A Study of Gamma Tocopherol-enriched Supplement on Lower Airway Responses to Inhaled Wood Smoke in Healthy Adults (SmokeyT)

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6.0. PROTOCOL

A PHASE II RANDOMIZED, DOUBLE BLINDED, PLACEBO CONTROLLED STUDY OF GAMMA TOCOPHEROL-ENRICHED SUPPLEMENT ON LOWER AIRWAY RESPONSE TO INHALED WOOD SMOKE

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Study Product: Gamma E Gems (J.R. Carlson Laboratories, Inc., 600 W University Drive Arlington Heights, IL 60004)

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List of Abbreviations

5-NO_2-γT: 5-nitro-gamma tocopherol

γT: gamma tocopherol

γ-CEHC: 2,7,8-trimethyl-2-(B-carboxy-ethyl)-6-hydroxychroman

αT: alpha tocopherol

α-TTP: alpha tocopherol transfer protein

ANC: absolute neutrophil count

AUC: area under the curve

BAR: brachial artery reactivity

BSA: body surface area

COX-2: cyclooxygenase-2

CYP3A: cytochrome P450 3A

FA: filtered air

FMD: flow mediated dilation

FDR: fractional disappearance rate

FEF_{25-75}: forced expiratory flow during mid portion (25%-75%) of forced vital capacity

FEV_1: forced expiratory volume in 1 second

FRC: functional residual capacity

FVC: forced vital capacity

GSTM1: glutathione-S-transferase mu 1

HF: high frequency

HRV: heart rate variability

IDS: investigational drug service

LDMS: lab data management system

LF: low frequency

LPS: lipopolysaccharide

LVS: left ventricular strain

mT-γT: mixed tocopherol-γT

NSBR: nonspecific bronchial reactivity

O_3: ozone

PBMC: peripheral blood mononuclear cell

PC_{20}: provocative concentration resulting in 20% reduction in FEV_1

PF: peak flow

PGD_2/PGE_2: prostaglandin D_2/prostaglandin E_2

PM: particulate matter

PPM: parts per million

PMN: polymorphonuclear leukocytes

PNN50: mean number of times per hour in which the change in consecutive normal sinus (NN) intervals exceeds 50 milliseconds

QTVI: QT variability index

SDNN: standard deviation of normal to normal R-R intervals

TEOM: tapered element oscillating microbalance

T_H1: T helper type 1

VAPS: versatile air pollution sampler

VLF: very low frequency

WSP: wood smoke particles

Study Summary

Title	A phase II randomized, double blinded, placebo-controlled study of gamma tocopherol-enriched supplement on lower airway responses to inhaled wood smoke in healthy adults
Short Title	Gamma tocopherol for wood smoke-induced airway inflammation
IRB Number	17-2303
Phase	II
Study Duration	3 years
Study Center(s)	University of North Carolina Hospitals in Chapel Hill, North Carolina, 27599-7310
Objectives	To determine the efficacy of 1100 mg gamma tocopherol-enriched supplement for mitigating inhaled wood smoke particle-induced airway inflammation in healthy adults with no more than mild asthma
Number of Subjects	35 subjects

Diagnosis and Main Inclusion Criteria	Healthy adult volunteers with no more than mild asthma
Study Product, Dose, Route, Regimen	Gamma E Gems (J.R. Carlson Laboratories, Inc., 600 W University Drive Arlington Heights, IL 60004).)
Statistical Methodology	Change in sputum % neutrophils (%PMNs) [Δ postWSP-preWSP (γT) vs Δ postWSP-preWSP (placebo)]. Statistically significant differences will be determined using two-sample tests, either parametric (t-test) or non-parametric (Wilcoxon signed-rank test) depending on whether the normality assumption is met.

1. Introduction

This document is a protocol of a human research study. This study is to be conducted according to US and international standards for Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

1.1 Background

Asthma is among the most prevalent chronic diseases in the U.S. (1) and represents a source of significant burden to patients and healthcare systems. Acute exacerbation of asthma is characterized by airway inflammation, bronchoconstriction, increased production of airway mucous, and decreased mucociliary clearance with formation of mucous plugs (2, 3). While corticosteroids are often the treatment of choice, their use is associated with a number of adverse effects, particularly when administered systemically. Currently there is an unmet need for non-steroidal treatment options for asthma, and Vitamin E has been proposed as one such treatment to combat inflammatory features of asthma.

Among the isoforms of vitamin E that can be tested in asthma are α-tocopherol (αT), the predominant isoform found in over the counter Vitamin E supplements, and gamma tocopherol (γT), the predominant isoform found in foods. Intervention trials of αT in humans with asthma have been generally disappointing (4, 5). However, γT and its primary metabolite 2,7,8-trimethyl-2-(B-carboxy-ethyl)-6-hydroxychroman (γ-CEHC) have been shown to have a number of anti-inflammatory activities not observed with αT and its metabolites (6-10). These actions include scavenging reactive nitrogen species to form 5-nitro-y-tocopherol (5-NO2-γT) (6) and inhibition of cyclooxygenase-2 (COX-2) and 5-lipooxygenase, reducing inflammatory eicosanoid production (7-9).

Particulate matter (PM) is a leading cause of respiratory tract and cardiovascular disease in the United States and world-wide. Wood smoke particles (WSP) derived from wildland and other fires account for a significant fraction of ambient air PM. Health effects associated with WSP include acute bronchitis, asthma exacerbation, pneumonia, cough and systemic inflammation. While these effects are seen in both healthy and asthmatic individuals, many studies indicate that asthmatics have increased susceptibility to the effects of WSP. Our center has developed a 500 μg/m3 WSP exposure protocol (levels similar to those encountered by firefighters and residents

in close proximity to wildland burn sites) that induces airway and systemic inflammation in healthy volunteers. As with other pollutants, these inflammatory responses modulate non-specific bronchial reactivity (NSBR), inflammatory cell recruitment to the airways (primarily neutrophils), and potentially cardiovascular function.

Our team has focused on gamma tocopherol as a nutritional intervention to prevent inflammatory responses to air pollutants such as WSP. Building on animal and in vitro preclinical studies, we have established that 1400 mg/day of oral γT-enriched supplement for 7 and 14 days in healthy volunteers and mild asthmatics, respectively, inhibited neutrophil influx into the airways, reduced production of sputum mucins, and improved mucociliary clearance following challenge with inhaled endotoxin, another common component of PM (10, 11). The findings occurred in the context of significantly increased plasma concentrations of γT and its active metabolite γ-CEHC. Given our findings in these early phase clinical trials, γT supplementation is an attractive approach to prevent WSP-induced adverse health effects. We propose to use γT supplementation in a human model of WSP inhalation to mitigate key features of airway inflammation: inflammatory cell recruitment, production of inflammatory cytokines and mucous, and changes in airway physiology.

1.2. Investigational Agent

Gamma E Gems 548 mg softgels, (J.R. Carlson Laboratories, Inc., 600 W University Drive Arlington Heights, IL 60004.)

1.3. Dose Rationale and Risk/Benefits

Gamma tocopherol will be administered in softgel form, with each softgel containing 874.1 mg of tocopherols, , including:

Alpha Tocopherol:	141.0 mg/sg (softgel)
Beta Tocopherol:	18.1 mg/sg
Gamma Tocopherol:	548.0 mg/sg
Delta Tocopherol:	167.0 mg/sg

Subjects will consume two softgels by mouth once daily for 7 days. This dosing regimen was chosen based on the results of our previous early phase clinical trials examining the impact of gamma tocopherol on LPS-induced airway inflammation in healthy adults (11) and adults with asthma (26). These studies tested a 7 and 14 day course of treatment, respectively, and found similar plasma concentrations of γT and active metabolites in both studies. Furthermore, we showed in both studies that γT significantly reduced LPS-induced sputum neutrophilia compared to placebo. Based on our previous findings, we will now study the efficacy of γT for mitigating WSP-induced airway inflammation.

