Brief report

# A pilot randomized clinical trial of γ-tocopherol supplementation on wood smoke–induced neutrophilic and eosinophilic airway inflammation

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**Background: Air pollutants, including particulates from wood smoke, are a significant cause of exacerbation of lung disease. γ-Tocopherol is an anti-inflammatory isoform of vitamin E that has been shown to reduce allergen-, ozone-, and endotoxin-induced inflammation.**

**Objective: The objective of this study was to determine whether γ-tocopherol would prevent experimental wood smoke–induced airway inflammation in humans.**

**Methods: This was a randomized, placebo-controlled clinical trial testing the effect of a short course of γ-tocopherol–enriched supplementation on airway inflammation following a controlled exposure to wood smoke particulates.**

**Results: Short-course γ-tocopherol intervention did not reduce wood smoke–induced neutrophilic airway inflammation, but it did prevent wood smoke–induced eosinophilic airway inflammation.**

**Conclusion: γ-Tocopherol is a potential intervention for exacerbation of allergic airway inflammation, but further study examining longer dosing periods is required. (J Allergy Clin Immunol Global 2023;2:100177.)**

***Key words:*** *γ-Tocopherol, wood smoke particles, air pollution, eosinophils, neutrophils, asthma, environmental lung disease*

*Abbreviations used*

| | |
|---|---|
| αT: | α-Tocopherol |
| γT: | γ-Tocopherol |
| γ-CEHC: | 2, 7, 8-Erimethyl-2S-(γ-carboxyethyl)-6-hydroxychromane |
| IRB: | Institutional review board |
| $O_3$: | Ozone |
| %PMNs: | Differential count of PMNs in sputum |
| WSP: | Wood smoke particle |

## INTRODUCTION

The vitamin E isoform γ-tocopherol (γT) and its metabolite 2, 7, 8-trimethyl-2S-(γ-carboxyethyl)-6-hydroxychromane (γ-CEHC) have antioxidant and anti-inflammatory properties.[1] In preclinical rodent studies, γT inhibits airway inflammation following allergen,[2] endotoxin,[3] and ozone ($O_3$)[4] challenge. In humans, γT doses of 1080 to 1214 mg given in schedules of 1 dose every 12 hours for 3 doses to 14 daily doses have been found to increase plasma γT and γ-CEHC levels.[3,5-7] γT administration reduces *ex vivo* activation of PBMCs with endotoxin-[6,7] and IgE-mediated basophil responses.[8]

Endotoxin is a component of particulate air pollution, and our first clinical studies assessed the effects of γT on endotoxin-induced airway inflammation. Oral treatment of healthy volunteers with a preparation containing 1080 mg of γT per day for 1 week increased plasma γT and γ-CEHC levels and reduced neutrophil and eosinophil airway responses to endotoxin.[3] In volunteers with asthma, daily dosing for 2 weeks with 1214 mg of γT increased plasma γT and γ-CEHC levels, decreased endotoxin-induced neutrophilic airway inflammation, and reduced constitutive levels of sputum eosinophils and MUC5AC.[5]

Wood smoke particles (WSPs) from wildfires exacerbate asthma and other lung diseases and induce cardiovascular pathology.[9] We have developed a WSP challenge protocol in which volunteers between 18 and 45 years of age undergo a 2-hour controlled exposure to 500 μg/m$^3$ of WSPs in an institutional review board (IRB)-approved screening WSP protocol after providing informed consent (ClinicalTrials.gov identifier NCT02767973; University of North Carolina IRB approval no. 15-1775).[10] Approximately 65% of volunteers are responsive to WSPs, as defined by at least a 10% increase in the differential count of sputum PMNs 24 hours after challenge.[11,12] The homozygous null genotype for glutathione-s-transferase mu

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1 (GSTM1) and asthma status enhance response to WSPs.[12] We now use this WSP challenge protocol to assess the effects of γT supplementation on WSP-induced airway inflammation. As WSPs also induce cardiovascular responses, we assessed brachial artery flow–mediated dilation as a measure of vascular endothelial function and echocardiographic measures of left ventricular mechanics.[11,12]

