*Original Research*
*Brief communication*

# Greater γ-tocopherol status during acute smoking abstinence with nicotine replacement therapy improved vascular endothelial function by decreasing 8-iso-15(S)-prostaglandin $F_{2\alpha}$

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## Abstract

Nicotine replacement therapy (NRT) improves the long-term success rate of smoking cessation, but induces oxidative stress and inflammatory responses that may delay the restoration of vascular endothelial function (VEF). No studies have examined co-therapy of NRT-assisted smoking abstinence with γ-tocopherol (γ-T), a vitamin E form with antioxidant and anti-inflammatory activities, on improvements in VEF. In a randomized, double-blind, placebo-controlled study, healthy smokers ($25 \pm 1$ y old; mean ± SEM) received NRT and abstained from smoking for 24 h with placebo ($n = 12$) or oral administration of γ-T-rich mixture of tocopherols (γ-TmT; $n = 11$) that provided 500 mg γ-T. Brachial artery flow-mediated dilation (FMD), and biomarkers of nitric oxide metabolism, antioxidant status, inflammation, and lipid peroxidation [8-iso-prostaglandin $F_{2\alpha}$ stereoisomers (8-iso-15(R)-$PGF_{2\alpha}$ and 8-iso-15(S)-$PGF_{2\alpha}$)] were measured prior to and after 24 h of smoking abstinence. Smoking abstinence with NRT regardless of γ-TmT similarly decreased urinary naphthol ($P < 0.05$) without affecting plasma cotinine. γ-TmT increased plasma γ-T by 4-times and the urinary metabolite of γ-T, γ-carboxyethyl-chromanol, by three times. Smoking abstinence with γ-TmT, but not smoking abstinence alone, increased FMD without affecting plasma nitrate/nitrite or the ratio of asymmetric dimethylarginine/arginine. Urinary 8-iso-15(S)-$PGF_{2\alpha}$ decreased only in those receiving γ-TmT and was inversely correlated to FMD ($R = -0.43$, $P < 0.05$). Circulating markers of inflammation were unaffected by smoking abstinence or γ-TmT. Short-term NRT-assisted smoking abstinence with γ-TmT, but not NRT-assisted smoking abstinence alone, improved VEF by decreasing 8-iso-15(S)-$PGF_{2\alpha}$, a vasoconstrictor that was otherwise unaffected by NRT-assisted smoking abstinence.

**Keywords:** Vascular endothelial function, vitamin E, smoking cessation, flow-mediated dilation, nicotine replacement therapy

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## Introduction

Cigarette smoking is the foremost preventable cause of premature mortality and a leading risk factor for cardiovascular disease (CVD).[1] Despite the known risks, 22% of Americans continue to smoke. Vascular dysfunction, an early event leading to CVD, occurs in smokers[2,3] in an oxidative stress-dependent manner[2,4–8] due to reactive oxygen and nitrogen species (ROS/RNS) from cigarette smoke and activated inflammatory cells.[9–12] Smoking abstinence decreases morbidity,[13] but the success rate for quitting unassisted (i.e. 'cold-turkey') is 3–5%, and increases to 24% with the use of pharmacological aids and behavioral support.[14] Although nicotine replacement therapy (NRT) curbs tobacco addiction,[15] studies in rodents show that nicotine induces oxidative stress and inflammation[16] and impairs vascular endothelial function (VEF) independent of cigarette smoking.[17] Thus, complementary therapies that attenuate nicotine-induced ROS/RNS and vascular dysfunction are needed to fully realize the benefits of NRT-assisted smoking cessation.

Vitamin E describes eight lipophilic tocopherols (T) and tocotrienols.[18] γ-T is the most abundant dietary form, and it

and/or its physiologic metabolite γ-carboxyethyl-hydroxychroman (γ-CEHC) has anti-oxidative, anti-nitrative, anti-inflammatory, and vasoprotective activities that have received limited attention compared to α-T, the major circulating form of vitamin E.[19–22] Short-term administration of a γ-T-rich mixture of tocopherols (γ-TmT) during smoking cessation in the absence of NRT decreases TNFα and myeloperoxidase (MPO) while improving VEF beyond that of smoking cessation alone.[21] We therefore hypothesized that improvements in γ-T status during NRT-assisted smoking abstinence would improve VEF by decreasing oxidative stress and inflammation. We measured brachial artery flow-mediated dilation (FMD), and oxidative stress and inflammatory markers, during a randomized, double-blind, placebo-controlled study examining NRT-assisted smoking abstinence with γ-TmT administration.

