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## Gamma tocopherol-enriched supplement reduces sputum eosinophilia and endotoxin-induced sputum neutrophilia in volunteers with asthma

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### Abstract

**Background**—We and others have shown that the gamma tocopherol ( $\gamma$ T) isoform of vitamin E has multiple anti-inflammatory and antioxidant actions and that  $\gamma$ T supplementation reduces eosinophilic and endotoxin (LPS)-induced neutrophilic airway inflammation in animal models and healthy human volunteers.

**Objective**—To determine if  $\gamma$ T supplementation reduces eosinophilic airway inflammation and acute neutrophilic response to inhaled LPS challenge in volunteers with asthma.

**Methods**—Participants with mild asthma were enrolled in a double-blinded, placebo controlled crossover study to assess the effect of 1200 mg of  $\gamma$ T daily for 14 days on sputum eosinophils, mucins and cytokines. We also assessed the effect on acute inflammatory response to inhaled LPS

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challenge following  $\gamma$ T treatment, focusing on changes in sputum neutrophilia, mucins and cytokines. Mucociliary clearance was measured using gamma scintigraphy.

**Results**—Fifteen subjects with mild asthma completed both arms of the study. Compared to placebo,  $\gamma$ T notably reduced pre-LPS challenge sputum eosinophils and mucins, including MUC5AC, and reduced LPS-induced airway neutrophil recruitment 6 and 24-hours after challenge. Mucociliary clearance was slowed 4-hours post-challenge in the placebo group but not in the  $\gamma$ T treatment group. Total sputum mucins (but not MUC5AC) were reduced at 24-hours post-challenge during  $\gamma$ T treatment compared to placebo.

**Conclusion**— $\gamma$ T supplementation for 14 days reduced inflammatory features of asthma, including sputum eosinophils and mucins, as well as acute airway response to inhaled LPS challenge when compared to placebo. Larger scale clinical trials are needed to assess the efficacy of  $\gamma$ T supplements as a complementary or steroid-sparing treatment for asthma.

### Keywords

asthma; vitamin E; tocopherol; endotoxin; lipopolysaccharide; neutrophil; eosinophil; mucin; mucociliary clearance

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## INTRODUCTION

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. Environmental pollutant exposure is a known trigger for asthma exacerbations, which are characterized by airway inflammation, bronchoconstriction, increased production of airway mucous, and decreased mucociliary clearance with formation of mucous plugs (2, 3). Endotoxin (the main component of which is lipopolysaccharide, or LPS) is commonly encountered in ambient air particulate matter as well as in domestic and occupational settings and has been linked to asthma severity (4–6). Endotoxin is a potent stimulator of the innate immune response (7), signaling through Toll-like receptor 4 on airway macrophages to stimulate production of inflammatory cytokines and eicosanoids, recruitment of granulocytes, and production of gel-forming airway mucins, including mucin 5AC (MUC5AC) (8).

Airway inflammation during acute exacerbations of asthma is often characterized by increase in both airway eosinophils and neutrophils (9). Neutrophilic airway inflammation is particularly evident in viral asthma exacerbations as well as in some chronic asthma phenotypes (10). Our group has shown that inhaled LPS challenge induces airway neutrophilia in human volunteers, and now employ this procedure as a model of acute exacerbation of airway disease against which potential therapies can be tested (11–13). We have previously demonstrated that inhaled fluticasone propionate administered for two weeks decreased sputum eosinophilia subsequent LPS-induced acute airway neutrophilia in asthmatics (14). In subsequent studies, we have shown that treatment with the IL-1 receptor antagonist, anakinra (15), and the vitamin E isoform, gamma tocopherol ( $\gamma$ T) (16) also attenuated LPS-induced airway neutrophilia in healthy volunteers.

Our hypothesis that vitamin E supplementation decreases airway inflammation in asthma and allergy airway disease was inspired by epidemiologic evidence suggesting that increased



the U.S. Food and Drug Administration (IND 13004), and is listed on ClinicalTrials.gov, NCT02104505.

