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γ-Tocopherol abolishes postprandial increases in plasma methylglyoxal following an oral dose of glucose in healthy, college-aged men☆,☆☆

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Abstract

Postprandial hyperglycemia contributes to the risk of cardiovascular disease in part by increasing concentrations of the reactive dicarbonyl methylglyoxal (MGO), a byproduct of glucose metabolism. Oxidative stress increases MGO formation from glucose in vitro and decreases its glutathione-dependent detoxification to lactate. We hypothesized that the antioxidant γ-tocopherol, a form of vitamin E, would decrease hyperglycemia-mediated postprandial increases in plasma MGO in healthy, normoglycemic, college-aged men. Participants (n=12 men; 22.3±1.0 years; 29.3±2.4 kg/m^2) received an oral dose of glucose (75 g) in the fasted state prior to and following 5-day ingestion of a vitamin E supplement enriched in γ-tocopherol (500 mg/day). γ-Tocopherol supplementation increased (P<.0001) plasma γ-tocopherol from 2.22±0.32 to 7.06±0.71 μmol/l. Baseline MGO concentrations and postprandial hyperglycemic responses were unaffected by γ-tocopherol supplementation (P>.05). Postprandial MGO concentrations increased in the absence of supplemental γ-tocopherol (P<.05), but not following γ-tocopherol supplementation (P>.05). Area under the curve for plasma MGO was significantly (P<.05) smaller with the supplementation of γ-tocopherol than without (area under the curve $_{0–180\ min}$, −778±1010 vs. 2277±705). Plasma concentrations of γ-carboxyethyl-hydroxychroman, reduced glutathione and markers of total antioxidant capacity increased after supplementation, and these markers and plasma γ-tocopherol were inversely correlated with plasma MGO (r=−0.48 to −0.67, P<.05). These data suggest that short-term supplementation of γ-tocopherol abolishes the oral glucose-mediated increases in postprandial MGO through its direct and indirect antioxidant properties and may reduce hyperglycemia-mediated cardiovascular disease risk.

Keywords: Methylglyoxal; γ-Tocopherol; Hyperglycemia; Oxidative stress

1. Introduction

At least 12% of Americans are estimated to have diabetes [1], which increases cardiovascular disease (CVD)-related mortality risk by threefold [2]. An additional ~14% of Americans also have impaired glucose tolerance (IGT) [1], resulting in 30% greater CVD-related mortality risk even in the absence of elevated fasting glucose [3]. Suppressing postprandial hyperglycemia with an α-glucosidase inhibitor reduces CVD-related events and myocardial infarction among nondiabetics with IGT [4]. However, such approaches are associated with adverse gastrointestinal side effects, thereby limiting compliance [5]. Thus, it remains of critical importance to develop safe and effective strategies that reduce postprandial hyperglycemia-mediated increases in CVD risk.

Postprandial hyperglycemia likely contributes to CVD risk by inducing oxidative stress [6,7]. Postprandial hyperglycemia increased lipid peroxidation markers in type 2 diabetics [6] and decreased plasma antioxidants in type 2 diabetics, nondiabetics with IGT and normoglycemic individuals [8,9]. Postprandial oxidative stress following glucose administration contributes to endothelial dysfunction in healthy nondiabetics [7]. Thus, postprandial hyperglycemia may enhance CVD risk, even in normoglycemic individuals.

Emerging evidence implicates the highly reactive dicarbonyl and glycolytic byproduct methylglyoxal (MGO), a precursor to advanced glycation end-products (AGE), in hyperglycemia-mediated oxidative stress and CVD risk [10]. Plasma MGO is greater in diabetics [11] and increases postprandially in diabetics in proportion to blood glucose [12]. MGO decreases glucose tolerance in rodents [13,14], suggesting that postprandial MGO production in normoglycemic individuals could result in glucose intolerance and consequently greater MGO accumulation. MGO also induces mitochondrial damage, resulting in the generation of reactive oxygen species (ROS) [15] and impaired endothelial function [10], thus contributing to CVD risk.

Macrophages treated with H_2O_2 or the ROS-generator dinitrophenyl chloride has greater MGO accumulation [16]. Given this association in vitro, dietary antioxidants may regulate MGO production in vivo. We therefore investigated the extent to which dietary supplementation with a vitamin E preparation rich in γ-tocopherol regulates increases in MGO following an oral dose of glucose in

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college-aged men. γ-Tocopherol is a form of vitamin E that functions as a lipophilic antioxidant but is also metabolized rapidly to its water-soluble physiological metabolite, γ-carboxyethyl-hydroxychroman (γ-CEHC) [17], which has anti-inflammatory [18] and antioxidant [19] activities. We hypothesized that γ-tocopherol would reduce postprandial increases in MGO by increasing antioxidant status and reducing hyperglycemia-induced oxidative stress. To our knowledge, this investigation provides the first evidence that improvements in γ-tocopherol status attenuate glucose-induced postprandial increases in MGO, without altering postprandial hyperglycemia.