## Methods

### Study design

This protocol was approved by the Institutional Review Boards at University of Connecticut and The Ohio State University. Healthy male and female cigarette smokers (≥10 cigarettes/day; ≥1 year) were randomized to abstain from smoking with the use of NRT while taking placebo ($n = 12$) or γ-TmT ($n = 11$) for 24 h. Transdermal nicotine patches (NicoDerm CQ Step 1; GlaxoSmithKline, Philadelphia, PA, USA) provided 20 mg nicotine for 24 h. Placebo was a corn oil capsule (0.07 mg α-T and 0.18 mg γ-T) and γ-TmT contained 500 mg γ-T, 62 mg α-T, 24 mg β-T and 6 mg δ-T (provided by Dr. Jose Llobrera, Designs for Health, Inc., Suffield, CT, USA). Participants visited the study center in the fasted state (10–12 h) prior to (pre) and after 24 h of smoking abstinence (post). They ingested placebo or γ-TmT capsules with dinner the night prior to their post-visit to achieve near-maximal plasma γ-T concentrations during their visit.[23] FMD assessment, and plasma and 24 h urine collection were performed as described.[21] Lastly, participants completed four-day food records for three days prior to their pre-visit and 24 h prior to their post-visit. These were analyzed using the 2010 Nutrition Data System for Research (University of Minnesota, Minneapolis, MN, USA).

### Flow-mediated dilation and carotid artery intima media thickness

Brachial artery FMD was assessed by high-frequency ultrasonography.[21] Brachial artery FMD is expressed as change from baseline (Δ mm; post-occlusion peak diameter – baseline diameter) and percent change from baseline (%; Δ mm/baseline diameter (mm) × 100). Statistical analysis for absolute (Δ mm) and relative (%) FMD responses was performed in parallel and the results did not differ qualitatively. For simplicity, only results for FMD (%) are reported for regression analyses. To control for possible confounding factors, FMD measurements for each participant were performed at the same time of day during the pre- and post-visits, and participants were required to avoid exercise, caffeine, and alcohol for 24 h prior to any FMD measurements. Additionally, FMD measurements for female participants were completed between days 7 and 14 following the completion of menses to account for changes in vascular reactivity that occur throughout the menstrual cycle.[24] Finally, to define atherosclerotic risk, carotid intima media thickness (cIMT) was assessed at pre-visit as described.[21]

### Nitric oxide (NO•) metabolism

Plasma arginine, the substrate for NO• synthase (NOS)-mediated NO• synthesis, and asymmetric dimethylarginine (ADMA), an endogenous competitive inhibitor of NOS, were measured by HPLC-FL.[25] Plasma nitrate and nitrite ($NO_x$), the end-metabolites of NO•, were measured by colorimetric assay (Cayman Chemical, Ann Arbor, MI, USA).

### Plasma cotinine and urinary naphthol

Plasma cotinine[21] and urinary 2-naphthol,[26] the metabolite of naphthalene, a polycyclic aromatic hydrocarbon found in cigarette smoke,[27] were measured by HPLC-FL.

### Antioxidants, oxidative stress, and inflammation

Plasma vitamin E (as α- and γ-T) and urinary α- and γ-CEHC were measured by UHPLC-MS methods, and plasma vitamin C and uric acid by HPLC-Coularray.[21] CEHCs were normalized to urinary creatinine, which was measured by clinical assay (Pointe Scientific, Canton, MI, USA). Urinary 8-iso-prostaglandin $F_{2\alpha}$ stereoisomers (8-iso-15(R)-$PGF_{2\alpha}$ and 8-iso-15(S)-$PGF_{2\alpha}$) and the metabolites of 8-iso-15(S)-$PGF_{2\alpha}$, 2,3-dinor-5,6-dihydro-8-iso-$PGF_{2\alpha}$ (2,3-dinor-F1) and 2,3-dinor-8-iso-$PGF_{2\alpha}$ (2,3-dinor-F2) were measured by LC-MS/MS.[28] Plasma malondialdehyde (MDA) was measured by HPLC-FL.[21] Plasma-oxidized LDL (oxLDL; Mercodia, Winston Salem, NC, USA), MPO, C-reactive protein (CRP; BioCheck, Foster City, CA, USA), soluble intracellular adhesion molecule-1 (sICAM-1), and monocyte chemoattractant protein-1 (MCP-1; R&D Systems, Minneapolis, MN, USA) were measured by ELISA.