### Study Design

Subjects were randomized to 120 mg  $\gamma$ T or placebo (safflower oil) treatment for 14 days (Figure 1), a study period similar to that we used to assess the effect of fluticasone propionate on airway response to LPS challenge in asthmatics. The  $\gamma$ T supplement consisted of geltabs each containing 612 mg  $\gamma$ -tocopherol, 7 mg  $\alpha$ -tocopherol, 28 mg  $\beta$ -tocopherol, and 8 mg  $\delta$ -tocopherol (Callion Pharma, Inc), and subjects were instructed to take 2 geltabs once daily with a meal to maximize bile secretion and enhance absorption. Medication bottles were returned and any leftover pills were counted on the day of LPS challenge to ensure adherence. Twenty four to 48 hours prior to LPS challenge, subjects presented for sputum induction and gamma scintigraphy to measure mucociliary clearance (MCC). On day 14 of dosing, subjects underwent inhaled LPS challenge with 20,000 endotoxin units (EU) of Clinical Center Reference Endotoxin (CCRE), with MCC measurement performed 4-hours post-challenge and sputum induction at 6 and 24-hours post-challenge. Sputum was analyzed for granulocytes, inflammatory cytokines, and mucin content as previously described (31–34). After a minimum three-week washout to allow for clearance of inflammatory cells from the airways, subjects were crossed over to the alternate treatment group. Venipuncture was performed at regular intervals for assessment of PT, aPTT, and INR.

### Randomization and Masking

The randomization list was prepared by a biostatistician using SAS© and provided to the UNC Investigational Drug Service. Only the pharmacist and statistician had access to the randomization schedule. Subjects were randomized to treatment groups 1:1 using permuted blocks of four.  $\gamma$ T and safflower oil (placebo) geltabs were identical in appearance and were dispensed as a 7-day supply from the Investigational Drug Service to the study staff. Subjects returned for a follow up visit to receive the additional 7-day supply of investigational drug for that period.

### Endotoxin inhalation challenge

CCRE, referred to as LPS, was provided by the National Institutes of Health Clinical Center. All doses were prepared by the Investigational Drug Service and inhaled by subjects as a nebulized preparation using a DeVilbiss Ultraneb 99 ultrasonic nebulizer (12, 16).

### Sputum induction, processing, and mediator measurement

Each subject provided seven induced sputum samples (Figure 1): screening (prior to placebo or active treatment), 24–48 hours prior to each LPS challenge session (post-treatment sputum), and 6 and 24-hours after each LPS challenge session (post-challenge sputum). Induced sputum samples were processed as previously described (31–34). Cell viability (trypan blue exclusion) and total cell counts were assessed in a Neubauer hemacytometer, and differential cell counts were performed on cytocentrifuged cells stained with a modified Wright stain (Hema-Stain-3; Fisher Scientific, Hampton, NH). Cytokines from sputum

supernatants were measured using multiplex technology (Meso Scale Discovery/MSD, Gaithersburg, MD, USA). Each sample was analyzed with the V-PLEX Human Proinflammatory Panel II kit. Even though ability to provide adequate sputum for analysis was an entrance criterion, there were instances during the study in which a subject was not able to provide a sputum sample or provided a poor-quality sputum sample. In these instances, these volunteers were excluded from analysis of the effect of active treatment on airway inflammation. These volunteers were included in assessment of MCC and safety endpoints for the study.

### **Gamma scintigraphy for measurement of mucociliary clearance**

The procedure used for measuring MCC in humans has been described in detail previously (35, 36). Briefly, volunteers inhaled an aerosol of  $^{99m}\text{Tc}$ -sulfur colloid ( $^{99m}\text{Tc}$ -SC) using a slow inhalation (80 mL/sec), large particle (9.5  $\mu\text{m}$  mass median aerodynamic diameter) method (37). Immediately following inhalation of the radioaerosol (duration of less than five minutes), an initial deposition scan was recorded (sum of two two-minute images) and then continuous two-minute images were recorded for a period of two hours to monitor clearance of particles from the lung as the subject remained seated in front of the gamma camera. Subjects returned the following day after the radiolabeled aerosol exposure to obtain a 30-minute scan of 24-hour lung activity/retention.

A whole lung region of interest bordering the right lung (created from a Co57 transmission lung scan (37)) was used to determine, by computer analysis, the whole lung retention (Rt) (decay and background corrected) as a fraction of the initial counts in the right lung, over the two-hour clearance period at 10-minute intervals. MCC was calculated and expressed as average clearance in percent over the two-hour period (35). Because measures of MCC can be influenced by the initial, regional lung deposition of the inhaled radioaerosol, we also calculated 1) the central to peripheral (C/P) ratio of activity and 2) the skew of the counts/pixel vs. number of pixels histogram for the initial two-minute image from each study visit (35, 38).

### **Analysis of serum tocopherols and $\gamma$ -CEHC**

$\alpha\text{T}$ ,  $\gamma\text{T}$ ,  $\delta\text{T}$ , and 5- $\text{NO}_2$ - $\gamma\text{T}$  were measured by a HPLC assay with electrochemical detection (39), and  $\gamma$ -CEHC was analyzed using liquid chromatography tandem mass spectrometry (LC-MS/MS) as previously described (40).