2. Materials and methods

2.1. Subjects

The study protocol was approved by the institutional review board for the protection of human subjects at the University of Connecticut, and all participants provided written consent before enrolling. Otherwise healthy, nonsmoking men (n=12; Table 1) were selected for this study on the basis of age (18–35 years), stable body weight (>2 months), body mass index (19–40 kg/m^2), nonuse of dietary supplements (>2 months), fasting blood cholesterol (<200 mg/dl) and glucose (<100 mg/ dl) and resting blood pressure (<140/90 mm Hg). In addition, participants had stable exercise patterns (<5 h/week of aerobic activity), were free of diabetes and other metabolic diseases and did not use any medications.

2.2. Materials

High performance liquid chromatography (HPLC) grade solvents and the following chemicals were purchased form Fisher Scientific (Pittsburgh, PA, USA): ascorbic acid, butylated hydroxytoluene, diethylenetriaminepentaacetic acid (DTPA), potassium hydroxide (KOH), lithium perchlorate, potassium phosphate, sodium acetate and sodium phosphate ($NaPO_4$). 2,4,6-Tri(2-pyridil)-*s*-triazine, 5-methylquinoxaline (5-MQ), dansyl chloride, iron chloride ($FeCl_3$), fluorescein, MGO, *o*-phenylenediamine), Trolox and *RRR*-α-, β-, γ- and δ-tocopherols were purchased from Sigma-Aldrich (St. Louis, MO, USA). 2,2′-Azobis-2-methyl-propanimidamide was from Cayman Chemical (Ann Arbor, MI, USA).

2.3. Study design

Each participant visited the study center in the fasting state (10–12 h) on two occasions separated by ≥7 days. For 5 consecutive days preceding the second visit, participants ingested an encapsulated vitamin E supplement containing 500 mg γ-tocopherol, 60 mg α-tocopherol, 170 mg δ-tocopherol and 9 mg β-tocopherol, which was kindly provided by Brent Flickinger (Archer Daniels Midland, Decatur, IL, USA). Participants were instructed to ingest supplements with their evening meal to ensure maximal plasma γ-tocopherol concentrations during study procedures [20]. During each visit, participants completed a standard clinical oral glucose tolerance test where they ingested a glucose solution (75 g glucose prepared in 240 ml water). Blood samples were collected prior to (0 min) and following the ingestion of glucose at 15, 30, 45, 60, 90, 120, 150 and 180 min.

2.4. Sample handling

A catheter was inserted into the antecubital vein, and blood samples were collected into evacuated tubes containing 0.05 ml 15% (wt/vol) EDTA, sodium heparin or lithium heparin (Becton Dickinson, Franklin Lakes, NJ, USA). Plasma was obtained by centrifugation (4°C, 15 min, 500×*g*; Eppendorf 5810R, Hamburg, Germany) and snap frozen in liquid nitrogen. For ascorbic acid and glutathione measurements, an aliquot of heparinized plasma was mixed (1:1) with 10% perchloric acid (PCA) containing 1 mM DTPA, centrifuged (5 min, 15,000×*g*, 4°C; Eppendorf 5415R), and the collected supernatant was frozen in liquid nitrogen. All samples were stored at −80°C until analysis.

Table 1
Participant characteristics

Parameter	Means±S.E.
Age (years)	22.3±1.0
Height (m)	1.81±0.01
Weight (kg)	93.63±7.69
BMI (kg/m^2)	29.3±2.4
Systolic blood pressure (mm Hg)	117.3±1.9
Diastolic blood pressure (mm Hg)	79.3±1.8
Glucose (mmol/l)	5.46±0.20
Insulin (μlU/ml)	22.0±3.9
HOMA-IR	5.5±1.2
Triglyceride (mmol/l)	0.67±0.09
Cholesterol (mmol/l)	3.81±0.14

BMI, body mass index; HOMA-IR, homeostatic model of assessment-–insulin resistance.

2.5. Dietary analysis

Participants completed a 3-day dietary record before each visit, and all records were reviewed with participants for accuracy with a dietitian. Intakes of dietary nutrients were analyzed using Food Processor for Windows 7.9 (ESHA Research, Salem, OR, USA).

2.6. Clinical chemistries

Plasma glucose was analyzed enzymatically using a commercially available glucose hexokinase reagent kit (Pointe Scientific Inc., Canton, MI, USA). Plasma insulin was measured using a sandwich enzyme-linked immunosorbent assay kit (Diagnostic Systems Laboratories, Inc., Webster, TX, USA) and performed in accordance with the manufacturer's instructions. The homeostatic model of assessment was calculated to assess insulin resistance using fasting plasma glucose and insulin concentrations as described [21]: [(fasting glucose (mmol/l) * fasting insulin (μlU/ml)/22.5]. Plasma triglyceride (Pointe Scientific Inc., Canton, MI, USA) and total cholesterol (Thermo Diagnostics, Middletown, VA, USA) were measured spectrophotometrically using enzymatic kits in accordance with the manufacturer's specifications.