### Statistical analysis

A power calculation was performed using FMD responses to determine appropriate sample sizes (Power and Sample Size Calculation Version 3.0.43). In the absence of studies examining γ-T during NRT-mediated smoking cessation, we estimated an additional 1% increase in FMD responses following γ-TmT supplementation in combination with smoking cessation. This increase is of physiological relevance because a 1% increase in FMD corresponds to a 14% decrease in future CVD events.[24] Based on a power calculation of previously published data of FMD responses during smoking cessation,[29] we estimated that a minimum of eight participants per group would be needed to detect differences with 80% power ($P < 0.05$).

Data (means ± SEM) were analyzed by SPSS Version 15.0 (IBM, Armonk, NY, USA). Student's independent *t*-tests were used to evaluate pre-data between placebo and

γ-TmT groups. Initial analysis was performed using three-way repeated measures ANOVA to define main effects for gender, time, γ-TmT, and their interaction. Because no gender effects were observed, two-way repeated measures ANOVA with Bonferroni correction was performed to assess effects due to time, γ-TmT, and their interaction. Pearson correlation coefficients (r) for study variables at pre-visit were determined by linear regression. Multiple linear regression, controlling for within-subject repeated measures, was used to calculate correlation coefficients ($R$) as described.[30] $P \leq 0.05$ indicates statistical significance for all analyses.

## Results

### Participants and compliance

Participants were generally healthy despite their smoking history (Table 1) and cIMT indicated no evidence of plaque or increased CVD risk.[31] Baseline brachial artery diameter, FMD, smoking frequency, and smoking burden did not differ between groups (Table 1). Baseline plasma cotinine and urinary naphthol were also similar between groups and correlated to smoking burden ($P < 0.05$; $r = 0.45$–$0.53$). Cotinine was unaffected by smoking abstinence consistent with NRT administration, whereas naphthol decreased regardless of γ-TmT administration (Figure 1a). No group differences occurred in dietary intakes (Table 2).

**Table 1** Participant characteristics

| | Placebo | γ-TmT |
|---|---|---|
| M/F | 8/4 | 7/4 |
| Age (y) | 23.2 ± 1.4 | 26.5 ± 2.4 |
| Smoking frequency (cigarettes/day) | 15.2 ± 1.5 | 12.5 ± 1.5 |
| Smoking burden (pack-y) | 5.55 ± 1.42 | 5.95 ± 2.31 |
| Cotinine (nmol/L) | 139 ± 24 | 214 ± 43 |
| Naphthol (μmol/L) | 3.36 ± 0.73 | 3.15 ± 0.55 |
| Body mass index (kg/m$^2$) | 25.6 ± 1.5 | 27.5 ± 1.1 |
| Systolic blood pressure (mm Hg) | 123 ± 3 | 125 ± 3 |
| Diastolic blood pressure (mm Hg) | 72 ± 2 | 74 ± 2.6 |
| Total cholesterol (mg/dL) | 156 ± 10 | 144 ± 8 |
| Triglyceride (mg/dL) | 79.9 ± 11.4 | 94.7 ± 17.6 |
| Glucose (mg/dL) | 89.4 ± 3.4 | 89.5 ± 2.3 |
| Blood urea nitrogen (mg/dL) | 12.8 ± 1.1 | 13.2 ± 0.7 |
| Creatinine (mg/dL) | 0.84 ± 0.04 | 0.85 ± 0.05 |
| Sodium (mEq/L) | 140 ± 0.4 | 141 ± 1 |
| Potassium (mEq/L) | 4.72 ± 0.10 | 4.47 ± 0.15 |
| Chloride (mEq/L) | 104 ± 1 | 104 ± 1 |
| Bicarbonate (mEq/L) | 20.6 ± 0.8 | 21.8 ± 0.6 |
| Calcium (mg/dL) | 9.45 ± 0.12 | 9.30 ± 0.11 |
| Albumin/globulin | 1.98 ± 0.08 | 2.11 ± 0.09 |
| Total bilirubin (mg/dL) | 0.97 ± 0.18 | 0.63 ± 0.09 |
| Alkaline phosphatase (U/L) | 69.5 ± 6.0 | 62.3 ± 5.2 |
| Aspartate aminotransaminase (U/L) | 20.0 ± 1.8 | 25.2 ± 3.1 |
| Alanine aminotransaminase (U/L) | 20.4 ± 5.5 | 31.7 ± 7.8 |
| Lactate dehydrogenase (U/L) | 187 ± 20 | 205 ± 24 |
| Baseline brachial artery diameter (mm) | 3.80 ± 0.14 | 4.06 ± 0.29 |
| Average left cIMT (mm) | 0.50 ± 0.02 | 0.49 ± 0.03 |
| Average right cIMT (mm) | 0.50 ± 0.03 | 0.50 ± 0.02 |