### **Analysis of sputum mucins**

To measure total mucins, a 100  $\mu\text{L}$  aliquot of induced sputum was solubilized in 6MGuHCl and subjected to differential refractometry (tREX, Wyatt Technology, Goleta, CA) coupled with size exclusion chromatography as described previously (41). Individual concentration of MUC5AC was measured by labeled mass spectrometry method using deuterium labeled MUC5AC peptide standards.

### **Statistical analysis**

We employed methods similar to those used in our initial study of the effect of  $\gamma\text{T}$  in healthy volunteers (16). The primary endpoints of the study were airway eosinophilia (defined as the

difference in sputum eosinophils present in post-treatment samples) and LPS-induced airway neutrophilia (defined as the change in induced sputum neutrophils (PMNs) from post-treatment to 6-hours post-challenge), comparing  $\gamma$ T treatment to placebo.

In planning this study, we were guided by the results of our previous study of  $\gamma$ T-enriched supplementation on airway PMN response to LPS challenge in 13 healthy volunteers (16). Based on these data, we estimated that a sample size of 30 volunteers would be adequate for this study, with an *a priori* plan to undertake an interim analysis after 15 volunteers completed this study. As planned and approved by IRB and FDA review, the interim analysis would lead us to stop the study due to demonstration of futility or statistically significant support of the hypotheses that  $\gamma$ T inhibits airway eosinophilia and LPS-induced neutrophilia, or continuation of the study to n=30 due to inconclusive interim results.

For initial post-treatment vs. post-challenge comparisons of sputum endpoints and MCC within each treatment group, paired t-tests or Wilcoxon signed rank tests (depending on whether the normality assumption was met) were employed. Data that were not normally distributed were transformed using Box-Cox transformation. Given the crossover design of our study, we next determined the  $\gamma$ T treatment effect (compared to placebo) on post-treatment sputum endpoints and on LPS-induced changes (post-challenge – post-treatment) in sputum endpoints using a linear mixed model approach described by Jones and Kenward (42) that considers the above individual tests in a global, unified way where all data are used at the same time. SAS PROC MIXED was used to fit the linear mixed model. Criterion for significance was taken to be  $p < 0.05$ .

## RESULTS

### Subject Demographics

Twenty-three subjects with mild asthma were enrolled and underwent randomization. Based on frequency of daytime and nighttime symptoms and use of rescue albuterol, 22 subjects were classified as having mild intermittent asthma. One subject was classified as mild persistent asthma and was using montelukast daily at the time of enrollment. However, this subject withdrew from the study prior to inhaled LPS exposure. No subjects were using inhaled corticosteroids at the time of enrollment or at any point during the study. The majority of participants were atopic (74%) based on the results of skin prick testing. Demographic characteristics of the study population are shown in Table 1. Fifteen subjects completed both arms of the crossover study (Figure E1 in the article's Online Repository at [www.jacionline.org](http://www.jacionline.org)), with 13 providing adequate sputum for assessment of the primary sputum inflammatory endpoints for both treatment periods.

### $\gamma$ T supplementation increased serum $\gamma$ -tocopherol and $\gamma$ -CEHC concentrations

Serum  $\gamma$ T and  $\gamma$ -CEHC concentrations rose significantly from baseline values in the active treatment group only ( $p < 0.0001$  for both) (Table 2). Conversely,  $\alpha$ T concentrations decreased from baseline in the active treatment group ( $p = 0.003$ ).



### **$\gamma$ T treatment reduced post-treatment sputum eosinophils and mucins**

Using the linear mixed model approach, we found that  $\gamma$ T treatment significantly reduced post-treatment sputum %eosinophils ( $p=0.04$ ) and eosinophils/mg of sputum ( $p=0.01$ ) compared to placebo (Figure 2a–b). Likewise,  $\gamma$ T treatment significantly reduced post-treatment total mucins ( $p=0.03$ ) and MUC5AC content ( $p<0.0001$ ) compared to placebo (Figure 2c–d).

### **$\gamma$ T treatment attenuated LPS-induced sputum neutrophilia**

Inhaled LPS challenge significantly increased sputum percent neutrophils (%PMNs) ( $p=0.003$ ) and neutrophils per mg (PMNs/mg) of sputum ( $p=0.01$ ) at 6 hours compared to post-treatment sputum during the placebo period. The increase during the placebo period (%PMNs:  $20.1\% \pm 16.5$  SD,  $p<0.01$ ; PMNs/mg  $384.1 \pm 531.2$  SD,  $p<0.01$ ) was greater than that seen during the active period (% PMNs  $11.7\% \pm 20.7$  SD,  $p=0.04$ ; PMNs/mg  $236.2 \pm 692.7$  SD,  $p=0.2$ ). Linear mixed modeling demonstrated that  $\gamma$ T treatment (compared to placebo) significantly attenuated sputum %PMNs at both 6 ( $p=0.04$ ) and 24 hours ( $p=0.02$ ) after LPS challenge (Figure 3a–b). There was no effect of inhaled LPS challenge or  $\gamma$ T treatment on any measure of sputum eosinophilia following LPS challenge.