## RESULTS AND DISCUSSION

In this study, we first identified 23 responsive volunteers between 18 and 45 years of age by using our WSP challenge protocol.[10] These WSP-responsive volunteers then provided informed consent and were evaluated for this IRB-approved, double-blinded, placebo-controlled crossover study of the effects of γT-enriched supplementation on airway inflammation following a 2-hour exposure to 500 μg/m$^3$ of WSPs (NCT03444298, University of North Carolina IRB approval no. 17-2303). There was a washout period of at least 4 weeks between each arm. The primary end point was change in differential counts of PMNs (%PMNs) in the sputum from baseline to 6 hours and 24 hours after initiation of the exposure to WSPs. The absolute sputum levels of PMNs (cells/mg of sputum) at 6 hours and 24 hours were obtained as a secondary end point. The exploratory outcomes included sputum concentrations of eosinophils and inflammatory cytokines (IL-1β, IL-6, IL-8, and TNF-α), spirometry measurements, flow-mediated dilation of the brachial artery as an indicator of endothelial dysfunction, and echocardiographic assessments of left ventricular function.

Of the 23 WSP-responsive volunteers, 7 were excluded on the basis of entrance criteria, with 5 being lost to follow-up before crossover into the second arm and 11 completing the entire protocol (see the Consolidated Standards of Reporting Trials [CONSORT] protocol flowchart [Fig 1]). The median participant age was 28.2 years, with 6 participants self-identifying as female, 5 self-identifying as male, 8 self-identifying as White, and 3 self-identifying as Black. The methods used to perform WSP challenge and process sputum are listed in the Online Repository (at www.jaci-global.org) and have been described previously.[12-14]

The original dosing scheme for this study was guided by our dosing study showing that 3 doses of γT increased plasma γT and γ-CEHC levels, with reduction of *ex vivo* endotoxin responses of PBMCs.[6] However, when we found that similar supplementation dosing with γT has no effect on $O_3$-induced neutrophilic inflammation,[15] we modified our dosing schedule so that volunteers would receive 1 dose of γT per day for 7 days before WSP challenge. We were then confronted with an 18-month pause in the study because of the coronavirus disease 2019 (COVID-19) pandemic, during which our stock of γT expired and our original vendor was unable to provide replacement stock. We opted to substitute our stock with a commercially available γT-enriched preparation, which yielded a daily dose of 1154 mg of γT (92% of the previous daily dose). The γT dosing is fully described in the Online Repository.

Of the 11 volunteers who completed the study, the first 4 received the short course of γT (4 doses given every 12 hours before exposure to WSPs), 4 received a 7-day course with the original stock of γT, and 3 received a 7-day course with the commercially available stock. Plasma levels of γT and γ-CEHC (but not α-tocopherol [αT]) obtained immediately after WSP challenge were significantly increased following active dosing