Note: No significant differences ($P > 0.05$) were observed between groups, and all participants had blood chemistry values that were within the normal clinical reference range. Values expressed as means ± SEM.
cIMT: carotid intima media thickness, γ-TmT: γ-tocopherol-rich mixture of tocopherols.

Plasma γ- and α-T, and urinary γ- and α-CEHC, were unaffected in participants receiving placebo, whereas γ-TmT administration increased γ-T (Figure 1b) and γ-CEHC (Table 3) by approximately 3–4 times. γ-TmT administration decreased α-T by 10% (Figure 1b), while increasing α-CEHC by 62% (Table 3).

### Brachial artery flow-mediated dilation and NO• metabolism

Pre-occlusion brachial artery diameters at pre and post were unaffected by smoking abstinence and γ-TmT (placebo: 3.80 ± 0.14 vs. 3.82 ± 0.15 mm; γ-TmT: 4.06 ± 0.29 vs. 4.06 ± 0.28 mm). FMD increased only in participants receiving γ-TmT (Figure 1c). FMD was correlated to γ-T and γ-CEHC ($R = 0.43$–$0.55$, $P < 0.05$). Plasma arginine, ADMA, and ADMA/arginine, an index of NO• bioavailability,[32] and plasma $NO_x$ were unaffected by smoking abstinence or γ-TmT (Table 3).

### Antioxidants, oxidative stress, and inflammation

Plasma vitamin C, uric acid, MDA, oxLDL, and inflammatory markers (MPO, CRP, MCP-1, sICAM-1) were unaffected by smoking abstinence and γ-TmT (Table 3). Urinary 8-iso-15(S)-$PGF_{2\alpha}$, but not urinary 8-iso-15(R)-$PGF_{2\alpha}$, total 8-iso-$PGF_{2\alpha}$ (sum of 8-iso-15(R)- and 8-iso-15(S)-$PGF_{2\alpha}$), or metabolites of 8-iso-15(S)-$PGF_{2\alpha}$ (i.e. 2,3-dinor-F1 and 2,3-dinor-F2) decreased following smoking abstinence only in the γ-TmT group (Table 3). Urinary 8-iso-15(S)-$PGF_{2\alpha}$ was also inversely correlated to FMD ($R = -0.43$, $P < 0.05$).

## Discussion

This study demonstrated that short-term NRT-assisted smoking abstinence with γ-TmT, but not NRT-assisted smoking abstinence alone, improved FMD in association with lowered 8-iso-15(S)-$PGF_{2\alpha}$. γ-TmT administration during NRT-assisted smoking abstinence decreased urinary 8-iso-15(S)-$PGF_{2\alpha}$, suggesting that γ-TmT increases VEF by attenuating activities of this vasoconstrictor.[33] γ-TmT increased plasma γ-T and urinary γ-CEHC without affecting pro-inflammatory proteins, suggesting that γ-TmT improves VEF independent of inflammation. These findings provide the first evidence that greater γ-T status improves VEF during smoking abstinence by specifically decreasing 8-iso-15(S)-$PGF_{2\alpha}$ that was otherwise unaffected by smoking abstinence.