### **$\gamma$ T effects on airway mucin production and mucociliary clearance**

MUC5AC content was significantly increased from post-treatment levels in both treatment groups 6 hours after inhaled LPS challenge [ $p=0.001$  (placebo),  $p=0.0004$  (active)]. By 24 hours post-LPS challenge, total sputum mucins decreased in both treatment groups compared to prior to LPS challenge, though not significantly. Using linear mixed modeling to assess for a treatment effect, we detected significantly less total sputum mucins during the active treatment period compared to placebo (Figure 3c,  $p=0.03$ ). We found no significant difference in MUC5AC concentrations between the treatment groups at the same time point (**data not shown**).

As an exploratory measure, we assessed how  $\gamma$ T intervention may impact MCC. MCC was measured prior to and 4 hours after LPS challenge. There were no differences in regional deposition indices (C/P or skew) nor in 24-hour retention between post-treatment and post-challenge measurements for either treatment period. MCC was significantly slowed following LPS challenge compared to post-treatment measurements for the placebo treatment period (MCC =  $16.3 \pm 9.3\%$  post-challenge vs.  $21.4 \pm 6.9\%$  post-treatment,  $p < 0.01$ ) (Figure 4). In contrast, there was no such slowing of MCC by LPS challenge during active treatment (MCC =  $20.2 \pm 8.0\%$  post-challenge vs.  $21.4 \pm 9.7\%$  post-treatment,  $p=0.6$ ). However, for this new endpoint, the sample size was not adequate to definitively ascribe a treatment effect for  $\gamma$ T on LPS-induced slowing of MCC when accounting for period and carryover effects.

### **$\gamma$ T treatment did not impact LPS-induced changes in sputum $T_H1$ cytokines**

During the placebo period, sputum IL-1 $\beta$  and IL-8 concentrations were significantly increased 6 hours post-LPS challenge compared to post-treatment values ( $p=0.002$  and  $p=0.01$ , respectively), while no significant LPS-induced increase was observed during the

active treatment period ( $p=0.07$  and  $p=0.40$ , respectively). There was no significant LPS-induced change in sputum IL-6 concentration during either treatment period. Compared to placebo treatment, we did not detect a significant  $\gamma$ T treatment effect on LPS-induced inflammatory cytokine concentrations in sputum following LPS challenge.

### Adverse events

No serious adverse events occurred during the study period. The most commonly reported symptoms were gastrointestinal in nature. During the active treatment period, 21.7% of subjects reported nausea and 26% reported diarrhea or loose stools, compared to 8.7% and 4.3% during the placebo treatment period, respectively. These symptoms were typically transient and tended to occur during the first or second day of treatment then self-resolved. One subject chose to discontinue study participation due to intolerable diarrhea during the active treatment period. No significant change in PT, aPTT, or INR was observed, and there were no reported bleeding events during the study. After completion of 14 days of active treatment, no clinically or statistically significant changes were seen in FEV<sub>1</sub> or FEV<sub>1</sub>/FVC from measurements taken during the initial baseline visit.

## DISCUSSION

The primary goal of this proof-of-concept study was to determine if  $\gamma$ T supplementation in adults with asthma decreases airway eosinophilia as well as the inflammatory response to inhaled LPS, a model of neutrophil-predominant asthma exacerbation. Our results demonstrate that asthmatics treated with  $\gamma$ T supplementation for 14 days had significantly reduced eosinophils in sputum when compared to placebo treatment. These findings suggest that  $\gamma$ T may reduce baseline T<sub>H</sub>2-mediated airway inflammation, which could be beneficial for eosinophilic asthma phenotypes. We also found that  $\gamma$ T treatment was associated with lower post-treatment sputum mucins compared to placebo, including the inducible mucin glycoprotein, MUC5AC, which has been found in high concentrations in mucous plugs from fatal asthma cases (43). *Ex vivo*  $\gamma$ T treatment was previously found to inhibit IL-13-induced secretion of eotaxin from airway epithelial cells, a potent chemotactic factor for eosinophils (44). Given that mucin production is enhanced by IL-13, similar mechanisms may account for the impact of  $\gamma$ T on mucin production as well.