2.7. Plasma MGO

Plasma MGO was measured as described [22], with minor modifications. Briefly, 500 μl EDTA plasma and MGO standards prepared in parallel were mixed with 100 μl PCA (0.45 M final concentration), 20 μl *o*-phenylenediamine (10 mM final concentration) and 5 μl of the internal standard 5-MQ (11.5 nM final concentration) and incubated in the dark for 24 h. Following incubation, samples were centrifuged (15 min, 15,000×*g*, 4°C; Eppendorf 5415R) to separate precipitated proteins, and 30 μl of the supernatant was injected onto a Shimadzu Prominence HPLC system (Columbia, MD, USA). Separation was performed isocratically at 1 ml/min on a Nova-Pak C_{18} column (3.9×150 mm, 4 μm; Waters, Franklin, MA, USA) using water/acetonitrile (82.4:17.6) containing 5 mM NaH_2PO_4 as the mobile phase. 2-Methylquinoxaline, the MGO derivative, was detected at 317 nm and quantified using peak area relative to 5-MQ as the internal standard.

2.8. Plasma total antioxidant status

Total antioxidant status was measured using the oxygen radical absorbance capacity (ORAC) and ferric reducing ability of plasma (FRAP) assays. The ORAC assay was performed as described previously [23]. Briefly, EDTA plasma was diluted (1:300) in 75 mM potassium phosphate buffer (pH 7.4) and mixed with 150 μl fluorescein (81.6 nM). Following a 10-min incubation, 25 μl of 153 mM 2,2′-azobis-2-methyl-propanimidamide was added to initiate oxidative degeneration of fluorescein. Fluorescein intensity was measured every 1 min for 80 min using a Molecular Devices M2 microplate reader (485:520 nm, excitation/emission). Areas under the curve (AUC) for Trolox standards and samples were determined using the trapezoidal rule, and ORAC values were calculated following linear regression between Trolox concentration and AUC.

FRAP concentration was determined as described previously [24]. In brief, EDTA plasma was diluted fourfold with Chelex-treated water, and 40 μl of the diluted plasma was mixed with 300 μl of FRAP working solution (50 ml of 300 mM sodium acetate buffer, 5 ml 2,4,6-tri(2-pyridil)-*s*-triazine prepared in 40 mM HCl and 5 ml of 20 mM $FeCl_3$). Following incubation (15 min, 37°C), the absorbance of the samples and Trolox were measured at 593 nm. FRAP values were calculated following linear regression between Trolox concentration and absorbance.

2.9. Plasma vitamin E

Vitamin E (as α-, β-, γ- and δ-tocopherol) was measured by HPLC-Coularray (ESA Inc., Chelmsford, MA, USA), as described previously with minor modifications [25]. In brief, 350 μl plasma was mixed with 2 ml 1% ascorbic acid prepared in ethanol (wt/vol), 1 ml purified water and 500 μl saturated KOH (79.2 g KOH dissolved in 74.2 ml water). Following saponification (30 min, 70°C), samples were rapidly chilled and mixed with water (1 ml), and 4.54 mM butylated hydroxytoluene (25 μl) that was prepared in reagent alcohol. Tocopherols were extracted with hexane (2 ml); the extract was dried under nitrogen and dissolved in a known volume of 1:1 methanol/ethanol. The injected sample was separated isocratically (0.6 ml/min) on a Luna C18(2) column (150 ×3 mm I.D., 3 μm; Phenomenex, Torrence, CA, USA) and detected at potential settings of 350, 450, 525 and 600 mV. The mobile phase was 98:2 methanol/water containing 10 mmol/l lithium perchlorate.

Table 2
Dietary intakes of selected nutrients[a]

	Glucose	Glucose+γ-tocopherol
Energy (kcal)	2639±187	2450±124
Fat (g)	96.4±6.3	92.5±4.6
Protein (g)	105.5±8.0	100.7±6.3
Carbohydrate (g)	334.0±24.5	307.5±21.2
Vitamin C (mg)	102.5±22.2	101.4±28.1
α-Tocopherol (mg)	6.2±1.3	4.8±1.0
Zinc (mg)	10.3±1.6	9.3±0.8

[a] Data are means±S.E. No significant differences were observed between trials.