We have discussed the potential benefits of γT supplementation for protection against ambient air pollutant-induced airway inflammation in healthy volunteers and adults with mild asthma in section 1.1. In terms of risk, the most significant safety concern for Vitamin E supplements in general is increased bleeding risk, and most trials have used supplements containing predominantly αT as opposed to γT. The Alpha Tocopherol Beta Carotene cancer prevention

study, a randomized, double blind, placebo controlled trial of αT and b-carotene supplementation in middle aged male smokers, found that alpha tocopherol supplementation was associated with increased risk of fatal subarachnoid hemorrhage (13). To our knowledge, there are no published studies of γT supplementation that show an increased risk of bleeding. In our own previous studies, we have not observed any significant bleeding events nor any changes in PT, aPTT, or INR with γT supplementation at doses similar to the proposed study.

An additional area of concern was raised by findings from the Coronary Artery Risk Development in Young Adults (CARDIA) study, a population-based observational study. In this study, higher plasma γT levels in young adults (not taking Vitamin E supplements) were associated with lower lung function measurements (FEV_1 and FVC) (14). However, others have shown that increased *dietary* vitamin E intake had protective effects on lung function in adults (15). Plasma γT levels have not been significantly associated with risk of asthma or asthma outcomes. However, increased dietary vitamin E, which is predominantly gamma tocopherol (γT), has been associated with decreased occurrence of asthma in both children (16-18) and adults (19).

Based on our own previous studies, the most common adverse effects of γT are diarrhea, flatulence, abdominal bloating, and nausea. In our most recent randomized, placebo controlled crossover study evaluating 14 days of γT-enriched supplementation (1400 mg daily) in 23 adult volunteers with asthma (26), diarrhea was reported in 6 participants (26%) on active treatment, compared to 1 participant (4.3%) on placebo. Nausea was reported in 6 participants (26%) on active treatment, compared to 2 participants (8.7%) on placebo. Bloating and flatulence were reported in 2 participants (8.7%) and 1 participant (4.3%) on active treatment, respectively, with no reports of these symptoms in subjects receiving placebo. One participant withdrew from the study due to intolerable gastrointestinal symptoms. In all other participants, gastrointestinal symptoms were transient in nature, beginning after the first or second dose of study drug and self-resolving despite continued treatment for the 14-day period.

2. Study Objective

The primary objective of this study is to examine the effectiveness of gamma tocopherol for mitigating inhaled WSP-induced airway inflammation in healthy adults and mild asthmatics, specifically airway inflammatory cell recruitment and cytokine production, mucous production, and non-specific bronchial reactivity.

3. Study Design

3.1. General Design

This study is designed to evaluate the effectiveness of gamma tocopherol for mitigating features of airway inflammation induced by inhaled WSP by examining airway inflammatory cell recruitment and cytokine production, and mucous production,. We anticipate 3 years will be required for completion of study visits for all participants.

Upon successful completion of two screening protocols (one as a general screen to determine eligibility for airway challenge studies and a second to identify those individuals who are deemed "responders" to WSP inhalation, defined as ≥10% increase in sputum PMNs following exposure), participants will be enrolled to participate in a randomized, double blinded, placebo-controlled, crossover study consisting of 8 visits. Participants will be randomized to γT or placebo treatment followed by chamber exposure with WSP. After a 4-week washout period, participants will cross over to the alternate treatment group.

Following randomization to placebo or gamma tocopherol treatment, subjects will return for baseline (V0) and training visits (V1). Participants will consume 1400 mg of γT-enriched supplement or placebo once daily for 7 days. Participants will undergo WSP exposure (V2), then return the following day for a 24 hour post-exposure visit (V3). After a 4 week wash out period, subjects will present for a review of medical history and adverse events and cross over to the alternate treatment (V4). They will undergo a second WSP exposure (V5) followed by a 24 hour post-exposure visit (V6). Five to 10 days after the exposure, participants will return for a study completion visit (V7).

3.2. Primary Study Endpoint

The primary endpoint will be change in sputum %PMNs [Δ (postWSP (4 hr) – preWSP)[γT] vs Δ (postWSP (4 hr)– preWSP)[placebo]] and change in sputum %PMNs [Δ (postWSP (24 hr) – preWSP)[γT] vs Δ (postWSP (24 hr)– preWSP)[placebo]].

3.3. Secondary Study Endpoints

The secondary endpoints of the study will be change in peripheral blood absolute neutrophil count (ANC) in cells $x10^9$/L [Δ (postWSP (4 hr) – preWSP)[γT] vs Δ (postWSP (4 hr)– preWSP)[placebo]] and [Δ (postWSP (24 hr) – preWSP)[γT] vs Δ (postWSP (24 hr)– preWSP)[placebo]].

Exploratory endpoints of interest include:

1. Role of asthma as a modifier of γT treatment effect on WSP-induced airway inflammation
2. Spirometry measurements – forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), FEV_1/FVC ratio
3. Secretion of sputum mucins (total sputum mucins, MUC5AC, MUC5B) will be determined by Western blot
4. Serum cytokine concentrations via Mesoscale:
    a. T_H1 cytokines: IL-1b, IL-6, IL-8, TNFa
5. Sputum cytokine concentrations via Mesoscale:
    a. T_H1 cytokines: IL-1b, IL-6, IL-8, TNFa
6. Flow mediated dilation (FMD) using brachial artery ultrasound
7. Left ventricular strain (LVS)
8. Serum concentrations of gT aT gCEHC and aCEHC.

3.4. Primary Safety Endpoints

Safety Endpoints: Safety endpoints include respiratory symptoms such as wheezing or shortness of breath requiring albuterol, significant bleeding events, or significant change in blood coagulation markers (PT, aPTT) such that these measurements are outside of normal range, and adverse events (AEs) and severe adverse events (SAEs). Exploratory safety endpoints include GI symptoms with γT vs Placebo. Adverse event data will be collected through 7 days post study drug treatment for both treatment periods. UNC Chapel Hill Human Research Protection Program Standard Operating Procedures will be followed for reporting adverse events/unanticipated problems to the Biomedical IRB. All SAEs will be reported to the CDER of the FDA as well as to the UNC Biomedical IRB within 24 hours of recognition of the event. Adverse events will be reported to both the FDA and UNC IRB on no less than an annual basis, or when the protocol is completed.

3.4.1. Criteria for safety prior to initiation of wood smoke inhalation challenge

(Subjects not meeting these criteria the morning of challenge will not proceed with wood smoke inhalation challenge):

1. FEV_1 of at least 75% of predicted (without use of bronchodilating medications for 12 hours) consistent with lung function of persons with no more than mild intermittent or mild persistent asthma.
2. Baseline oxygen saturation of at least 93%.
3. No history of viral respiratory tract symptoms within 4 weeks of challenge.
4. No current symptoms of rhinorrhea, sneezing, nasal or ocular pruritus consistent with allergic rhinoconjunctivitis.

3.4.2. **Criteria for safety of a given individual following challenge which would suspend the individual from further participation in the study will include**:

1. No greater than 25% relative decrease in FEV_1 or FVC from WSP pre-challenge values, which does not improve within the first 2 hours of challenge.
2. FEV_1 must recover without treatment to within 90 % of baseline within 24 hours.
3. A symptom score no greater than 45 (representing a ranking of "moderate" on each of 15 criteria, with a maximum score of 60, see section 5.2.8. below) with improvement to a score of 15 or less within 6 hours without therapy.
4. Specifically, no symptom score greater than "moderate" (3 on a scale of 0-4) for shortness of breath or cough at 24 hours post-challenge.
5. Oxygen saturation of ≥93% throughout the exposure period.
6. If albuterol therapy is needed more than once during an observation period.
7. Any significant abnormalities in PT and aPTT during the treatment periods will be assessed and reported to CDER, IRB, and the funding agency.