We also found that  $\gamma$ T treatment attenuated neutrophilic airway response to inhaled LPS challenge. Neutrophilic airway inflammation is often less responsive to corticosteroid treatment (45), and there is a great unmet need for non-steroidal therapies that target this specific type of inflammation. While we found no significant difference in sputum mucins between the treatment periods at 6 hours post-LPS challenge, total mucins were significantly lower at 24 hours with  $\gamma$ T treatment, which could suggest faster recovery from mucin hypersecretion following an acute inflammatory challenge.

We observed a significant impairment of MCC following inhaled LPS challenge during the placebo treatment period but not during the  $\gamma$ T treatment period. We have previously found a slowing of MCC by LPS challenge in healthy non-smokers (35) and a trend towards slowing in mild asthmatics that was confounded by regional deposition differences between baseline and post-LPS challenge MCC (unreported) (38). While this study was not powered



to detect a significant treatment effect of  $\gamma$ T on MCC, our results do suggest that  $\gamma$ T reduces LPS-induced slowing of MCC, and warrants further study. The mechanism by which inhaled LPS slows MCC is not understood, but may be related to quantity or quality of sputum mucins, epithelial tethering of mucins, direct effects on ciliary function, or a combination of these factors (43).

The  $\gamma$ T supplement used in our study given daily over a 2-week period resulted in significantly increased serum concentrations of  $\gamma$ T and its primary active metabolite  $\gamma$ -CEHC but with reduced serum  $\alpha$ T concentrations by an unknown mechanism. This finding is consistent with previous studies of  $\gamma$ T supplementation effects on reducing  $\alpha$ T plasma concentrations (46), including one conducted by our group that demonstrated reduced  $\alpha$ T concentrations in serum following a 3-dose regimen of an identical  $\gamma$ T supplement administered over a 24-hour period (47). It is unknown whether continued  $\gamma$ T supplementation would result in further decline in  $\alpha$ T concentrations, nor is it known what the long-term physiologic consequences of this decline would be.

While our work has consistently demonstrated a beneficial effect of  $\gamma$ T on airway inflammation, others have proposed a pro-inflammatory role for  $\gamma$ T based primarily on human observational or animal model studies. In a cross-sectional study of young adults enrolled in the Coronary Artery Risk Development in Young Adults (CARDIA) cohort, higher serum  $\alpha$ T levels were associated with higher lung function values (FEV<sub>1</sub> and FVC), while higher serum  $\gamma$ T levels were associated with lower FEV<sub>1</sub> and FVC values (48). While the results of this epidemiological study are intriguing, the correlation between serum  $\gamma$ T levels and lung function may reflect  $\gamma$ T as a risk factor or biological marker for lung function. Furthermore, others have shown that *dietary* vitamin E, at least 70% of which is comprised of  $\gamma$ T, was associated with increased FEV<sub>1</sub> in older adults (49) and may be protective against adult-onset asthma (20). It is important to emphasize that these studies were not intervention trials, and several potential confounding factors could have influenced their results, including differences in intake of dietary fats. For example,  $\gamma$ T-rich oils tend to have higher levels of polyunsaturated fatty acids, which may contribute to certain disease states, whereas  $\alpha$ T-rich oils contain predominantly monounsaturated fatty acids, which have more health benefits (50). Although further studies are needed to address the long-term impact of  $\gamma$ T supplementation on airway inflammation, our 2-week dosing regimen with  $\gamma$ T had no impact on spirometry measurements.

There are very few published human trials of  $\gamma$ T supplementation in the context of airway inflammation prevention and/or treatment. Vitamin E has been studied for prevention and treatment of many chronic health conditions (51–54), yet human trials in asthma have yielded conflicting results and have focused on treatment with  $\alpha$ T, the most abundant tocopherol isoform in widely available supplements. In contrast to the results presented here, studies utilizing murine models found that  $\gamma$ T supplementation exacerbated eosinophilic inflammation, while  $\alpha$ T supplementation conferred protection (55, 56). It is possible that these conflicting reports reflect species-dependent differences in the anti-inflammatory effects of  $\gamma$ T. Previous work from our group demonstrates that  $\gamma$ T supplementation reduces airway eosinophilia in humans (16) and rodents (27, 28). This is in agreement with our current study, in which short term dosing with  $\gamma$ T exhibits acute anti-eosinophilic and anti-

inflammatory properties in human subjects. These results, coupled with evidence that  $\gamma$ T has unique anti-inflammatory properties compared to  $\alpha$ T (including the ability to scavenge reactive nitrogen species (24) and inhibit cyclooxygenase-2 (COX-2) and 5-lipoxygenase (25)) supports the use of  $\gamma$ T-enriched Vitamin E preparations as a potential intervention for acute exacerbation, pollution induced disease, and possibly even chronic allergic diseases. These findings support conducting larger trials with  $\gamma$ T supplementation in volunteers with asthma to further evaluate its role in modulating features of asthma.