2.10. Plasma ascorbic acid and uric acid

Plasma ascorbic acid and uric acid were analyzed by HPLC-Coularray as described previously [26]. Briefly, PCA-treated plasma (20 μl) was mixed with 20 μl of 1 mM DTPA in phosphate-buffered saline, 12 μl of 2.5 M potassium phosphate buffer and 148 μl mobile phase (92.5:7.5 water/methanol, 40 mM sodium acetate, 508 μM DTPA, 1.5 mM Q12, pH 4.75). Ascorbic acid standard was prepared fresh in Chelex-treated water and verified spectrophotometrically ($\varepsilon^{265\ nm}$=14,500 $M^{-1}\cdot cm^{-1}$) [26]. Prepared uric acid standard was purchased from Pointe Scientific, Inc. (Lincoln Park, MI, USA). Standard or sample was injected onto the HPLC system set to 150, 275, 400 and 525 mV and separated isocratically at 1 ml/min on a Supelcosil LC-8 column (4.6×150 mm, 3 μm; Sigma-Aldrich).

2.11. Plasma glutathione and cysteine

Reduced (GSH) and oxidized (GSSG) glutathione, cysteine (CYS), and cystine (CYSS) were measured by HPLC-FL as described previously [27]. Briefly, PCA-treated plasma was mixed with internal standard (γ-glutamylglutamate) and derivatized with dansyl chloride. Following incubation (24 h, room temperature), samples were centrifuged and the supernatant was injected onto an HPLC-FL system equipped with two florescence detectors (Jasco Inc., Easton, MD, USA) connected in series. Both detectors were set to 335:515 nm (excitation/emission), but at different sensitivity settings in order to measure each thiol redox pair, which differ substantially in concentration. Mobile phase A was 80% methanol, and mobile phase B was 640 ml methanol, 125 ml glacial acetic acid, 50 ml water and 200 ml acetate stock (272 g sodium acetate trihydrate, 378 glacial acetic acid and 122 ml water). Samples were separated at 1 ml/min on a 3-aminopropyl column (250 ×4.6 mm, 5 μm; Thermo Fisher Scientific, Waltham, MA, USA) using the following binary gradient: 20% B for 10 min, linear gradient to 99% B over 12 min and held for 10 min, then back to 20% B over 2 min, and equilibrated at 20% B for 14 min. Thiols were quantified using peak area relative to the internal standard. The Nernst equation was used to calculate the redox potential (E_h) of the GSH/GSSG and CYS/CYSS couples as described [28]. For GSH/GSSG, E_h =−264 mV+30log([GSSG]/[GSH]^2), and for CYS/CYSS, E_h =−250 mV+30log([CYSS]/[CYS]^2).

2.12. Statistical analysis

Data are expressed as means±S.E. GraphPad Prism (version 5; San Diego, CA, USA) was used for all analyses except multiple linear regression, which was performed using SPSS (version 18; Chicago, IL, USA). Time- and treatment-dependent changes in glucose and methylglyoxal were evaluated using two-way repeated-measures analysis of variance with a Bonferroni correction to evaluate pairwise differences. Differences between dietary intakes, AUC or baseline values between trials were assessed with the Student's *t* test. Multiple linear regression, controlling for within-subject repeated measures, was used to calculate correlation coefficients as described by Bland and Altman [29]. All results were considered statistically significant at an α level of $P<.05$.

3. Results

3.1. Participants, dietary intakes and plasma vitamin E

On the basis of body mass index, participants were classified as overweight but had resting blood pressure and fasting plasma glucose, total cholesterol and triglyceride that were otherwise within normal clinical limits (Table 1). Dietary intakes of macronutrients and selected micronutrients during the two study phases are shown in Table 2. No differences were observed between trials for total energy and macronutrients or intakes of vitamin E, vitamin C or zinc, indicating that participants maintained similar dietary patterns throughout the study. Vitamin E supplementation did not affect plasma α-tocopherol concentrations but increased plasma γ-tocopherol by more than threefold ($P<.0001$), demonstrating good compliance with the supplementation protocol. Plasma β-tocopherol remained below quantitative limits, and although δ-tocopherol concentrations increased, they accounted for only ~3% of the total plasma tocopherol concentration. Consistent with greater plasma γ-tocopherol, plasma γ-CEHC, the physiological metabolite of γ-tocopherol, increased by more than ninefold ($P<.001$) (Table 3).

3.2. Plasma glucose

Fasting glucose was not significantly different between trials (Fig. 1). Regardless of γ-tocopherol supplementation, plasma glucose increased by 15 min postprandially following the oral glucose challenge and returned to baseline concentrations at 150 min. γ-Tocopherol supplementation had no effect on postprandial glucose AUC or on AUC following data transformation to examine percent change from baseline (data not shown).