Criteria for safety within the entire protocol (failure of which would result in suspension of further study until consultation with the CDER of the FDA and the UNC IRB) will include the following:

1. The study will be suspended if 3 of the first 10 participants fail the individual safety criteria outlined above. If this occurs, the study will remain suspended until consultation with the CDER of the FDA.
2. Once 10 subjects have been enrolled, study suspension will occur if more than 25% of all participants fail the individual safety criteria outlined above
3. No occurrence of any SAE

Subjects will be under direct supervision for 6 hours from the start of WSP challenge and will have contact information for after-hours access to a study physician. In the event of an unexpected event, such as wheezing or bronchospasm, the physician will follow the usual medical standard of care to treat the subject.

4. Subject Selection and Withdrawal

Preliminary results from our screening WSP exposure study have shown that approximately 57% of healthy volunteers were responsive to the inflammatory effects of WSP. Based on this experience, we estimate we will need to screen 50 volunteers to identify 28 who are responsive to inhaled WSP. Anticipating at attrition rate of 20%, we will need to identify 35 volunteers for the intervention study, requiring an overall screen of a minimum of 64 volunteers. There will be no gender or ethnic restrictions. The target completion goal is 28 healthy volunteers and mild asthmatics. Individuals who meet all of the following criteria are eligible for enrollment as study participants.

4.1. Inclusion Criteria

1. Age 18-45 years, inclusive, of both genders
2. Negative pregnancy test for females who are not s/p hysterectomy with oopherectomy
3. FEV_1 of at least 75% of predicted (without use of bronchodilating medications for 12 hours), consistent with lung function of persons with no more than mild intermittent or mild persistent asthma.
4. Oxygen saturation of ≥93% and blood pressure within the following limits: (Systolic between 150-85 mmHg, Diastolic between 90-50 mmHg).
5. Ability to provide an induced sputum sample.
6. Subject must demonstrate a ≥ 10% increase in sputum neutrophils following inhaled WSP exposure, when compared to baseline sputum (to be completed in a separate protocol).
7. Ability/willingness to discontinue inhaled corticosteroids, montelukast, and cromolyn for 2 weeks without increased symptoms or increased need for beta agonist rescue medication prior to screening and through the course of the study.
8. Documented vaccine to Covid 19

4.2. Exclusion Criteria

Patients who meet *any* of these criteria are *not* eligible for enrollment as study participants:

1. Clinical contraindications:
    a. Any chronic medical condition considered by the PI as a contraindication to the exposure study including significant cardiovascular disease, diabetes, chronic renal disease, chronic thyroid disease, history of chronic infections/immunodeficiency.
    b. Viral upper respiratory tract infection within 4 weeks of challenge.

c. Any acute infection requiring antibiotics within 4 weeks of exposure or fever of unknown origin within 4 weeks of challenge.
d. Abnormal physical findings at the baseline visit, including but not limited to abnormalities on auscultation, temperature of 37.8° C, Systolic BP > 150mm Hg or < 85 mm Hg; or Diastolic BP > 90 mm Hg or < 50 mm Hg, or pulse oximetry saturation reading less than 93%.
e. Physician directed treatment for an asthma exacerbation, including requirement for oral corticosteroids, within the preceding 12 months.
f. Moderate or severe asthma
g. Asthma symptoms more than 2 times a week, which would be characteristic of a person with moderate or severe persistent asthma.
h. Daily requirement for albuterol due to asthma symptoms (cough, wheeze, chest tightness) which would be characteristic of a person with moderate or severe persistent asthma (not to include prophylactic use of albuterol prior to exercise)
i. Requirement for asthma controller therapy greater than low dose ICS or leukotriene receptor antagonist. Persons requiring medium or high-dose ICS, ICS/LABA, ICS plus leukotriene receptor antagonists, omalizumab or other biologic therapies for asthma control will not be enrolled.
a. Nighttime symptoms of cough or wheeze greater than 1x/week at baseline (not during a clearly recognized viral induced asthma exacerbation) which would be characteristic of a person of moderate or severe persistent asthma.
b. History of intubation for asthma
c. If there is a history of allergic rhinitis, subjects must be asymptomatic of allergic rhinitis at the time of study enrollment.
d. Mental illness or history of drug or alcohol abuse that, in the opinion of the investigator, would interfere with the participant's ability to comply with study requirements.
e. Cigarette smoking > 1 pack per month
f. Unwillingness to use if sexually active (IUD, implant, sterilization, or when used in combination: birth control pills/patch and barrier methods). (i.e., those that, alone or in combination, result in a failure rate of <1% per year when used consistently and correctly). Note that male condom use, hormonal contraceptive pills, or hormonal contraceptive patches used alone have estimated failure rates that do not meet these criteria.
g. Abnormal PT or aPTT values at screening or during the treatment period. Normal values will be those published by the clinical lab (Labcorp, INC).
h. Use of immunosuppressive or anticoagulant medications including routine use of NSAIDS. Oral contraceptives are acceptable, as are Antidepressants and other medications may be permitted if, in the opinion of the investigator, the medication will not interfere with the study procedures or compromise safety and if the dosage has been stable for 1 month.
i. Orthopedic injuries or impediments that would preclude bicycle or treadmill exercise.
j. Inability to avoid NSAIDS, Multivitamins, Vitamin C or E or herbal supplements.
k. Allergy/sensitivity to study drugs or their formulations
l. Known hypersensitivity to methacholine or to other parasympathomimetic agents

m. Unwillingness to avoid coffee, tea, cola drinks, chocolate, or other foods containing caffeine after midnight on the days that methacholine challenge testing is to be performed.
n. Positive test for Covid 19 in the past 90 days

2. Pregnant/nursing women and children (< 18 years as this is age of majority in North Carolina) will also be excluded since the risks associated with woodsmoke exposure to the fetus or child, respectively, are unknown and cannot be justified for this non-therapeutic protocol. Individuals over 45 years of age will not be included due to the increased possibility of co-morbidities and need for prohibited medications.
3. Inability or unwillingness of a participant to give written informed consent.

4.3. Withdrawal or Termination

Participants will be terminated early from the study for the following reasons:

1. The participant elects to withdraw consent from all future study activities including follow up.
2. The participant is "lost to follow up" (i.e., no further follow up is possible because attempts to reestablish contact with the participant have failed).
3. The participant dies.
4. The participant develops a medical condition or is started on a new medication(s) not previously mentioned in the list of prohibited medications that, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant's ability to comply with study requirements or that may impact the quality of the data obtained from the study.
5. The participant meets any of the individual stopping rules as delineated.
6. The study is terminated for any reason.

Participants with early termination from this study will not be replaced.

5. Study Procedures

5.1. Schedule of Events

Each subject will have 8 study visits (excluding the general screening visit and wood smoke exposure screening visit to identify responders, which are under separate protocols):

5.1.1. Screening Visits

General Screening: To identify non-asthmatic and asthmatic subjects suitable for WSP exposure, subjects will complete a general screening protocol (UNC IRB approved study #98-0799, *Database and Screening Protocol for Research Studies of the Center for Environmental Medicine & Lung Biology*).

Screening WSP exposure: This protocol will identify subjects who experience a ≥ 10% increase in sputum neutrophilia after WSP exposure, compared to baseline sputum (UNC IRB approved study #15-1775, *To identify persons who are susceptible to WSP-induced inflammation and examine the role of GSTM1 and other factors in this susceptibility*). This protocol involves a detailed review of the subject's medical history and medication use, lung function (spirometry), collection of an induced sputum sample, and WSP exposure.