Nicotine, independent of cigarette smoking, impairs VEF.[17] We showed that smoking cessation without NRT or γ-TmT increases FMD.[21] In the present study, NRT was provided to maintain participants' nicotine levels, and had no effect on FMD, suggesting that its short-term use limits smoking abstinence-mediated restoration of VEF.

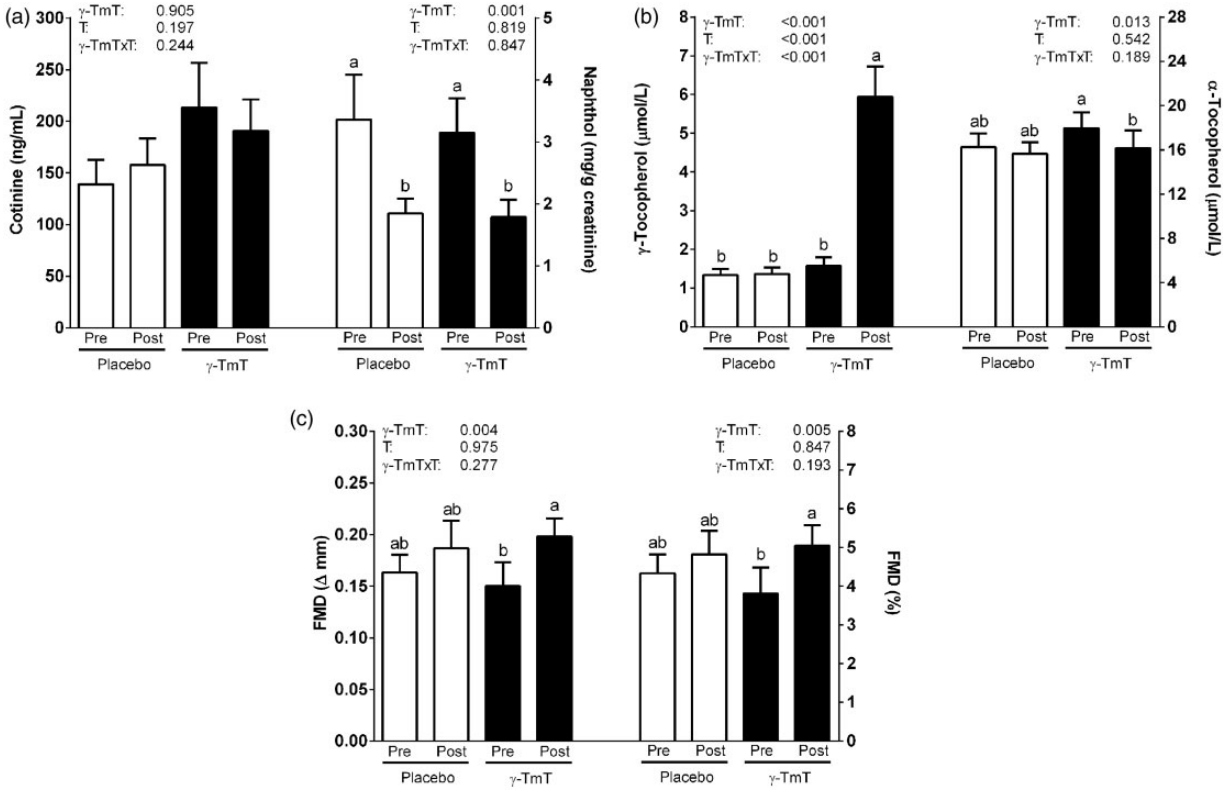


**Figure 1** Plasma cotinine and urinary naphthol, plasma tocopherols, and flow-mediated dilation (FMD) responses in healthy smokers following 24 h smoking abstinence with ($n = 11$) or without ($n = 12$) γ-tocopherol-rich mixture of tocopherols (γ-TmT). (a) Plasma α- and γ-tocopherol concentrations, (b) plasma cotinine and urinary naphthol concentrations, and (c) FMD responses expressed as absolute change and percent change from baseline diameter in smokers following smoking abstinence with or without oral administration of γ-TmT. T: time; γ-TmT × T: γ-TmT × time interaction. Bars are means ± SEM. Means not sharing a common superscript are significantly different ($P < 0.05$)