3.3. Plasma MGO

Fasting MGO was not significantly different between trials ($P=.50$; Fig. 2A). Glucose ingestion increased postprandial plasma MGO (time main effect, $P<.001$), and MGO concentrations were significantly greater at 45 and 90 min compared with baseline concentrations. Following γ-tocopherol supplementation, hyperglycemia-induced increases in plasma MGO were unaffected throughout the postprandial period except that MGO at 180 min was significantly lower than baseline concentrations. To better define postprandial MGO responses resulting from the glucose challenge and to control for interindividual variations in baseline MGO, plasma MGO was transformed to percent change from baseline (Fig. 2B). Thus, all subsequent analyses pertaining to plasma MGO are expressed as percent change from baseline. Transformed MGO responses had significant main effects due to time ($P<.01$), supplementation ($P<.05$) and a time×supplementation interaction ($P<.05$). In the absence of γ-tocopherol supplementation, plasma MGO concentrations were significantly greater at 45–90 min compared with baseline. In striking contrast, postprandial MGO concentrations following γ-tocopherol supplementation remained unchanged relative to baseline at all time points. In addition, a significant difference between trials was observed at 90 min, indicating that MGO response during the control trial was greater ($P<.05$) compared with the γ-tocopherol trial (Fig. 2B). γ-Tocopherol supplementation also decreased ($P<.05$) postprandial AUC for plasma

Table 3
Plasma antioxidants and markers of oxidative stress[a]

	Glucose	Glucose+γ-tocopherol	*P*
γ-Tocopherol (μmol/l)	2.22±0.32	7.06±0.71	<.0001
α-Tocopherol (μmol/l)	16.1±1.4	17.7±1.9	NS[b]
δ-Tocopherol (μmol/l)	0.13±0.03	0.79±0.11	<.0003
γ-CEHC (μmol/l)	0.35±0.09	3.24±0.58	<.001
Vitamin C (μmol/l)	33.7±5.0	39.2±4.8	<.01
Uric Acid (μmol/l)	358.0±13.56	371.7±12.68	NS
FRAP (μmol/l)	980.4±29.9	1052.0±23.4	<.05
ORAC (μmol/l)	3612.0±308.7	4115.0±354.7	<.05
Cys (μmol/l)	13.3±1.4	12.9±0.6	NS
CYSS (μmol/l)	75.7±8.1	71.1±8.2	NS
Cys/CYSS	0.19±0.02	0.21±0.03	NS
Cys/CYSS (mV)	−80.7±2.2	−82.0±1.6	NS
GSH (μmol/l)	1.39±0.10	1.53±0.08	<.05
GSSG (μmol/l)	0.12±0.02	0.10±0.02	NS
GSH/GSSG	13.97±2.31	31.16±8.93	<.05
GSH/GSSG (mV)	−111.9±2.0	−119.9±3.6	<.05

[a] Data are means±S.E.
[b] Not significant.

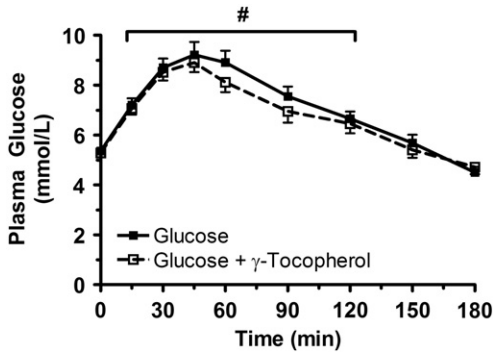

Fig. 1. Postprandial plasma glucose responses following an oral glucose tolerance test. Data are means±S.E. #, significantly different from baseline in both trials ($P<.05$).

MGO (Fig. 2C) such that AUC was positive during the control trial and negative during the supplemented trial. Moreover, plasma γ-tocopherol concentrations were inversely correlated to plasma MGO AUC ($r=-0.58$, $P<.05$), supporting a finding that improvements in γ-tocopherol concentrations suppress MGO formation (Fig. 2D). Collectively, these data demonstrate that a vitamin E preparation high in γ-tocopherol abolishes postprandial increases in plasma MGO that otherwise result from acute hyperglycemia.

3.4. Markers of antioxidant status and oxidative stress

To define the relation of antioxidant status to MGO response, we measured several plasma antioxidants and oxidative stress biomarkers (Table 3). No significant differences were observed between trials at baseline ($t=0$ min) for α-tocopherol, uric acid or markers of cysteine redox status. In contrast, ORAC and FRAP increased by 14% and 7% ($P<.05$), respectively, following γ-tocopherol supplementation. Plasma vitamin C concentrations also increased by 16%. Lastly, plasma GSH increased significantly, which resulted in a greater GSH/GSSG ratio and a less oxidized redox potential of the GSH/GSSG couple. Multiple linear regression, controlling for within-subject repeated measures, was performed to calculate correlation coefficients and regression slopes. Regression responses are depicted in Figs. 2–3 by juxtaposing the adjusted regression line to the raw data. Plasma γ-tocopherol was associated with plasma concentrations of γ-CEHC ($r=0.90$, $P<.001$), FRAP ($r=0.68$, $P<.001$), ORAC ($r=0.62$, $P<.05$) and GSH ($r=0.56$, $P<.05$) (Fig. 3). These same antioxidant markers were, in turn, associated with responses in plasma MGO AUC