This early phase clinical trial has several limitations. Our participants were predominantly female which could reduce the generalizability of our results. The study population was somewhat heterogeneous with both atopic (74%) and non-atopic (26%) participants, and based on our safety criteria to undergo inhaled LPS challenges, had mild asthma. Given that the supplementation period only lasted two weeks, the longer-term effect of reduced serum  $\alpha$ T levels noted with  $\gamma$ T supplementation will have to be further studied for safety and efficacy in treating chronic airway inflammation. Our dosing regimen was generally well-tolerated, though early, transient gastrointestinal symptoms occurred in about one-fourth of participants studied. Additionally, we saw no prolongation of blood coagulation measurements, and no significant bleeding events were reported. The occurrence of early GI side effects and potential need for both long term and short term treatment regimens suggests that dose ranging studies be done. Finally, the impact of BMI on driving treatment responses to LPS will have to be further evaluated, given that we have previously shown that increased BMI is associated with sputum neutrophil recruitment to inhaled LPS among atopic subjects with asthma (57).

In conclusion, we have shown that a 14-day course of  $\gamma$ T supplementation resulted in reduced eosinophilic inflammation of the airways and reduction in sputum mucins including MUC5AC. Additionally,  $\gamma$ T supplementation reduced LPS-induced neutrophilic airway inflammation and mucinous content of sputum following inhalation challenge and was associated with reduced impact of LPS on MCC. Overall, our results with two weeks of  $\gamma$ T supplementation were similar to the effects of two weeks of treatment with inhaled fluticasone propionate on both post-treatment sputum eosinophilia and acute neutrophilic response to inhaled LPS challenge (14). Taken together, these observations indicate that  $\gamma$ T has potential to treat multiple features of asthma, including airway inflammation, mucous production, and clearance of mucous from the airways, and should be studied further in larger-scale clinical trials to investigate the efficacy of  $\gamma$ T for improving asthma outcomes.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations

<b>αT</b>	α-Tocopherol
<b>γT</b>	γ-Tocopherol
<b>γ-CEHC</b>	2,7,8-trimethyl-2-(β-carboxyethyl)-6- hydroxychroman
<b>PMN</b>	neutrophil
<b>COX</b>	cyclooxygenase
<b>LPS</b>	lipopolysaccharide
<b>IL</b>	interleukin
<b>LC-MS</b>	liquid chromatography tandem mass spectrometry
<b>INR</b>	international normalized ratio
<b>PT</b>	prothrombin time
<b>aPTT</b>	activated partial thromboplastin time
<b>MCC</b>	mucociliary clearance
<b>PGE2</b>	prostaglandin E2
<b>5-NO<sub>2</sub>-γT</b>	5-nitro-gamma- tocopherol
<b>MUC5AC</b>	mucin 5AC

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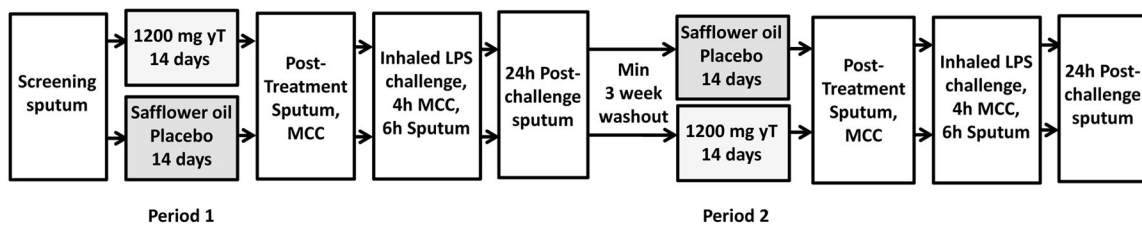
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**KEY MESSAGES**

- $\gamma$ T supplementation in mild asthmatics reduced sputum eosinophils and mucins compared to placebo in a fashion similar to that of inhaled fluticasone propionate and may have a role in reducing  $T_H2$ -mediated inflammation.
- $\gamma$ T reduced the neutrophilic inflammatory response to inhaled LPS challenge compared to placebo and may represent a useful therapy for neutrophil-predominant asthma exacerbation.