**Table 2** Dietary intakes from 4-day dietary records of smokers who abstained from smoking with ($n = 11$) and without ($n = 12$) oral administration of γ-tocopherol-rich mixture of tocopherols (γ-TmT) prior to the start of the study

| | Placebo | γ-TmT |
|---|---|---|
| Total energy (kcal) | 2217 ± 174 | 2268 ± 262 |
| Energy from carbohydrate (%) | 44.4 ± 2.5 | 44.8 ± 2.9 |
| Energy from protein (%) | 14.8 ± 0.9 | 14.7 ± 1.2 |
| Energy from fat (%) | 37.0 ± 2.2 | 39.9 ± 2.2 |
| Total saturated fat (g) | 31.2 ± 2.2 | 36.4 ± 5.0 |
| Monounsaturated fat (g) | 32.1 ± 2.5 | 34.3 ± 4.2 |
| Polyunsaturated fat (g) | 19.2 ± 1.5 | 23.5 ± 3.7 |
| Cholesterol (mg) | 251 ± 19 | 251 ± 35 |
| Vitamin A (RAE) | 5466 ± 2133 | 4930 ± 1744 |
| β-Carotene (μg) | 2511 ± 1308 | 2015 ± 941 |
| Vitamin C (mg) | 98.2 ± 21.5 | 76.8 ± 21.9 |
| Vitamin D (μg) | 3.4 ± 0.5 | 3.2 ± 0.7 |
| α-Tocopherol (mg) | 8.0 ± 0.9 | 10.1 ± 1.7 |
| γ -Tocopherol (mg) | 13.5 ± 1.5 | 15.9 ± 2.5 |
| Vitamin K (μg) | 64.4 ± 8.3 | 76.6 ± 13.2 |
| Magnesium (mg) | 256 ± 23 | 242 ± 24 |
| Selenium (μg) | 116 ± 9 | 102 ± 10 |
| Zinc (mg) | 11.1 ± 1.2 | 12.1 ± 2.0 |
| Sodium (mg) | 4307 ± 503 | 3788 ± 473 |

Note: Values are means ± SEM. No significant differences ($P > 0.05$) were observed between groups.

In contrast, γ-TmT use during NRT-assisted smoking abstinence improves VEF.

NRT-assisted smoking abstinence in combination with γ-TmT, but not NRT-assisted smoking abstinence alone, decreased urinary 8-iso-15(S)-$PGF_{2\alpha}$. This $F_2$-isoprostane is generated by non-enzymatic free radical peroxidation of arachidonic acid, and is recognized as an oxidative stress biomarker and vasoconstrictor.[33] Antagonism of thromboxane $A_2$ ($TXA_2$)/prostaglandin $H_2$ (TP) receptors inhibit 8-iso-15(S)-$PGF_{2\alpha}$-induced vasoconstriction *in vitro*.[33] Likewise, 8-iso-15(S)-$PGF_{2\alpha}$ induces vasoconstriction in wild-type mice but not in TP-receptor-deficient mice.[34] In CVD patients, circulating 8-iso-15(S)-$PGF_{2\alpha}$ is increased[35] and TP receptor antagonists improve their FMD responses.[36,37] Although 8-iso-15(S)-$PGF_{2\alpha}$ binds to TP receptors, the mechanism by which it induces vasoconstriction is unclear. 8-iso-15(S)-$PGF_{2\alpha}$ is suggested to mediate endothelium-dependent vasoconstriction in porcine periventricular and retinal vessels by stimulating $TXA_2$ release.[38,39] Furthermore, inhibition of $TXA_2$ synthase abolishes 8-iso-15(S)-$PGF_{2\alpha}$-induced vasoconstriction.[38] In turn, $TXA_2$ induces vasoconstriction by activating TP receptors on vascular smooth muscle cells (i.e. endothelium-independent) or by decreasing eNOS-mediated NO• synthesis.[40] We showed that γ-TmT decreased 8-iso-15(S)-$PGF_{2\alpha}$ without affecting ADMA/arginine or $NO_x$,