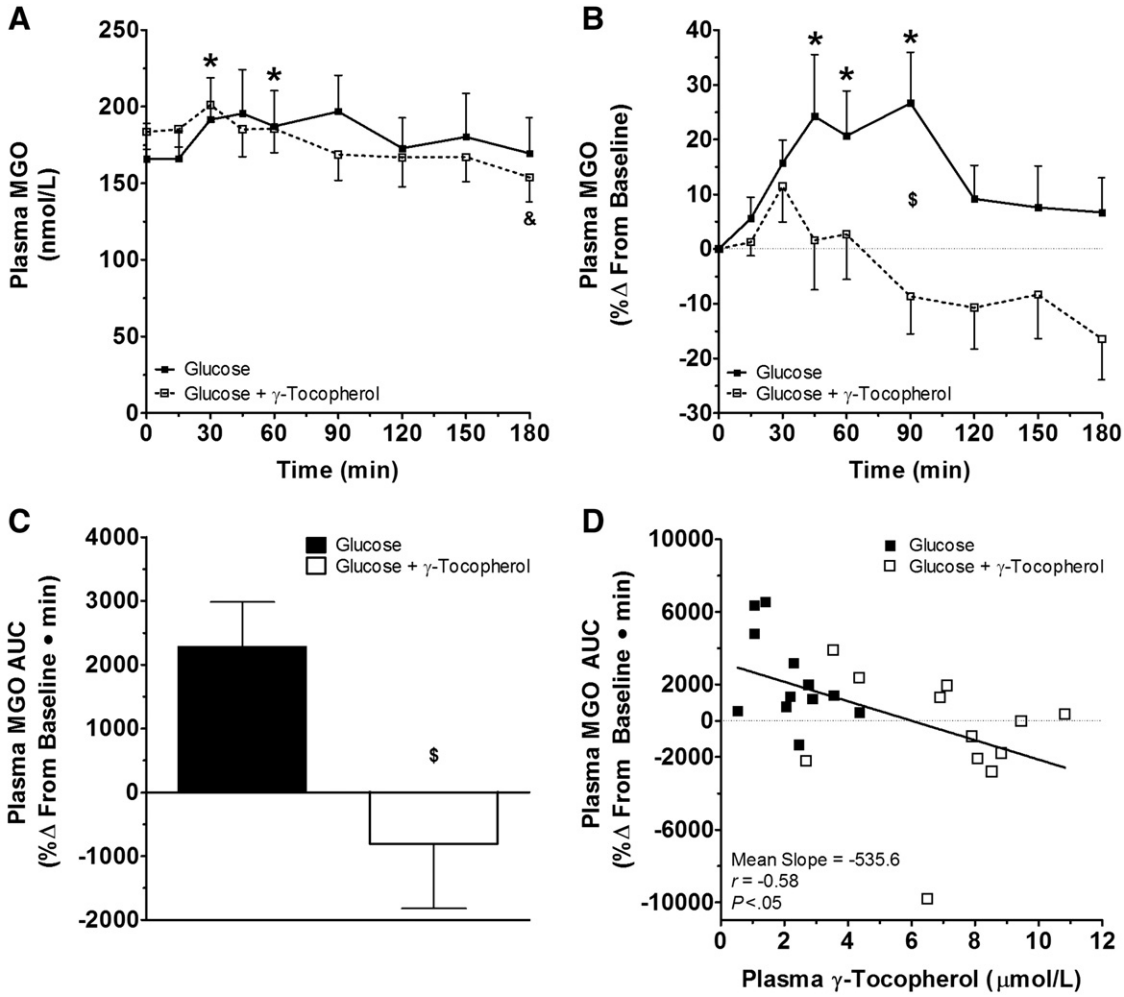

Fig. 2. Postprandial plasma MGO following an oral glucose challenge. (A) Postprandial time course of plasma MGO. (B) Postprandial plasma MGO concentrations transformed to percent change relative to baseline MGO concentrations. (C) AUC for percent change in plasma MGO relative to baseline throughout the postprandial period. (D) Multiple linear regression, controlling for within-subject repeated measures, was performed to determine the relation between plasma γ-tocopherol and plasma MGO AUC. Data in panels A, B and C are means±S.E. The line represents the obtained regression slope juxtaposed to the data. *, Significantly different from baseline in the control trial. &, Significantly different from baseline in the γ-tocopherol trial. $, Significantly different between control and γ-tocopherol trial. Data are significant at an α level of $P<.05$.