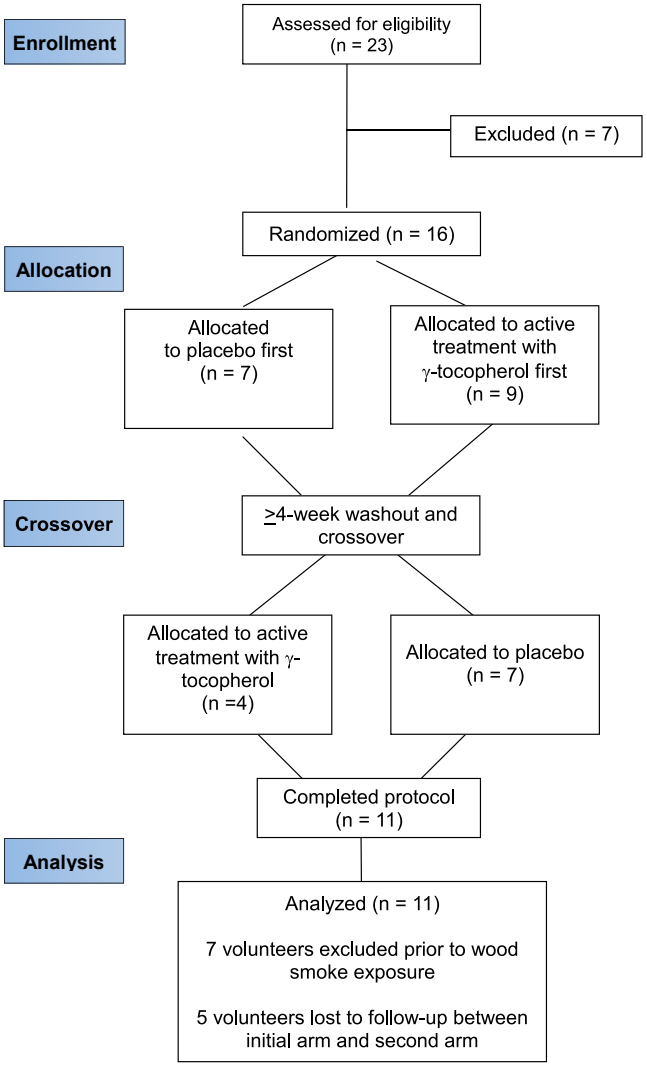


**FIG 1.** CONSORT diagram of study volunteer flow through the study protocol.

with γT-enriched preparations versus placebo, regardless of the specific dosing schedule used, which is consistent with our previous studies (Fig 2).[3,5] The active treatment doses used were 4 doses of 1253 mg of γT given over 2 days (n = 4) or daily doses of 1154 to 1253 mg of γT given over 7 days (n = 7).

We used an intent-to-treat analysis of primary, secondary, and exploratory end points from all 11 volunteers. Our analyses methods using statistical approaches that we have used previously are described in the Online Repository.[12,16,17] WSPs induced the expected increases in sputum %PMNs at 6 and 24 hours, with no observed differences in sputum %PMNs between the γT and placebo treatment periods (our primary end points). The numbers of PMNs per mg of sputum at 6 and 24 hours (our secondary end points) were not significantly different from baseline following exposure to WSPs in the γT or placebo treatment periods (Fig 3).

As we found that γT supplementation reduced endotoxin-induced airway neutrophilic inflammation, we anticipated similar results with WSP-induced responses. Thus, the failure of γT supplementation to reduce the effects of WSPs and $O_3$ were unexpected. As our study showed that 2 weeks of γT

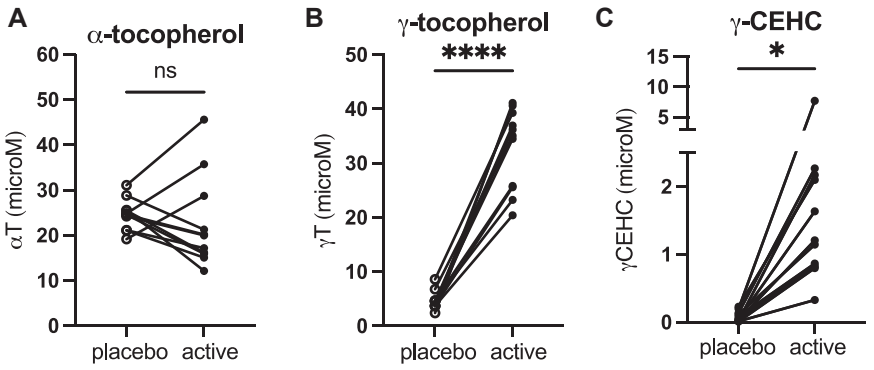


**FIG 2.** Comparison of plasma levels of αT (**A**), γT (**B**), and γ-CEHC (**C**) immediately following exposure to WSPs following placebo and active γT dosing, expressed in micromolar concentrations. Compared with placebo, γT supplementation resulted in significantly increased ($P < .001$ and $P = .01$, respectively) levels of γT and γ-CEHC, with no significant change in αT level.