**Table 3** Antioxidant status, oxidative stress and inflammation markers of smokers who abstain from smoking with ($n = 11$) and without ($n = 12$) oral administration of γ-tocopherol-rich mixture of tocopherols (γ-TmT) prior to (pre) and after 24 h smoking abstinence (post)

| | Placebo | | γ-TmT | | | | |
|---|---|---|---|---|---|---|---|
| | Pre | Post | Pre | Post | T | γ-TmT | T × γ-TmT |
| Arginine, ADMA, and $NO_x$ | | | | | | | |
| Arginine (μmol/L) | 79.7 ± 4.2 | 81.8 ± 4.4 | 74.7 ± 3.7 | 73.0 ± 3.4 | 0.893 | 0.219 | 0.221 |
| ADMA (nmol/L) | 744 ± 49 | 742 ± 42 | 864 ± 52 | 874 ± 54 | 0.753 | 0.079 | 0.681 |
| ADMA/Arginine (nmol/μmol) | 11.5 ± 0.8 | 11.1 ± 0.7 | 11.3 ± 0.8 | 11.0 ± 0.8 | 0.166 | 0.896 | 0.787 |
| $NO_x$ (μmol/L) | 17.5 ± 2.5 | 14.2 ± 1.2 | 22.2 ± 3.0 | 18.3 ± 2.2 | 0.017 | 0.140 | 0.840 |
| Antioxidants and oxidative stress | | | | | | | |
| α-CEHC (nmol/L) | 13.6 ± 3.0 | 12.1 ± 2.2 | 15.4 ± 3.3 | 25.0 ± 4.7* | 0.117 | 0.078 | 0.037 |
| γ-CEHC (nmol/L) | 69 ± 10 | 76 ± 10 | 106 ± 16 | 296 ± 61* | 0.012 | <0.01 | 0.018 |
| Vitamin C (μmol/L) | 44.2 ± 3.7 | 44.6 ± 3.7 | 43.0 ± 4.6 | 45.8 ± 5.0 | 0.391 | 0.999 | 0.502 |
| Uric Acid (μmol/L) | 306 ± 19 | 304 ± 20 | 289 ± 18 | 291 ± 17 | 0.993 | 0.573 | 0.757 |
| Malondialdehyde (μmol/L) | 1.08 ± 0.04 | 1.08 ± 0.05 | 1.06 ± 0.03 | 1.08 ± 0.04 | 0.794 | 0.798 | 0.551 |
| Oxidized LDL (U/L) | 48.8 ± 3.7 | 50.1 ± 3.9 | 61.9 ± 7.4 | 58.1 ± 5.3 | 0.486 | 0.153 | 0.152 |
| Total 8-iso-$PGF_{2\alpha}$ (ng/g creatinine) | 523 ± 37 | 479 ± 37 | 676 ± 59 | 639 ± 71 | 0.181 | 0.030 | 0.905 |
| 8-iso-15(R)-$PGF_{2\alpha}$ (ng/g creatinine) | 267 ± 26 | 250 ± 25 | 367 ± 41 | 396 ± 63 | 0.808 | 0.026 | 0.367 |
| 8-iso-15(S)-$PGF_{2\alpha}$ (ng/g creatinine) | 256 ± 22 | 229 ± 20 | 309 ± 26 | 242 ± 24* | 0.005 | 0.257 | 0.200 |
| 2,3-dinor-F1 (ng/g creatinine) | 1431 ± 178 | 1275 ± 134 | 1507 ± 168 | 1551 ± 226 | 0.636 | 0.437 | 0.407 |
| 2,3-dinor-F2 (ng/g creatinine) | 2162 ± 166 | 2015 ± 174 | 2158 ± 339 | 2416 ± 322 | 0.663 | 0.566 | 0.124 |
| Inflammation | | | | | | | |
| MPO (ng/mL) | 19.8 ± 2.5 | 15.9 ± 1.6 | 18.6 ± 1.9 | 17.8 ± 1.9 | 0.101 | 0.880 | 0.260 |
| CRP (mg/L) | 1.25 ± 0.46 | 1.28 ± 0.52 | 1.71 ± 0.64 | 1.79 ± 0.68 | 0.682 | 0.560 | 0.855 |
| MCP-1 (pg/mL) | 58.1 ± 4.0 | 53.7 ± 4.8 | 54.9 ± 3.7 | 53.0 ± 2.3 | 0.167 | 0.700 | 0.580 |
| sICAM-1 (pg/mL) | 10.7 ± 0.6 | 10.8 ± 0.7 | 11.5 ± 1.1 | 11.3 ± 1.2 | 0.630 | 0.622 | 0.342 |