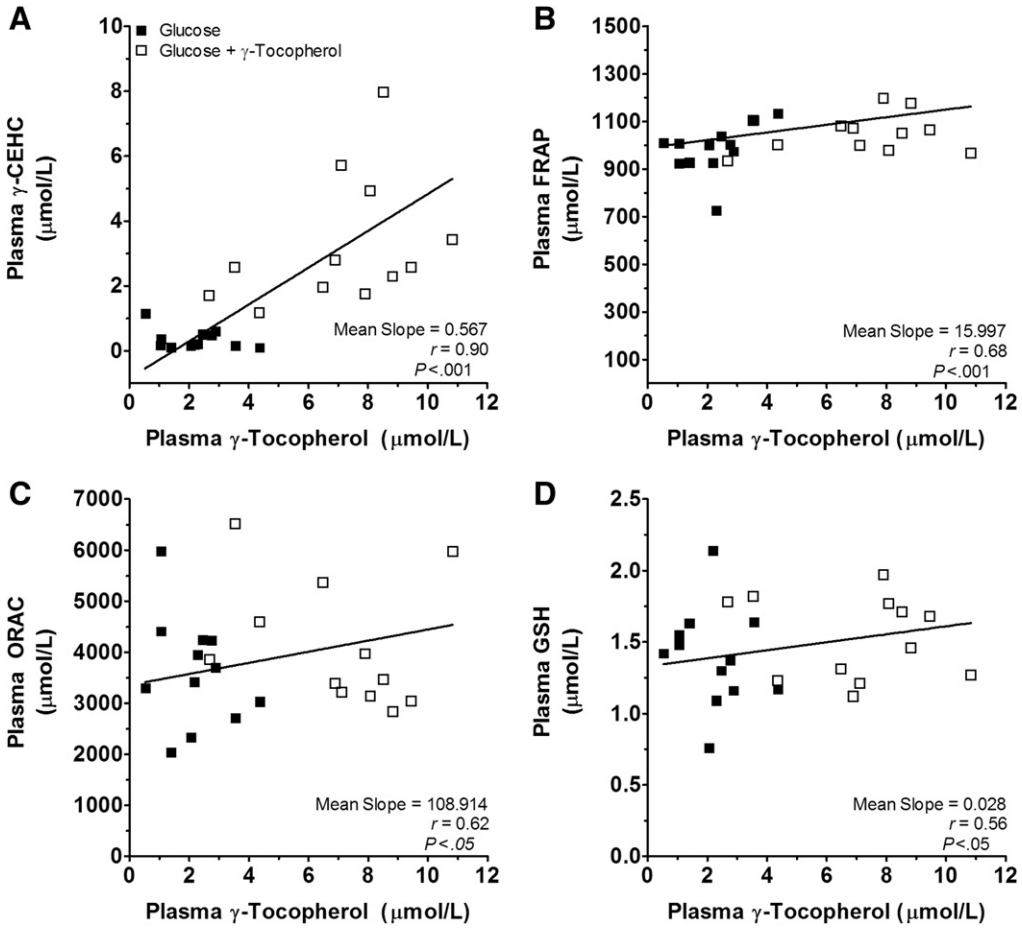

Fig. 3. Relations between plasma γ-tocopherol and antioxidant markers. Multiple linear regression, controlling for within-subject repeated measures, was performed to determine the relation between postprandial MGO AUC and baseline plasma concentrations of γ-CEHC (A), FRAP (B), ORAC (C) and GSH (D). The line represents the obtained regression slope juxtaposed to the data.

(Table 4), suggesting that MGO formation increases with lower antioxidant status. Plasma γ-tocopherol (r=0.61, P<.05) and γ-CEHC (r=0.52, P<.05) were both related to plasma vitamin C, but vitamin C only tended (r=−0.47, P=.052) to inversely correlate with plasma MGO AUC.

4. Discussion

Short-term administration of vitamin E supplements highly enriched in γ-tocopherol completely abolished postprandial increases in plasma MGO induced by an oral dose of glucose. The findings of this study are the first to demonstrate postprandial increases in MGO following administration of glucose in healthy human subjects and to demonstrate that γ-tocopherol mitigates this response. Although the supplement contained relatively small amounts of other tocopherols, supplementation had no effect on plasma α-tocopherol, while plasma δ-tocopherol represented only ~3% of total plasma tocopherol even after supplementation. By contrast, supplementation increased plasma γ-tocopherol by threefold (P<.0001) to 28% of total plasma tocopherol and increased its physiological metabolite γ-CEHC by ninefold (P<.001). Thus, it is likely that the mitigating effects on plasma MGO are due to the direct effects of γ-tocopherol or to its indirect effects mediated through γ-CEHC. Consistent with this conclusion, plasma concentrations of γ-tocopherol and γ-CEHC were inversely associated with plasma MGO AUC. Supplementation did not affect postprandial hyperglycemia, ruling out the possibility that the reduction in plasma MGO is mediated by reductions in plasma glucose. Supplementation did, however, increase markers of total antioxidant capacity as well as the antioxidants vitamin C and GSH, supporting an in vivo antioxidant activity of γ-tocopherol. Thus, γ-tocopherol may also have reduced plasma MGO indirectly by improving the status of other antioxidants. This is the first report showing that antioxidant supplementation reduces postprandial MGO.