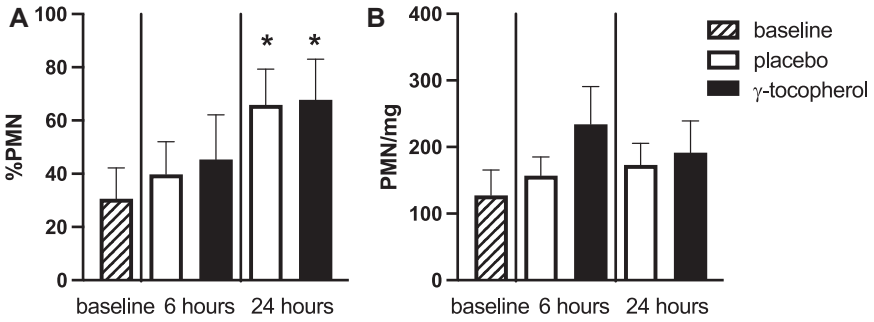


**FIG 3.** Comparison of γT dosing on sputum PMN outcomes 6 and 24 hours after initiation of controlled exposure to WSPs compared with baseline values. **A,** The effect of γT on WSP-induced %PMNs in sputum (the primary study end points). **B,** The effect of γT on WSP-induced PMN/mg of sputum (the secondary study end points). Asterisks represent a significant ($P \leq .05$) increase above baseline value for a given end point. Note that there was no significant effect of γT on WSP-induced %PMNs (the primary study end point).

supplementation in volunteers with asthma was effective, we hypothesize that a longer duration of γT dosing will inhibit WSP- and $O_3$-induced neutrophilic inflammation. However, it is also possible that γT is more effective against endotoxin-induced neutrophilic responses than against those triggered by WSPs and $O_3$.

In contrast, exposure to WSPs and γT treatment were associated with changes in sputum eosinophil levels. Of the 11 volunteers who completed the WSP exposures, 6 had sputum eosinophils, with 5 of these volunteers reporting an atopic disorder (including 3 who had allergic rhinitis; 1 who experienced an IgE-mediated reaction to penicillin; and 1 who had allergic rhinitis, mild asthma, and penicillin allergy). Both the sputum eosinophil differential counts and number of eosinophils per mg in sputum were increased at 6 hours after exposure to WSPs and returned to baseline levels by 24 hours after exposure with placebo treatment. These measures were not increased at 6 hours following γT treatment, suggesting a potential action of γT on WSP-induced eosinophil responses (Fig 4). Sputum levels of IL-1β, IL-6, IL-8, and TNF-α were an exploratory end point (shown in Fig 5). Other exploratory end points included forced vital capacity, $FEV_1$ value, brachial artery flow–mediated dilation, and measures of left ventricular function (not shown). All of the exploratory end points were unaffected by exposure to WSPs or γT treatment. The size of this study precluded assessment of the effect of the GSTM1 null genotype or asthma status on any of our study outcomes.

There are conflicting observations regarding the role of αT and γT in modulating allergic airway inflammation, as reflected in a number of reviews.[1,18-21] Mechanistic studies using murine models of asthma suggest that γT exposure during sensitization may skew toward a type 2 inflammation response whereas αT protects against type 2 inflammation. Epidemiologic studies also show that plasma γT levels are increased in groups with increased asthma whereas increased αT level is associated with less asthma and better lung function.[18-20,22]

However, mechanistic studies from our group and others support the hypothesis that γT inhibits acute eosinophilic or type 2 responses in the airway. Using a rat model, we reported that γT treatment inhibits allergen-, endotoxin-, and $O_3$-induced airway eosinophilia and mucous cell hyperplasia, which is consistent with our study of 2-week dosing with γT in persons with mild asthma.[2,4,23] Using *in vitro* techniques, we have reported that γT and γ-tocotrienol inhibits IL-13–induced production of eotaxin-3, a potent eosinophil chemoattractant, by lung epithelial cells.[24] Others have reported that γT dosing inhibits allergen-induced eosinophils in the airway, serum eotaxin, and IL-4.[25]