**Figure 1. Phase IIa crossover study design in volunteers with mild asthma (n=15)**

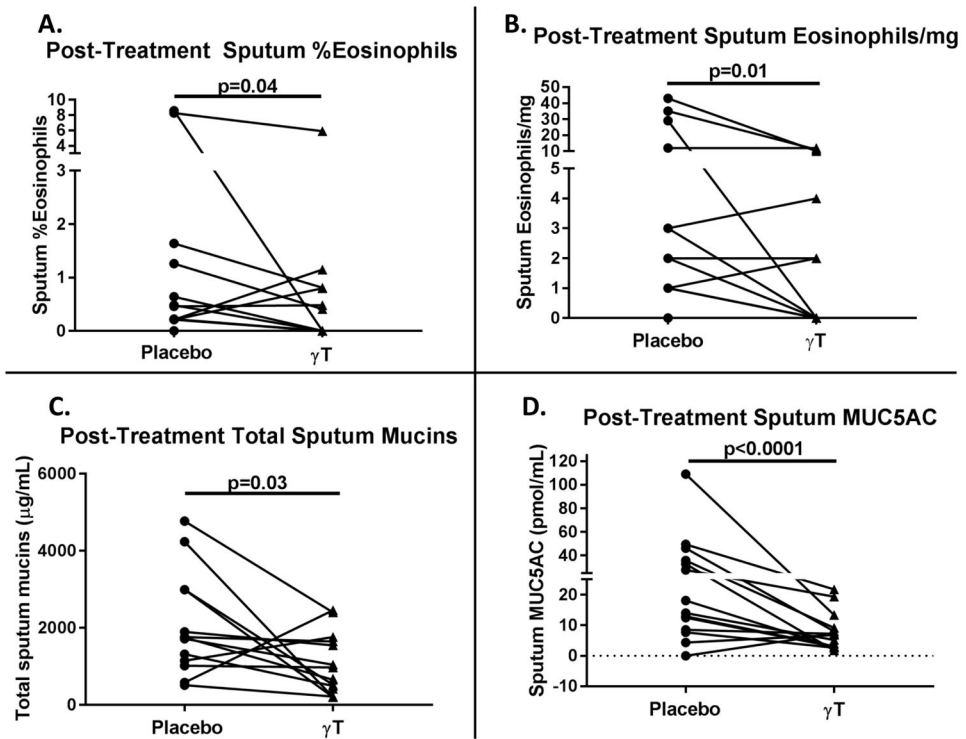
Randomized, placebo controlled crossover study of  $\gamma$ T supplement or safflower oil placebo in 15 subjects with mild asthma. Subjects were challenged with inhaled LPS followed 4 hours later by gamma scintigraphy to measure mucociliary clearance (MCC) and 6 hours later by sputum induction.

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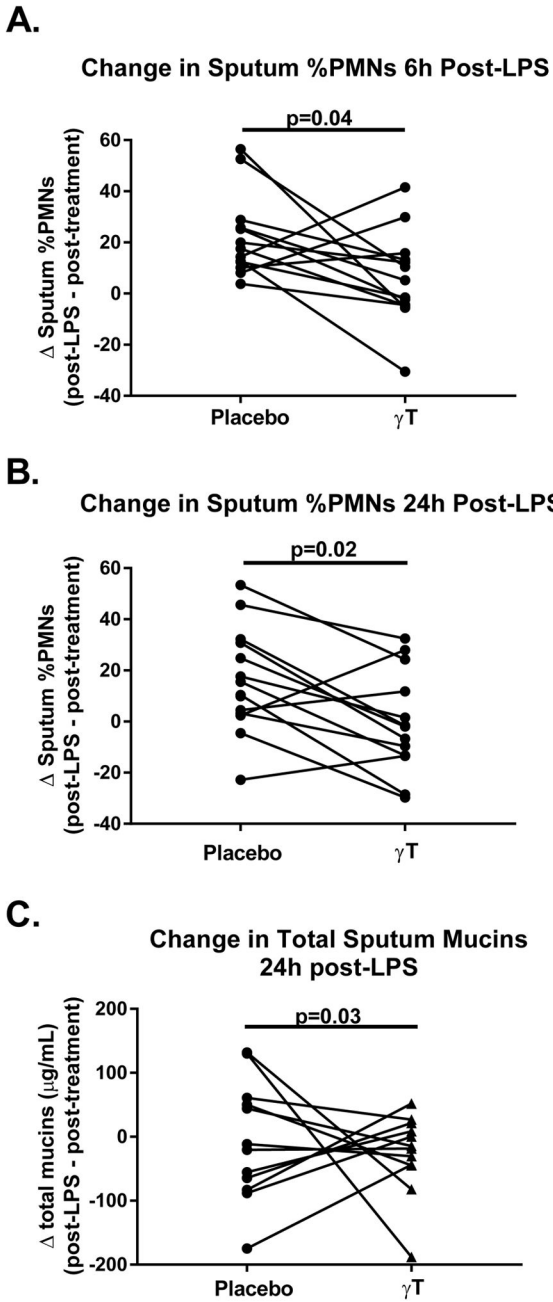
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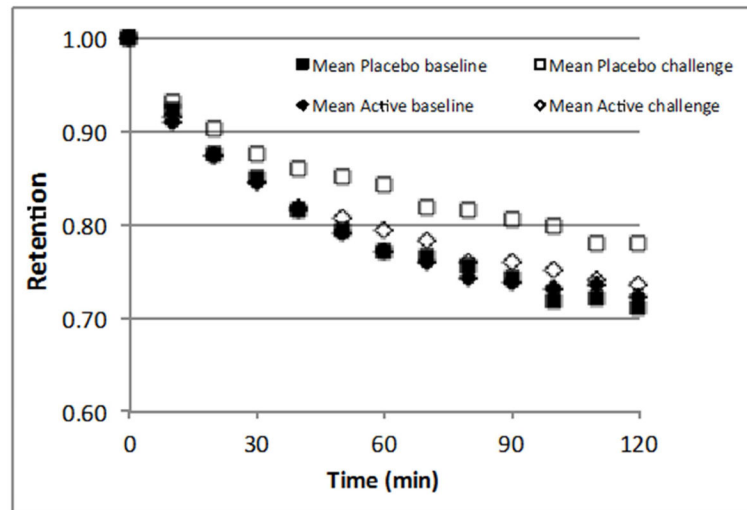