5.1.2. Visit 0: Baseline, determine eligibility (*at least 1 month after screening WSP exposure*):

1. Obtain informed consent.
2. Review any change in medical status since the last visit.
3. Vital signs, oxygen saturation, resting blood pressure
4. Urine pregnancy test for females
5. Physical exam
6. Obtain 12-lead EKG
7. Spirometry
8. Sputum induction
9. Venipuncture (CBC with differential, serum cytokines, serum C-reactive peptide, lipid panel, LDH, fibrinogen, PT, aPTT, INR, gT and metabolite levels).
10. HRV will be collected throughout the entire visit with a Spacelabs monitor. A 30 minute controlled rest will be performed at the end of visit.

5.1.3. Visit 1: Training/Dispense Study Drug (Period 1) *(≤ 1 month from baseline visit and at least 1 week prior to V2 Exposure Visit and up to 3 weeks) (we request participants to be fasting for this visit, therefore it is scheduled in the morning)*

1. Review any changes in medical status since the last visit.
2. Vital signs, oxygen saturation, and GI symptom questionnaire.
3. Urine pregnancy test for females.
4. Spirometry
5. Baseline flow mediated dilation (FMD) measurement
6. Baseline left ventricular strain (LVS) measurement
7. Volunteers will be trained on the cycle ergometer to reach an exercise level producing a minute ventilation of 20 L/min/m^2 body surface area (BSA). Subjects will be monitored with a standard 3 lead ECG during exercise.
8. Dispense study drug and symptom diary

5.1.4. Visits 2 (Period 1) and 5 (Period 2): WSP exposure

1. Vital signs, oxygen saturation, resting blood pressure
2. GI and WSP Exposure symptom questionnaire
3. Collect symptom diary
4. Urine pregnancy test for females
5. HRV will be collected throughout the entire visit with a Spacelabs monitor. The monitor will be worn until the 24 hours post exposure visit.
6. Spirometry
7. Venipuncture (CBC with differential, serum cytokines, serum C-reactive peptide, lipid panel, LDH, fibrinogen, gT and metabolite levels)
8. 500 µg/m3 WSP chamber exposure for 2 hours, with intermittent exercise and rest. Subjects will alternate 15 minutes of exercise on a cycle ergometer (intensity needed to achieve 20 L/min/m2 BSA determined at baseline visit) with 15 minutes of rest.
9. Symptom questionnaire (immediately prior to exit from the chamber)
10. Spirometry and resting blood pressure (immediately following exit from the chamber)
11. Lunch - low fat meal is provided. The meal must be the same during each exposure.
12. Obtain 30 minute controlled rest HRV, FMD, and LVS (90 minutes post-WSP exposure)

13. Venipuncture (CBC) with differential, serum cytokines, serum C-reactive peptide, lipid panel, LDH, fibrinogen) (4 hours post-WSP exposure)
14. GI and WSP Exposure Symptom questionnaire (4 hours post-WSP exposure)
15. Sputum induction (4 hours post-WSP exposure)

5.1.5. Visit 4: Dispense Study Drug (Period 2) (*following 4 week washout and at least 1 week prior to V5 Exposure Visit and up to 3 weeks)*

1. Review medical history and adverse events
2. Urine pregnancy
3. Vital signs, oxygen saturation, GI symptom questionnaire
4. Dispense study drug
5. Lung function testing for participants who have asthma

5.1.6. Visits 3 (Period 1) and 6 (Period 2): 24 hours post-exposure

1. Review any adverse events
2. Vital signs, resting blood pressure, oxygen saturation
3. WSP Exposure symptom questionnaire
4. Venipuncture (CBC with differential, serum cytokines, serum C-reactive peptide, lipid panel, LDH, fibrinogen, PT, aPTT, INR, gT and metabolite levels)
5. Spirometry
6. Sputum induction
7. .

At least a 4 week, but no more than an 8 week washout is required between Period 1 and Period 2.

5.1.7. Visit 7: Study completion, 5-10 days post-exposure

1. Review any adverse events
2. Vital signs, oxygen saturation, symptom questionnaire
3. If the participant has any health symptoms or concerns, they will receive a physical examination and evaluation by a study physician.

We will contact participants 1 month after participation to follow up and ask about health status.

If the participant develops an upper or lower respiratory tract illness between V0 and V1 or between periods, we will postpone the next study visit for a minimum of 4 weeks after resolution of symptoms. In this case, if V1 occurs >1 month from V0, or if V4 occurs >2 months from V3, to prioritize participant safety we will bring the subject in for an additional visit to collect safety labs, perform spirometry, and review interval history.

If the participant has an illness mid-dosing of the gamma tocopherol/placebo or fails to adhere to the dosing regimen, participants will return 4 weeks after they have taken their last dose or 4 weeks after resolution of symptoms. At that visit, gamma tocopherol/placebo will be re-dispensed. To prioritize participant safety we will review interval history. Subjects with an illness during dosing, we will additionally collect safety labs, and perform spirometry.

All subjects with a symptomatic Covid 19 infection will be deferred for 90 days. Baseline spirometry and safety labs will be assessed prior to any gamma tocopherol/placebo dosing and WSP exposure.

5.1.8. Table of study procedures

	General Screen	Screening WSP Exposure	V0 (Baseline)	V1 (Training)	V2 (period 1) V5 (period 2)	V4 (Dispense study drug)	V3 (period 1) V6 (period 2)	V7 (Completion visit)
Consent	X	X	X					
Review history/AE's	X	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X	X
Urine hCG (females only)	X	X	X	X	X	X		
Dispense study drug				X		X		
WSP Symptom Questionnaire		X			X		X	
GI symptom diary				X	X	X		
Spirometry (FEV_1)	X	X	X	X	X	X (asthmatic participants only)	X	
Allergy Skin Prick Test	X							
Physical Exam		X	X					
Venipuncture		X	X		X		X	
CV measure-ments: FMD, LVS				X (FMD, LVS only)	X		)	
Sputum Induction	X	X	X		X		X	
2 hour WSP chamber exposure		X			X			

5.2. Protocols for Measurements and Observations

5.2.1 Woodsmoke particle chamber exposure

We will employ the exposure protocol described by (20). We will heat whiteoak wood on an electric heating element (Brinkmann, Dallas, Texas, USA) in a Quadrafile 3100 woodstove (Colville, Washington, USA) to generate wood smoke. The smoke will be extracted from the chimney and injected into the chamber air stream, and wood smoke particle concentration will be

controlled using a tapered element oscillating microbalance (TEOM®; Thermo Fisher Scientific, Franklin, Massachusetts, USA) to measure the chamber concentration. A DustTrak 8530 aerosol monitor (TSI, Shoreview, Minnesota, USA) will also be used as an alternative monitor for independent verification of proper TEOM® operation. The particle number concentration will be measured with a Model 3022A CPC (TSI, Shoreview, Minnesota, USA) and the number and size distribution will be measured with a Model 3080L SMPS (TSI). The particle concentration will be verified by weighing filters obtained using a versatile air pollution sampler (VAPS; URG, Chapel Hill, North Carolina, USA). The chamber temperature and humidity will be controlled to approximately 22°C and 40%, respectively. The target average concentration of WSP we will use for this study will be 500 $\mu g/m^3$, with mild exercise to increase minute ventilation (20 $L/min/m^2$ BSA) for 15 minute periods, alternating with 15 minute rest periods across an overall 2 hour total exposure period.