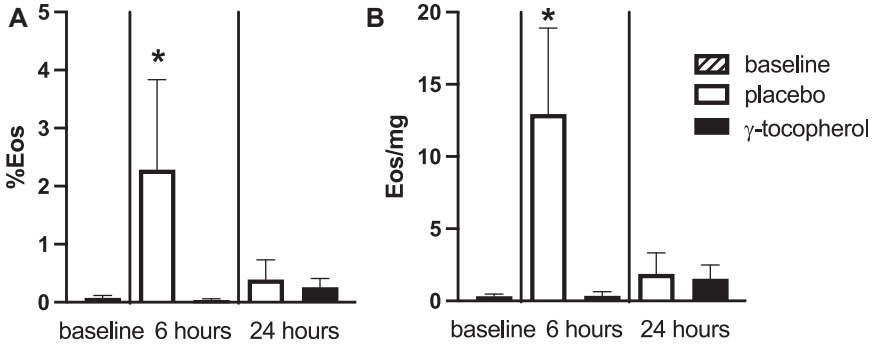


**FIG 4.** Comparison of γT dosing on sputum eosinophil outcomes 6 and 24 hours after initiation of controlled exposure to WSPs compared with baseline values. **A,** The effect of γT on WSP-induced differential count of eosinophils in sputum (%EOS) (an exploratory study end point). **B,** The effect of γT on WSP-induced EOS/mg of sputum (an exploratory study end point). Asterisks represent significant ($P \leq .05$) increase above the baseline value for a given end point. Note that WSP induced an increase in %EOS and EOS/mg of sputum at 6 hours only, with γT inhibiting this effect.

