences will be reported. If the randomization stratum is not statistically significant, sites and gestational age at randomization will be added in the analysis as additive variables. The 5-year completed population will be used for this analysis. These are children who had technically acceptable spirometry tests between their 5^{th} and 6^{th} birthday.

Secondary Outcomes:

Other additional Airway Function Tests measured at 5 Years of Age

Using a similar statistical analysis plan as for the primary outcome of FEF_{25-75} at the 5 year AFT, we will explore differences between infants in the two allocation groups for the additional spirometric outcomes of: FEF at 50% of expiration (FEF_{50}), FEF at 75% of expiration (FEF_{75}), forced expired volume in 1.0 seconds ($FEV_{1.0}$), forced vital capacity (FVC), and $FEV_{1.0}$ / FVC at the 5 year spirometry test. Inspiratory resistance (Ri) measured at 8 Hz by forced oscillometry testing and will be analyzed as above at 5 years of age.

VCSIP Renewal Analysis Plan for Clinical Secondary Outcomes

All analyses of secondary clinical outcomes will be performed using the intention-to-treat (ITT) paradigm, unless specifically stated.

We will adjust for the covariates that affect pulmonary function in infants: mother's race (as a binary variable – white versus non-white), infant's sex, and the randomization strata (study site and gestational age stratification at randomization). Infant length and corrected gestational age will not be included as covariates as they pertain to a point in time. To compare the incidence of current and recurrent wheeze and other clinical outcomes between the children of randomized smokers, we will use data obtained quarterly from the same validated respiratory questionnaire (RQ) that was administered during the VCSIP1 and from clinician reports.

A.Definition/Identification of Current Wheeze through Five Years of Age

The primary clinical outcome of the trial will be current wheeze. While the origins of asthma likely start perinatally, making a definite diagnosis of asthma is difficult even by 5 years of age, and wheezing illnesses may precede an asthma diagnosis. Two hundred and thirteen children were reconsented back into this renewal study from the VCSIP1 trial. To be included in this analysis of current wheeze, the child had to have at least one respiratory questionnaire completed after their fourth birthday.

The composite outcome of current wheeze will be defined as any report of wheeze or the use of an asthma medication or the diagnosis of asthma in the child between the 4th and up to the 6th birthday. This includes a positive response to any of the following questions obtained from the administered respiratory questionnaire (Question numbers below refer to numbering from this questionnaire)

- Parental report of wheeze on any respiratory questionnaire administered between 4th and 6th birthday. These questions start with the preface of "since the last time we talked, OR in the last 12 months:
  - Questions that will be summarized include:
    - Q1. "Has your child had wheezing or whistling in the chest?"
- Healthcare provider diagnosis of wheeze
  - Questions that will be summarized include:
    - Q2. "Has a health care provider (HCP) said your child has wheezy or asthmatic bronchitis?"
    - Q3. "Has a HCP said your child has asthma?"
    - Q14. "Has your child had bronchitis or bronchiolitis?"
    - Q18 "Has your child had to stay overnight in the hospital for any reason..?"
      - Bronchitis and bronchiolitis, wheezing illness, asthma or asthma exacerbation
    - Q 19. "…has your child been seen by a HCP…wheezing, asthma, or wheezy or asthmatic bronchitis…emergency room, doctor's office, urgent care or clinic?"
- Bronchodilator or steroid inhaler/oral use (from chart review and parental report)
  - Questions that will be summarized include:
    - Q4. "Has your child been given medication for wheezing, asthmatic bronchitis, asthma?"
    - Q10b. "Has your child been given any medicine for a cough…when he/she does not have a cold?"
    - Q10b1. "Which of the following types of medications for a cough without a cold: bronchodilators, steroid inhalers, leukotriene modifiers, steroid pills, .."
    - Q 17. "Has you child been given any medication other than those that we have already asked you about?"
    - Q18b. Nebulizer or inhaler treatment in hospital?

A positive answer to any of the above from the respiratory questionnaire administered between the child's fourth and sixth birthday will count as an incidence of wheezing. A negative response to *all* components will indicate that wheezing did not occur.

We will compare the occurrence of current wheeze between infants in the vitamin C and placebo groups using this composite variable in a generalized linear mixed model ANCOVA. Randomization and covariates as outlined above will also be included in this analysis. Refer to Appendix 1 for a break out of all variables included in the combined current wheeze variable.

APPENDIX 1 – Wheeze Outcome Variable

The composite wheeze variable consists of the following variables collected on the quarterly Infant Respiratory Questionnaire :

Field Label	Response Required/ Composite Notes (which to count)
PARENTAL REPORT	
1. Since [birth/the last time we talked] has your child had wheezing or whistling in his/her chest?	Any yes, ever. Include an overall count of individuals that answered yes as well as a second count of individuals that reported yes 2 or more times
14. Has child had any of the following illnesses?	14c. Bronchitis=1 (Yes) 14d. Bronchiolitis=1 (Yes)
HEALTHCARE PROVIDER	
2. Since [birth/the last time we talked] has a health care provider said that your child has wheezy or asthmatic bronchitis?	Response = 1 (Yes)
3. Since [birth/the last time we talked] has a health care provider said that your child has asthma?	Response = 1 (Yes)
18. Reason for hospitalization code	Response = 3 (Bronchitis) or 4 (Bronchiolitis) or 5 (Wheezing) or 8 (Asthma/asthma exacerbation)
19. Not counting hospitalizations, has [CHILD] been seen by a doctor or health care provider because of problems with wheezing, asthma, or wheezy or asthmatic bronchitis, since [birth/the last time we talked]? Include visits to an emergency room, a doctor's office, urgent care, or clinic.	Response = 1 (Yes)
MEDICATION USE	
4a. Since the last time we talked, OR in the last 12 months, which of the following types of medication has child been given?	4a1. Bronchodilator inhalers/nebulizers, pills, or syrups= 1 (Yes) 4a2. Steroid inhalers/nebulizers= 1 (Yes) 4a3. Leukotriene modifiers=1 (Yes) 4a4. Steroid pills or liquids= 1 (Yes) 4a6a. If other, specify medication types. Refer to wheeze_med___1=1 (Checked)
10b. Has child been given any medicine for a cough when he/she did not have a cold?	10b1a. Bronchodilator inhalers/nebulizers, pills, or syrups= 1 (Yes) 10b1b. Steroid inhalers/nebulizers= 1 (Yes) 10b1c. Leukotriene modifiers=1 (Yes) 10b1d. Steroid pills or liquids= 1 (Yes) 10b1f . If other, specify medication types. Refer to wheeze_med___1=1 (Checked)
17. Specify other medications [1]	refer to wheeze_med____1= 1 (Checked)

Field Label	Response Required/ Composite Notes (which to count)
18c. Nebulizer/Inhaler (breathing) Treatment in Hospital?	Response = 1 (Yes)
If Other medication was used, medication was	Response = 1 (Yes)

[1] Other medications: albuterol, albuterol nebulizer, pills, or syrups, steroid inhalers, nebulizers, leukotriene modifiers, steroid pills or liquids

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