5.2.2. Induced sputum

Sputum samples will be collected using a specific sputum induction protocol in which FEV_1 and FVC will be measured to determine the baseline FEV_1 and FVC values. Asthmatic individuals will be pretreated with 2 to 4 puffs of albuterol prior to the procedure, and post-albuterol spirometry will be collected. The FEV_1 values that match a 10% and 20% fall from baseline will be calculated. An ultrasonic nebulizer will be filled with 15 mL of 3% hypertonic saline (inhalation grade for respiratory use only) to begin the test. The nebulizer will be set to the maximum output setting and turned on. The subject will be instructed to breathe normally (i.e. tidal breaths) through the nebulizer mouthpiece for 7 minutes. The saline will be nebulized through the mouthpiece in a jet stream and inhaled. The nose will not be occluded for this procedure. Prior to expectoration, subjects will blow their nose, rinse their mouth with water, and clear their throat to avoid the inclusion of non-airway fluid samples. The subject will also be encouraged to come off the mouthpiece at any time to cough if a sputum sample from the lower airways (i.e. not from the back of the throat) is ready for expectoration. The sample will be coughed into a sterile specimen jar and capped. Following repeat measurement of FEV_1 after the first 7 minute inhalation period, the concentration of saline will be increased from 3% to 4%, provided the FEV_1 falls by <10% from the baseline value. The same procedure will be followed for the final 7 minute inhalation period using 5% hypertonic saline. The nebulization will be stopped at that point, or earlier, if a sputum sample of good quality is obtained (i.e. visible sputum plugs). The 4% and 5% saline solutions will be mixed for each induction by study staff just prior to induction and a volume of 15-20 mL will be used on each occasion.

5.2.3. Spirometry

Spirometry will be performed according to American Thoracic Society guidelines. Subjects will inhale as deeply as possible, then exhale as rapidly and completely as possible into the spirometer. Measurements obtained from each maneuver include the FVC, the FEV_1, the maximal mid-expiratory flow rate (FEF 25-75%) and the peak flow (PF). The largest FVC and FEV_1, from at least 3 acceptable trials will be selected for analysis and subject must meet the appropriate inclusion/exclusion criteria based on comparison of the subject's measurements to the predicted value for an individual of his/her gender, age, race and height with NHANES III as the predicted set.

5.2.4. Flow mediated dilation measured by brachial artery ultrasound:

Vascular endothelial function will be assessed by measuring flow-mediated dilation of the brachial artery. Ultrasound images of the right brachial artery proximal to the antecubital fossa will be acquired using a Philips EPIQ 7 ultrasound machine equipped with a high-frequency (L9-3) broadband linear-array transducer. The baseline images will be obtained after 10 minutes of supine rest. Flow-mediated (endothelium-dependent) dilation will be assessed by determining the change in arterial diameter in response to reactive hyperemia. Reactive hyperemia will be induced by inflating a pneumatic occlusion cuff placed around the upper forearm to a suprasystolic pressure (approximately 200 mmHg) for five minutes. Images of the artery will be recorded for two minutes after cuff deflation. Gated end-diastolic images will be stored in a digital format on a personal computer for subsequent offline quantification. Measurements will be performed using customized software (Brachial Tools, Medical Imaging Applications, LLC). Arterial diameter will be measured from the lumen-intimal interfaces of the proximal and distal arterial walls. Data from at least ten end-diastolic frames will be averaged for the baseline measurement and from at least three frames at maximum dilation during reactive hyperemia FMD will be calculated as the percent change in diameter from baseline.

5.2.5. Left ventricular strain

LV strain will be assessed by measuring global longitudinal strain using 2-dimensional speckle tracking echocardiography. Speckle tracking imaging is a relatively new technique with which myocardial mechanics can be quantified by tracking groups of intramyocardial speckles. Global longitudinal strain, the average fractional change in myocardial segment length, is a more sensitive index of LV contractility than ejection fraction or other standard measures. Apical 4-chamber, 2-chamber, and long axis 2-dimensional ultrasound images of the heart will be acquired using a Philips EPIQ 7 ultrasound machine and stored for subsequent off-line analysis. Global longitudinal strain will be quantified using Philips QLAB advanced quantification software.

5.2.7. WSP Exposure Symptom Questionnaire

Please indicate if you are experiencing any of the symptoms or restrictions listed below, using the following scale to indicate the severity. Circle the number.

0 = NONE (not present)
1 = TRACE/NOTICED (barely detectable)
2 = MILD/LIGHT (present, but not annoying)
3 = MODERATE (present, but somewhat annoying)
4 = SEVERE/HEAVY (present and very annoying and painful)

SYMPTOMS	NONE	TRACE	MILD	MODERATE	SEVERE
1. HEADACHE	0	1	2	3	4
2. IRRITATION OF THE NOSE	0	1	2	3	4
3. STUFFY NOSE/SINUS CONGESTION	0	1	2	3	4
4. RUNNY NOSE	0	1	2	3	4
5. DRY/SORE THROAT	0	1	2	3	4
6. PAIN on DEEP INSPIRATION	0	1	2	3	4
7. UNUSUAL FATIGUE OR TIREDNESS	0	1	2	3	4
8. EYE IRRITATION	0	1	2	3	4
9. SHORTNESS OF BREATH	0	1	2	3	4
10. SNEEZING	0	1	2	3	4
11. COUGH	0	1	2	3	4
12. WHEEZING/WHISTLING in CHEST	0	1	2	3	4
13. CHEST TIGHTNESS	0	1	2	3	4
14. SWEATING	0	1	2	3	4
15. Other_____	0	1	2	3	4

5.2.8. GI Symptom Questionnaire

Please indicate if you are experiencing any of the symptoms or restrictions listed below, using the following scale to indicate the severity. Circle the number.

0 = NONE	(not present)
1 = MILD	(minimally noticeable but would not cause you to stop normal activities such as going to work or school or physical exercise)
2 = MODERATE	(symptom level would not ordinarily cause you stop normal activities such as going to work or school although it might stop you from doing physical exercise)
3 = SEVERE	(symptom would cause you to consider not going to work or school or to seek medical attention such as calling a healthcare provider, going to student health, or going to the emergency room)

SYMPTOMS	**NONE**	**MILD**	**MODERATE**	**SEVERE**
1. INCREASE IN STOOLS	0	1	2	3
2. DIARRHEA	0	1	2	3
3. BLOATING/FULLNESS	0	1	2	3
4. STOMACH CRAMPING/PAIN	0	1	2	3
5. INCREASE IN PASSING GAS	0	1	2	3
6. VOMITING	0	1	2	3
7. NAUSEA	0	1	2	3
8. HEADACHE	0	1	2	3
9. EASY BRUISING OR BLEEDING	0	1	2	3
10. Other_____	0	1	2	3

6. Statistical Plan

6.1 Sample Size Determination

The primary endpoint of this study is **Δ sputum %PMNs** (post WSP – pre WSP [γT]) at 4 hour vs (post WSP – pre WSP [placebo]) at 4 hours, and (post WSP – pre WSP [γT]) at 24 hours vs (post WSP – pre WSP [placebo]) at 24 hours. Preliminary analyses from our screening study of the effects of 500 μg/m^3 WSP exposure on sputum neutrophilia in healthy volunteers and mild asthmatics demonstrated that, among "responders" (those participants with a ≥ 10% increase in sputum neutrophils following WSP exposure), the mean increase in sputum %PMNs was 28.5±13%. In our studies of γT vs placebo for mitigating LPS-induced change in sputum %PMNs in healthy volunteers (11) and mild asthmatics (26), we found a γT treatment effect size of 14.5% ±25.9. Assuming β=0.8 and α=0.05, a sample size of 28 healthy volunteers and mild asthmatics would be required to detect the same γT treatment effect size. To account for a 25% attrition rate with human studies, we will inflate the sample size to 35 healthy volunteers and mild asthmatics. Importantly, we anticipate that we will decrease subject variability by only recruiting persons with ≥ 10% increase in WSP-induced %PMNs in sputum during the screeing protocol (the so-called "responders").