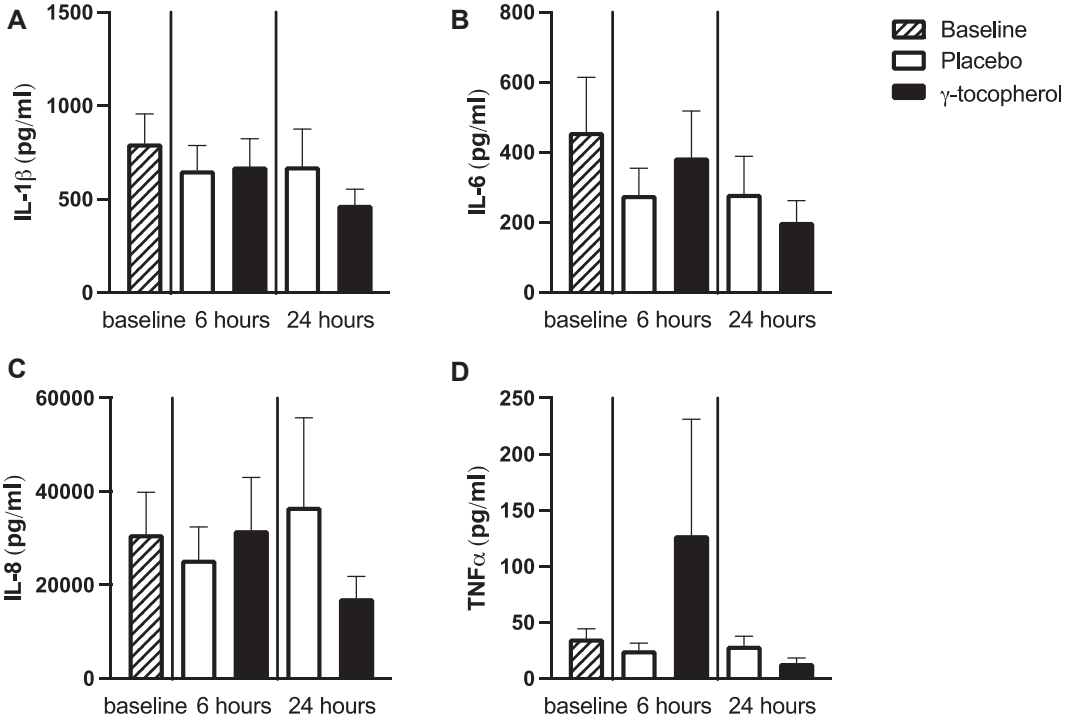


**FIG 5.** Comparison of γT dosing on sputum cytokine outcomes 6 and 24 hours after initiation of controlled exposure to WSPs compared with baseline values. **A,** The effect of γT on WSP-induced IL-1β level in sputum at baseline at 6 and 24 hours after exposure to WSPs. **B-D,** Results for IL-6, IL-8, and TNF-α, respectively. Results are shown as means ± SEs. There was no significant effect of either WSP or γT on sputum cytokine levels.

Our observations regarding WSP-induced airway eosinophilia in rodents are also consistent with the results of our human clinical trials showing that dosing with γT inhibited endotoxin-induced eosinophilic inflammation and reduced the constitutive levels of sputum eosinophils and MUC5AC in volunteers with allergic asthma.[3,5] Overall, our preclinical, *in vitro,* and now 3 human studies all support the hypothesis that γT supplementation reduces type 2 type/eosinophilic airway inflammation.

Taken together, our findings inform future studies of γT supplementation for anti-inflammatory effects in lung disease. In future phase II/III clinical trials, we would use a daily dose of γT of more than 1000 mg given for more than 14 days to assess the utility of

γT as an intervention for eosinophilic or neutrophilic airway diseases.

## DISCLOSURE STATEMENT

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**Clinical implications: Prior studies of supplementation with γT suggest that it is a promising nutraceutical intervention for pollutant-induced airway inflammation. In this pilot study of the effect of γT supplementation on wood smoke–induced airway inflammation, neutrophil influx was not affected but influx of eosinophils was reduced.**