**Figure 2.  $\gamma$ T reduced post-treatment sputum eosinophils and mucins (n=13)**

A) Sputum %eosinophils, B) sputum eosinophils/mg, C) total sputum mucins were reduced in post-treatment sputum samples during active treatment compared to placebo treatment.



**Figure 3.  $\gamma$ T attenuated LPS-induced sputum neutrophilia and mucin production (n=13)**  
 A) Sputum %PMNs at 6-hours and B) 24-hours post-challenge were significantly reduced during active treatment compared to placebo. C) Total sputum mucins at 24-hours post-challenge were significantly reduced during active treatment compared to placebo.



**Figure 4.  $\gamma$ T was associated with attenuation of LPS-induced changes in MCC (n=15)**  
MCC is represented as mean retention versus time at post-treatment and 4-hours post-challenge for each treatment group. Inhaled LPS challenge resulted in significant slowing of MCC after placebo treatment, but no significant effect on MCC was seen after  $\gamma$ T treatment.

**Table 1**

Demographic characteristics of enrolled study volunteers (N=23).

Age (years), median (range)	26 (20–47)
Sex (Female/Male)	19/4
Race	
Caucasian	15
African American	4
Asian	2
Native American	2
Ethnicity	
Hispanic	1
Non-Hispanic	22
Atopic, N (%)	17 (74%)
BMI (kg/m <sup>2</sup> ), median (range)	26 (20–42)
FEV1 (L), median (range)	3.3 (2.4–4.4)
FEV1 % predicted, median (range)	97 (83–109)

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**Table 2**

Serum concentrations of tocopherols and  $\gamma$ -CEHC from 18 volunteers with mild asthma.

	<b>Baseline</b>	<b>Placebo Treatment Period</b>	<b>Active Treatment Period</b>
$\gamma$ T ( $\mu$ M)	2.6 (1.43–6.22)	3.69 (1.65–8.82)	19.8* (2.49–50.15)
$\alpha$ T ( $\mu$ M)	25.41 (14.72–37.81)	25.22 (18.48–52.65)	19.04* (11.07–36.52)
$\delta$ T ( $\mu$ M)	0.09 (0.01–0.68)	0.12 (0.04–0.4)	0.26 (0.06–0.6)
$\gamma$ -CEHC ( $\mu$ M)	0.14 (0.05–0.45)	0.17 (0.07–1.4)	3.11* (0.08–7.82)
5-NO <sub>2</sub> - $\gamma$ T ( $\mu$ M)	0.01 (0–0.07)	0.02 (0–0.07)	0.01 (0–0.05)

Data represented as median (range).

\*  $p < 0.05$  comparing baseline concentrations to those obtained after 14 days of  $\gamma$ T supplementation. Analyses were performed using paired t tests for  $\gamma$ T and  $\alpha$ T data and by Wilcoxon Matched Pairs Signed Rank Test for  $\delta$ T and  $\gamma$ -CEHC data as they were not normally distributed.