6.2. Statistical Methods

Our analytic plan was developed in collaboration with Dr. Zhou, the biostatistician for the CEMALB, who will oversee all statistical analysis. For analysis of sputum and circulating PMN responses as well as cytokine changes within each treatment group, we will use (i) descriptive analysis, (ii) two-sample comparison test, and (iii) random effects regression analysis (Equation 1) designed to take advantage of the crossover design (22). Specifically, we will use the tabular and graphic methods to describe/explore the basic relationship between the groups. We will then follow up by **comparing the Δ sputum %PMNs (post WSP – pre WSP [γT]) vs (post WSP – pre WSP [placebo]) using two-sample tests, either parametric (T-Test) or nonparametric (Wilxocon signed-rank test), depending on whether the normality assumption is met. We will transform the data to achieve normality if needed.** Finally**,** we will employ a random effect regression modeling technique (**Equation 1**) that allows us to assess treatment effect while accounting for potential period effect and carry-over effects. This approach is similar to those we have previously described to assess genotype effects (including the GSTM1 null genotype) on response to pollutants (11, 23-25) and is shown below. In all analyses, criterion for significance will be $p \leq 0.05$. To address potential missing data, we will employ multiple imputation methods to address this issue when appropriate; using techniques we have previously described (24).

Linear mixed model approach for global unified analysis of data for secondary and exploratory outcomes:

$Y\_11k=\mu+\pi\_1+\tau\_1+\beta X\_k+s\_1k+\epsilon\_11k$; $Y\_12k=\mu+\pi\_2+\tau\_2+\lambda\_1+\beta X\_k+s\_1k+\epsilon\_12k$ $Y\_21k=\mu+\pi\_1+\tau\_2+\beta X\_k+s\_2k+\epsilon\_21k$; $Y\_22k=\mu+\pi\_2+\tau\_1+\lambda\_2+\beta X\_k+s\_2k+\epsilon\_22k$
μ is the grand mean, π1 and π2 are period effects, τ1 and τ2 are treatment effects, λ1 and λ2 are carryover effects for different sequences, ς1k and ς2k are individual random effects within each sequence, and ε's are random errors. Subscript k is for subjects.

6.3. Data Management

Study coordinators will be responsible for efforts and computations for database management. Data will be initially collected by study coordinators on paper documents. Clinical data such as health history will be collected by study staff as interview or directly measured, such as vital signs, spirometry, etc. All paper forms (source documentation) are maintained by a study coordinator or PI in a binder, 1 binder per subject. All data will be entered into REDCap by a study coordinator within 2 weeks of collection, and will be verified by a 2nd person within 4 weeks to ensure accuracy and completeness. REDCap is a secure web application developed at Vanderbilt University and can be used to collect virtually any type of data (including 21 CFR Part 11, FISMA, and HIPAA-compliant environments). It is specifically geared to support online or offline data capture for research studies and operations. Missing data will be noted and a comment made in the database to explain the missing data. Time stamps will be used when appropriate. At minimum, the visit number will be indicated.

Biological samples collected by coordinators will be delivered to the CEMALB lab by the coordinators in properly labeled containers. On receipt in the lab, samples are initially entered into a software program called LDMS (Lab Data Management System) which tracks samples. Processing of these samples is done per the SOP maintained in the CEMALB quality assurance plan, as some samples are processed immediately and others are processed in batches at later time points. This data is entered into REDCap at appropriate intervals, based on sample analysis.

We will use the data dictionary or REDCap codebook as our course for data codes. All data will be collected by qualified staff using CEMALB SOPs and will be entered into REDCap. Once the data is entered and confirmed by a 2nd person, it will be considered "locked" and will not be changed without documentation with the reason for the change. REDCap limits and monitors who can change data, a feature developed to ensure data integrity.

6.4. Randomization plan

The randomization schedule will be generated by Dr. Haibo Zhou (biostatistician) and provided to the investigational pharmacy. Only Dr. Zhou and the pharmacist will have access to the randomization schedule. Subjects will be allocated to begin the first period of the crossover study with placebo or γT treatment using permuted block randomization with a block size of 4 (2 placebo, 2 γT for the first treatment period of the protocol).

7. Monitoring and Risk Minimization

7.1. Definition of Adverse Event (AE) and Serious Adverse Event (SAE)

Adverse events and serious adverse events will be recorded and reported according to 21 CFR 312.32. An adverse event for a given volunteer will be defined as any untoward medical occurrence associated with the use of a drug whether or not considered drug related and will also include failure of any of the safety criteria outlined in section "3.4.". Additionally, minor upper respiratory tract infections occurring within 96 hours of the exposure will be considered adverse events. Other non-specified clinical illnesses or symptoms, which occur during the treatment period or within 96 hours of each challenge, will also be reported as an AE. Any decrease in lung function or increase in symptom score, as outlined in section "3.4," will be considered an

adverse event. Any symptoms that induce a volunteer to seek medical attention from any provider during study drug treatment or within 7 days of completing study drug treatment or within 96 hours of challenge will be considered an adverse event. A serious adverse event will also be defined as any event that requires hospitalization or results in life threatening illness or injury, permanent (or likely to be permanent) illness or injury, or death if these events occur during study drug treatment or within 7 days of completing study drug treatment or within 96 hours of challenge (or if the clinical scenario leading up to hospitalization, illness, injury or death begins during study drug treatment, within 7 days of completing study drug treatment or within 96 hours of a challenge).

7.1.1. Grading criteria

In addition to determining whether an adverse event fulfills criteria for a serious adverse event or not, the severity of adverse events experienced by study participants will be graded according to the criteria set forth in the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials except for those items listed in 7.1.2 below. This document provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all adverse events.

All adverse events whether or not listed in the Toxicity Grading Scale will be graded on a scale from 1 to 4 according to the following standards (A semi-colon indicates 'or' within the description of the grade.):

Grade 1 = Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2 = Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL (preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc).

Grade 3 = Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL (bathing, dressing and undressing, feeding self, using he toilet, taking medications, and not bedridden).

Grade 4 = Life-threatening consequences; or urgent intervention indicated.

7.1.2. Protocol specific potential AE's

Adverse events which have relative specificity for this protocol will be recorded and graded 1 to 4 according to the grade definition provided below:

	Mild (Grade 1)	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially life-threatening (Grade 4)**
Gastrointestinal distress (i.e. diarrhea, nausea, vomiting, flatulence)	Asymptomatic or mild symptoms; clinical or diagnostic observations only;	Moderate; minimal, local or noninvasive intervention indicated; limiting age appropriate	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of	Life-threatening consequences; urgent intervention indicated

	intervention not indicated	instrumental ADL	existing hospitalization; disabling; limiting self care ADL	
Coagulopathy with or without bleeding	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated	Moderate; minimal, local or noninvasive intervention indicated; limiting age appropriate instrumental ADL	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of existing hospitalization; disabling; limiting self care ADL	Life-threatening consequences; urgent intervention indicated

7.2. Risks to Subjects

7.2.1. Potential risks of gamma tocopherol

There is a rare chance that higher doses of αT may effect coagulation (PT and aPTT) and predispose to bleeding. It is possible that γT carries the same risk, though this has not been demonstrated in prior studies of γT. An observational study of un-supplemented individuals suggested that higher plasma γT levels were associated with reduced lung function, specifically FVC and FEV_1 (14). Finally, our prior studies with γT have demonstrated risk for mild gastrointestinal symptoms, including diarrhea, nausea, bloating, and flatulence that tended to occur early on during treatment and to self-resolve within a few days despite continued treatment. In our most recent randomized, placebo controlled crossover study evaluating 14 days of γT supplementation in adults with asthma, diarrhea was reported in 26% of subjects on active treatment, compared to 4.3% of subjects on placebo.