### REFERENCES

1. Jiang Q, Im S, Wagner JG, Hernandez ML, Peden DB. Gamma-tocopherol, a major form of vitamin E in diets: insights into antioxidant and anti-inflammatory effects, mechanisms, and roles in disease management. Free Radic Biol Med 2022;178:347-59.
2. Wagner JG, Jiang Q, Harkema JR, Ames BN, Illek B, Roubey RA, et al. Gamma-tocopherol prevents airway eosinophilia and mucous cell hyperplasia in experimentally induced allergic rhinitis and asthma. Clin Exp Allergy 2008;38:501-11.
3. Hernandez ML, Wagner JG, Kala A, Mills K, Wells HB, Alexis NE, et al. Vitamin E, gamma-tocopherol, reduces airway neutrophil recruitment after inhaled endotoxin challenge in rats and in healthy volunteers. Free Radic Biol Med 2013;60:56-62.
4. Wagner JG, Jiang Q, Harkema JR, Illek B, Patel DD, Ames BN, et al. Ozone enhancement of lower airway allergic inflammation is prevented by gamma-tocopherol. Free Radic Biol Med 2007;43:1176-88.
5. Burbank AJ, Duran CG, Pan Y, Burns P, Jones S, Jiang Q, et al. Gamma tocopherol-enriched supplement reduces sputum eosinophilia and endotoxin-induced sputum neutrophilia in volunteers with asthma. J Allergy Clin Immunol 2018;141:1231-1238 e1.
6. Burbank AJ, Duran CG, Almond M, Wells H, Jenkins S, Jiang Q, et al. A short course of gamma-tocopherol mitigates LPS-induced inflammatory responses in humans ex vivo. J Allergy Clin Immunol 2017;140:1179-1181 e4.
7. Wiser J, Alexis NE, Jiang Q, Wu W, Robinette C, Roubey R, et al. In vivo gamma-tocopherol supplementation decreases systemic oxidative stress and cytokine responses of human monocytes in normal and asthmatic subjects. Free Radic Biol Med 2008;45:40-9.
8. Mills K, Lay J, Wu W, Robinette C, Kesic MJ, Dreskin SC, et al. Vitamin E, gamma-tocopherol, diminishes ex vivo basophil response to dust mite allergen. Allergy 2014;69:541-4.
9. Cascio WE. Wildland fire smoke and human health. Sci Total Environ 2018;624:586-95.
10. Ghio AJ, Soukup JM, Case M, Dailey LA, Richards J, Berntsen J, et al. Exposure to wood smoke particles produces inflammation in healthy volunteers. Occup Environ Med 2012;69:170-5.
11. Burbank AJ, Vadlamudi A, Mills KH, Alt EM, Wells H, Zhou H, et al. The glutathione-S-transferase mu-1 null genotype increases wood smoke-induced airway inflammation. J Allergy Clin Immunol 2019;143:2299-302.e3.
12. Alexis NE, Zhou LY, Burbank AJ, Almond M, Hernandez ML, Mills KH, et al. Development of a screening protocol to identify persons who are responsive to wood smoke particle-induced airway inflammation with pilot assessment of GSTM1 genotype and asthma status as response modifiers. Inhal Toxicol 2022;34:329-39.
13. Lay JC, Peden DB, Alexis NE. Flow cytometry of sputum: assessing inflammation and immune response elements in the bronchial airways. Inhal Toxicol 2011;23:392-406.
14. Alexis NE, Huang YC, Rappold AG, Kehrl H, Devlin R, Peden DB. Patients with asthma demonstrate airway inflammation after exposure to concentrated ambient particulate matter. Am J Respir Crit Care Med 2014;190:235-7.
15. Burbank AJ, Hernandez ML, Robinette C, Wang T, Zhou H, Alexis N, et al. Short course gamma tocopherol did not mitigate effects of ozone on airway inflammation in asthmatics. Inhal Toxicol 2020;32:279-81.
16. Grizzle JE. The two-period change-over design an its use in clinical trials. Biometrics 1965;21:467-80.
17. Little R, Rubin D. Statistical analysis with missing data. 2nd ed. New York, NY: Wiley; 2014:200-20.
18. Cook-Mills J, Gebretsadik T, Abdala-Valencia H, Green J, Larkin EK, Dupont WD, et al. Interaction of vitamin E isoforms on asthma and allergic airway disease. Thorax 2016;71:954-6.
19. Cook-Mills JM, Averill SH, Lajiness JD. Asthma, allergy and vitamin E: current and future perspectives. Free Radic Biol Med 2022;179:388-402.
20. Cook-Mills JM, Abdala-Valencia H, Hartert T. Two faces of vitamin E in the lung. Am J Respir Crit Care Med 2013;188:279-84.
21. Jiang Q. Natural forms of vitamin E: metabolism, antioxidant, and anti-inflammatory activities and their role in disease prevention and therapy. Free Radic Biol Med 2014;72:76-90.
22. Abdala-Valencia H, Soveg F, Cook-Mills JM. gamma-Tocopherol supplementation of allergic female mice augments development of CD11c+CD11b+ dendritic cells in utero and allergic inflammation in neonates. Am J Physiol Lung Cell Mol Physiol 2016;310:L759-71.
23. Wagner JG, Harkema JR, Jiang Q, Illek B, Ames BN, Peden DB. Gamma-tocopherol attenuates ozone-induced exacerbation of allergic rhinosinusitis in rats. Toxicol Pathol 2009;37:481-91.
24. Wang Y, Moreland M, Wagner JG, Ames BN, Illek B, Peden DB, et al. Vitamin E forms inhibit IL-13/STAT6-induced eotaxin-3 secretion by up-regulation of PAR4, an endogenous inhibitor of atypical PKC in human lung epithelial cells. J Nutr Biochem 2012;23:602-8.
25. Wu YM, Xue ZW, Zhang LL, Gao NM, Du XM, Zhang XY, et al. Comparable function of gamma-tocopherols in asthma remission by affecting eotaxin and IL-4. Adv Clin Exp Med 2016;25:643-8.