Note: Values expressed as means ± SEM.
MPO: myeloperoxidase; CRP: C-reactive protein; MCP-1: monocyte chemoattractant protein-1; sICAM-1: soluble intracellular adhesion molecule-1; T: main effect of time; T × γ-TmT: time × γ-TmT interaction effect.
*Significantly different from pre ($P < 0.05$).

suggesting that γ-TmT likely improved VEF in a NO•-independent manner, although further study is needed to define the mechanisms involved. In addition, future studies should specifically measure the stereoisomer 8-iso-15(S)-$PGF_{2\alpha}$, because monitoring total 8-iso-$PGF_{2\alpha}$, which is more common, would have precluded any observation of γ-TmT-mediated decreases in 8-iso-15(S)-$PGF_{2\alpha}$ as a possible explanation for improved VEF.

The mechanism by which γ-TmT decreased 8-iso-15(S)-$PGF_{2\alpha}$ is unknown, but is likely independent of oxidative stress in the present study, consistent with the lack of decrease in oxLDL, MDA, 8-iso-15(R)-$PGF_{2\alpha}$, 2,3-dinor-F1, and 2,3-dinor-F2. Activity of 15-prostaglandin dehydrogenase, an enzyme that hydroxylates isoprostanes prior to β-oxidation,[41] is upregulated in α-T-deficient rabbits.[42] This suggests that γ-TmT-mediated decreases in α-T may have contributed to oxidative degradation of 8-iso-15(S)-$PGF_{2\alpha}$. Alternatively, decreases in 8-iso-15(S)-$PGF_{2\alpha}$ may reflect γ-T-mediated inhibition of phospholipase $A_2$, which hydrolyzes $F_2$-isoprostanes from phospholipids.[43,44] Regardless of the mechanism, which requires additional study, lowering of this vasoconstrictor would be expected to improve VEF, consistent with our observations.

The increasing use of brachial artery FMD to assess vascular function in clinical studies is attributed to its accuracy and sensitivity,[45] and prognostic value for predicting future CVD events.[24] However, subtle variations in FMD protocols critically impact FMD responses.[46] To ensure accuracy and reliability of our FMD measurements, numerous variables known to affect FMD were standardized according to current guidelines (i.e. occlusion duration, cuff placement, and positioning of the ultrasound probe, diurnal variations, dietary supplements, medication, exercise, and menstrual phase).[46] Although dietary intakes did not differ between treatment groups or visits, we cannot exclude possible effects of other vasoactive dietary components (e.g. nitrates and flavonoids) that are not included in food composition tables. Improvements in FMD in our study cannot be fully attributed to either γ-T or γ-CEHC alone because concentrations of both were increased by γ-TmT. We also cannot conclude whether improvements in FMD resulted from a synergistic or additive interaction between γ-TmT, smoking abstinence, and NRT. This study was specifically limited to young and healthy smokers to control for confounding factors affecting VEF, thus precluding any extrapolations to those with existing co-morbidities.

In conclusion, short-term NRT-assisted smoking abstinence with oral administration of γ-TmT, but not NRT-assisted smoking abstinence alone, improved VEF in association with decreases in the vasoconstrictor

8-iso-15(S)-$PGF_{2\alpha}$. Although NRT precluded the restoration of VEF by smoking abstinence at 24 h, FMD increases similarly following 1 year of smoking cessation with or without NRT,[47] suggesting that long-term smoking cessation improves VEF, despite NRT use. Chronic γ-TmT administration with NRT has not been studied and warrants investigation to better define γ-TmT as a complementary strategy to restore cardiovascular health in former smokers.

**Authors' contributions:** RSB, JSV, EM, and CM were responsible for the study design. RSB, EM, RP, YG, CM, and KDB were responsible for collecting and analyzing data. KDB and JSV reviewed the FMD analyses. BAP assisted with cIMT analysis and AWT and MGT assisted with analysis of urinary isoprostanes. EM and RSB wrote the initial draft of the manuscript and all authors contributed to the editing and review of this manuscript.

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