7.2.2. Potential risks of WSP

Concentrations of wood smoke exhaust proposed for this study (500 μg/m3) are below those reported for individuals using biomass burning for heating and food preparation and those living in the area of a forest fire or agricultural burning. No major adverse health effects were observed in a recently completed study which exposed healthy volunteers to concentrated Chapel Hill air particles at the EPA Human Studies facility (Physiological and Biochemical Changes Associated with Exposure to Air Pollution Particles; Andrew Ghio, MD, PI; IRB# 95-EPA-310)1. The mean particulate matter (PM) was 485 + 84 $\mu g/m^3$ during the WSP exposure (vs. "below the detectable limit" for filtered air (FA)), and analyses of chamber concentrations of total hydrocarbon and carbon monoxide during WSP exposure were also increased compared to FA (4.8 ± 0.5 vs. 2.0 ± 0.1 ppm and 2.7 ± 0.7 vs. 0.0 ± 0.0 ppm, respectively). However, these healthy subjects had no significant change in pulmonary function and no nasal irritation, rhinitis, inspiratory pain,

shortness of breath, cough, wheezing or chest tightness after exposure to WSP or filtered air. There were complaints of fatigue, headache and eye irritation, but these were noted by volunteers as frequently before as after both the filtered air and particle exposures. Pulmonary function testing demonstrated no significant changes from pre-exposure values following exposures to either filtered air or WSP.

There is potential for increased airway inflammation in asthmatics. Thus, we are limiting inclusion to mild asthmatics only. Seven participants with asthma have completed an ongoing screening protocol (UNC IRB approved study #15-1775, *To identify persons who are susceptible to WSP-induced inflammation and examine the role of GSTM1 and other factors in this susceptibility)* to identify mild asthmatics who experience a ≥ 10% increase in sputum neutrophilia after WSP exposure for inclusion into this study. In addition to the mild, transient symptoms experienced by healthy volunteers as noted above, these seven asthmatic participants have experienced transient chest tightness following WSP exposure. This symptom has not been accompanied by any significant changes in spirometry, and chest tightness has resolved spontaneously within a few minutes following exposure and without any intervention. We will assess for any symptoms following WSP exposure with the WSP exposure questionnaire immediately after exit from the chamber and again 4 hours later. Spirometry will also be performed immediately following exit from the chamber with additional spirometry to be performed for any persistent symptoms and/or physician discretion. If FEV1 drops by 20% or more immediately after wood smoke exposure, or if the subject has intolerable symptoms, albuterol will be given for rescue. Finally, our Center has previously performed inhaled challenges with ozone and LPS in the allergic asthmatic population. With ozone and LPS we have not experienced any serious adverse events, and we expect the response to the wood smoke challenge to be similar.

7.2.3. Potential Risks of the Induced Sputum Procedure

The saline solutions (3%, 4% and 5%) nebulized during sputum may cause throat irritation, but it is uncommon for the duration of discomfort to last more than a few minutes post procedure. The procedure also has a minimal risk of inducing bronchospasm, which is a potential concern for individuals with asthma. Thus, all volunteers with a history of asthma will be pre-treated with 2-4 puffs of albuterol prior to beginning sputum induction. Additional albuterol is employed as a rescue agent if needed, and a physician is available for all sputum induction procedures.

7.2.4. Potential Risks of Spirometry

Potential risks include possible lightheadedness or wheezing.

The adhesive patches used during monitoring may cause itching or skin irritation.

7.2.5. Potential Risks of Measuring Flow Mediated Dilation with Brachial Artery Ultrasound

There are no significant risks associated with ultrasound imaging of the brachial artery, or with the 5 minutes of reduced blood flow that is part of the test. Cutting off blood flow to the arm may result in mild discomfort or temporary sensations of tingling or numbness in the hand until the blood pressure cuff is released. About 1 in 200 patients develop a painless rash on the arm where the blood pressure cuff is placed; this disappears over several days.

7.2.6. Potential Risks of Measuring Left Ventricular Strain

There are no significant risks associated with ultrasound imaging to measure left ventricular strain.

7.2.7. Potential Risks associated with other study procedures

Bruising during the venipuncture and vasovagal response to phlebotomy are the anticipated risks of venipuncture. There are no anticipated risks for completion of questionnaires or collection of a urine sample from female subjects. Light bicycle exercise (used to increase minute ventilation) presents a potential, but minimal risk. Side effects of exercise include occasional muscle soreness, cramps or general fatigue. These effects are temporary and not typically harmful to volunteers. It is also possible that exercise might uncover a previously unidentified pre-existing cardiac condition that could present a health risk to a volunteer.

Additionally there is a risk of Breach of Confidentiality and Privacy.

7.3. Measures to Minimize Risk

7.3.1. Protections to Minimize Risk of Gamma Tocopherol

Tocopherols may affect coagulation. We will query volunteers for bruising and bleeding prior to enrollment and during the dosing period, and will monitor PT and aPTT values prior to the first dose of study drug and at the end of the dosing period. They may be checked at any time the subjects experiences abnormal bruising. Any participant with an abnormal baseline PT, aPTT or INR will be excluded from receipt of study treatment. Spirometry is performed at regular intervals throughout the γT dosing period to monitor for any changes in lung function. As to the incidence of gastrointestinal symptoms, we will recommend that γT supplements be taken with a high-fat meal to promote intestinal absorption of γT and mitigate many associated diarrhea or bloating. Participants will receive a GI symptom diary to be completed daily while taking the study drug and returned to study coordinators on the morning of the challenge visits (Visit 2 or 5). All female subjects, except those who have had a hysterectomy with oophorectomy, will undergo urine pregnancy testing prior to beginning treatment and prior to WSP exposure. A positive pregnancy test will exclude the subject, and they will be withdrawn if they become pregnant during the course of the study. Study medication, including placebo, will be dispensed by the Investigational Drug Service of UNC Hospitals.

7.3.2. Protections to Minimize Risk of Wood smoke exposure

Prior to enrollment, subjects will have completed either the CEMALB screening protocol at which time a detailed medical history will be obtained. Only otherwise healthy volunteers who meet study inclusion/exclusion criteria will be considered for this study, and the maximum age of participants will be 45 years in order to limit the likelihood of occult disease. Subjects will be deferred if they have had a viral upper respiratory tract infection within 4 weeks of exposure challenge. Also, unspecified illnesses, which in the judgment of the investigator increase the risks associated with woodsmoke, will be a basis for exclusion. All subjects will need to fulfill objective lung function and symptom criteria outlined above in section 3.4.1. prior to chamber entry. During wood smoke exposure, subjects will be monitored by direct observation or via closed-circuit television. Subjects will have ECG telemetry leads attached to monitor cardiac rate and rhythm during exercise throughout the exposure. Subjects will be aware that they can

terminate their exposure for any reason and still receive compensation for their participation up to that point. A physician familiar with the protocol will be available for all challenge procedures. The investigator or duty physician will end the exposure if the subject is found to be suffering from any major adverse effect. An emergency "crash cart" with standard emergency medications, IV fluids and a defibrillator are is also readily available at the CEMALB in the unlikely event of a medical emergency during any challenge or study visit.

For safety reasons, pulse oximetry will be performed during the exposures to wood smoke and filtered air, and the subject will be withdrawn from the chamber if the value is <90%. The risk of discomfort associated with exercise will be limited because ability and response to exercise will be assessed at the training visit, and subjects with orthopedic issues that limit ability to pedal a bicycle will be excluded. All female subjects, except those who have had a hysterectomy with oophorectomy, will undergo urine pregnancy testing on the morning (prior to) of each exposure. A positive pregnancy test will exclude the subject. Various engineering system controls will be used during woodsmoke exposures. A Tapered Element Oscillating Microbalance (TEOM®) will be used control and monitor wood smoke particle concentration in the chamber. If the concentration measured by the TEOM® exceeds a predetermined level, the exposure will be terminated and the subject removed from the chamber. A DataRAMTM particulate measurement device will also be used during exposure as an alternative monitor for independent verification of proper TEOM® operation. Additionally, gas analyzers continually monitor the chamber environment during exposures and will alarm if the carbon monoxide level exceeds 35 ppm.

7.3.3. Protections to Minimize Risks of the Induced Sputum Procedure

Throat irritation will be minimized by providing the subject with a drink (water, juice etc) and a snack after induction. All subjects with a history of asthma will be pretreated with 2-4 puffs of albuterol prior to procedure initiation. An additional albuterol multidose inhaler will be immediately available in the event the subject experiences symptoms of bronchoconstriction. A physician on duty in the facility will be available during sputum inductions. If the covering physician feels that the subject's respiratory status is such that providing an induced sputum sample would place them at increased risk for significant bronchospasm, sputum sampling will be deferred. Baseline FEV_1 and force vital capacity (FVC) will be measured before the start of the induction and at the end of each level (concentration) of saline inhalation. The FEV_1 values that match a 10% and 20% reduction from baseline will be calculated and recorded. Subjects who have a 10-20% decrease in FEV_1 after the 3% or 4% level of saline will not be advanced to a higher concentration of saline and will continue at the same level. If the FEV_1 drops by > 20%, the induction will be stopped and the subject will be treated with 2 puffs of albuterol. Additional albuterol may be given if needed.

7.3.4. Protections to Minimize Risks of Spirometry

Subjects will be seated in a non-rolling chair when spirometry is performed and standard methodology conforming to the American Thoracic Society guidelines for measurement of spirometry will be used. Subjects will be instructed to notify the study staff if they feel lightheaded, and albuterol will be available in the event the subject experiences any unanticipated bronchoconstriction.

7.3.5. Protection to Minimize Risks Associated with FMD

If any subject finds this procedure intolerable, he or she will be withdrawn. In the rare event of a rash in the area where circulation is restricted, the subject will be monitored until it resolves.

7.3.6. Protections to Minimize Risks Associated with Measuring Left Ventricular Strain
If any subject finds this procedure intolerable, he or she will be withdrawn.

7.3.7. Protections to Minimize Risks Associated with Other Study Procedures
In order to minimize harm from the procedure, venipuncture will only be performed by qualified personnel. Subjects will have access to juice and a small snack after blood draws and will have the opportunity to lie recumbent after the procedure. Subjects with a known serious vagal response to phlebotomy will not be enrolled. A fully stocked medical "crash" cart will be present and readily available in the unlikely event that a major vagal episode occurs. Subjects will be screened for orthopedic injuries or conditions which would increase the likelihood of injury. Additionally, subjects will be trained on the proper use of the bicycle, and will be monitored during bicycle use.

7.3.8. Protections for Minimizing Risks Associated with Breach of Confidentiality and Privacy
All phone conversations/interviews as well as collection of personal medical history will be carried out in private offices or private examination rooms in the CEMALB research facility. All physical examinations will be carried out in a private examination room. Access to subject's identity will be limited to the investigators and staff directly involved in the protocol. CRF's/worksheets generated as part of these studies will be kept in a locked room, and the database will not contain personal identifiers and will also be password protected and accessible only to the appropriate study coordinators and investigators. Subjects' names will not be used in any publication. Risks to Confidentiality will be also be minimized by labeling samples for in-house analysis with coded identifiers. The ID label for any samples sent outside of the UNC Health Care System will be also be coded and without any personal identifiers (i.e., no DOB, gender etc will be indicated on the tube or lab request form). Clinical blood samples (PT, aPTT, WBC) are typically sent to Labcorp INC, Burlington, NC, without identifiers. All subjects are discharged to home with 24 hour contact information for a physician who is familiar with the study.

7.4. Reporting of AEs and SAEs
All SAEs will be reported to the CDER of the FDA as well as to the UNC Biomedical IRB within 24 hours of recognition of the event. Adverse events will be reported to both the FDA and UNC IRB on no less than a quarterly basis, or when the protocol is completed. If criteria for suspension of the protocol are met, then the FDA and UNC IRB will be notified within 24 hours. All subjects with a non-fatal SAE will be evaluated medically by a study physician, in concert with their own physician as appropriate. Likewise all subjects with an adverse event will be examined and evaluated by a study physician. All assessments will include the same lung function and vital sign assessments outlined for challenge observation. Other assessments will be undertaken as needed. Any unspecified event, which in the judgment of the PI of the study, constitutes an unusual, unexpected or prolonged event (greater than 96 hours) will be reported to both the FDA and the UNC IRB.

7.5. Informed Consent

Investigators and study staff will explain all study procedures and the benefits and risks of the study to potential participants as part of obtaining informed consent. Subjects may withdraw their consent at any time during the study. If they withdraw from the study it will not impact the care they receive at UNC or its affiliated hospitals and clinics.

7.6. Confidentiality

Risks to subject confidentiality will be minimized by storing records with personal identifiers in an office in CEMALB which is locked when unattended by the study coordinators. All samples will be stored with codes only (no personal identifiers). The CEMALB is located in the US Environmental Protection Agency's Human Studies Facility on the UNC campus, which has a security guard and limited access 24 hours/day, 7 days/week.

All data and specimens will be coded with unique identifiers assigned to the subject at the time of enrollment, and actual identity cannot be ascertained exclusively from these data. Conversations and interviews with the subject, as well as collection of personal medical history, will be carried out in private examination rooms or private offices in the CEMALB research facility. All physical examinations will be carried out in a private examination room. Access to study records containing identifiable private information will be provided only to those individuals associated with the study who require access to the data to perform their duties. These individuals will have completed ethics training as required by the UNC Biomedical IRB and will be listed on the approved IRB protocol. Records containing PHI will be kept in a locked office.

7.7. Investigators, Facilities, and Institutional Review Board

Principal Investigator	David B. Peden, MD, MS CEMALB, 104 Mason Farm Road The University of North Carolina CB#7310 Chapel Hill, NC 27599-7310	Professor of Pediatrics & Director, Center for Environmental Medicine, Asthma and Lung Biology
Co-Investigator	Neil Alexis, PhD CEMALB, 104 Mason Farm Road The University of North Carolina CB#7310 Chapel Hill, NC 27599-7310	Assistant Professor of Pediatrics & Investigator, Center for Environmental Medicine, Asthma, and Lung Biology
Co-Investigator	Haibo Zhou, PhD 3104C McGavran-Greenberg Hall The University of North Carolina CB #7420 Chapel Hill, NC 27599	Professor of Biostatistics Director of the Biostatistics Core, CEMALB

Curriculum Vitae for investigators are appended to this application.

***Facilities*:** Volunteers for these studies will be recruited, screened and undergo challenge procedures at the Center for Environmental Medicine, Asthma and Lung Biology, CB#7310, 104 Mason Farm Road, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-7310. All necessary clinical research equipment, medical equipment, and laboratory equipment is located within the Center.

***Institutional Review Board*:** This study will be reviewed and approved by the Biomedical IRB for the UNC School of Medicine, The University of North Carolina at Chapel Hill CB# 7097, Medical School Building 52 Chapel Hill, NC 27599-7097 prior to subject enrollment.

8. References

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