MGO can be formed from ketone bodies, as a byproduct of threonine metabolism, or in small amounts as a byproduct of protein glycation, but the majority of MGO is derived from the spontaneous dephosphorylation of triose phosphates in the glycolytic pathway [30]. Since plasma ketones decline and plasma threonine is unaffected following oral glucose administration [31], the increase in plasma MGO observed in our study is best explained by increases in MGO from glucose-derived triose phosphates. The only known function of

Table 4
Relations between plasma antioxidant markers and MGO AUC[a]

	r	P
γ-CEHC	−0.48	<.05
FRAP	−0.52	<.05
ORAC	−0.67	<.01
GSH	−0.56	<.05

[a] Multiple linear regression, controlling for within-subject repeated measures, was performed to determine the correlation coefficient between postprandial MGO AUC and plasma antioxidant markers.

γ-tocopherol, like other vitamin E forms, is that of a chain-breaking antioxidant that terminates lipid peroxidation [32]. No evidence that it has a role in regulating glycolysis exists. However, consistent with its antioxidant function, γ-tocopherol can accumulate in the mitochondria [33] where it could help maintain low concentrations of cytosolic triose phosphates by suppressing ROS-induced damage to mitochondrial proteins. Low triose phosphate concentrations are normally maintained by the activity of nicotinamide adenine dinucleotide (NAD+)-dependent glyceraldehyde 3-phosphate dehydrogenase. However, mitochondrial lipid peroxidation results in carbonyl damage to mitochondrial membrane proteins [34] and impairs the oxidation of NADH to NAD+ by complex I, an effect that was rescued in vitro by α-tocopherol [35]. In addition, ROS deplete cellular NAD+ concentrations by inducing nuclear DNA strand breaks, thereby up-regulating poly (ADP-ribose) polymerase (PARP), a DNA repair enzyme that hydrolyzes NAD+ to form ADP-ribose [36,37]. PARP also directly impairs glyceraldehyde 3-phosphate dehydrogenase activity by covalently modifying the enzyme with ADP-ribose [36]. γ-Tocopherol inhibits superoxide formation [38] and likely facilitates NADH oxidation similar to α-tocopherol. Thus, γ-tocopherol may reduce triose phosphate accumulation by inhibiting ROS-mediated damage to the mitochondrial membrane, which would decrease hyperglycemia-induced MGO formation.

γ-Tocopherol may also have reduced postprandial MGO by enhancing its GSH-dependent detoxification. In support of this notion, γ-tocopherol supplementation increased plasma GSH, consistent with studies administering α-tocopherol [39]. In turn, plasma GSH was inversely related to MGO AUC responses. GSH is a rate-limiting substrate used for the detoxification of MGO to lactate [30]. Like α-tocopherol, γ-tocopherol spares GSH from oxidation by terminating membrane lipid peroxidation [40]. Unlike α-tocopherol, however, its oxidation product, γ-tocopheryl quinone, increases GSH biosynthesis by up-regulating a cystine transporter and increasing the bioavailability of cysteine bioavailability, which is the rate-limiting amino acid for GSH biosynthesis [41]. Thus, γ-tocopherol may have increased GSH concentrations through either pathway.

We also observed significant increases in plasma vitamin C following γ-tocopherol supplementation and a trend (P=.052) toward an inverse correlation between vitamin C and plasma MGO AUC. Vitamin C supplementation reduces PARP activation in humans [42]. Greater vitamin C could therefore have contributed to the decrease in postprandial MGO. Although the mechanism by which γ-tocopherol increases vitamin C is unknown, γ-tocopherol was shown in rats to increase plasma vitamin C as well as the ascorbate-to-dehydroascorbate ratio in kidney, suggesting that γ-tocopherol either supports the recycling of vitamin C or spares it from oxidation [43]. Mitochondrial recycling of dehydroascorbate contributes to cytosolic ascorbate [44] and may contribute to plasma ascorbate [45]. This process is dependent on complex III activity [46], which is impaired during lipid peroxidation but rescued by α-tocopherol [35]. γ-Tocopherol may therefore support vitamin C recycling through its direct antioxidant properties in the mitochondrial membrane. γ-CEHC also possesses water-soluble antioxidant activity [19] and may therefore interact with vitamin C in vivo as part of the antioxidant network [47] by directly recycling ascorbyl radicals or by quenching ROS that would otherwise oxidize ascorbate. In support, plasma concentrations of both γ-tocopherol and γ-CEHC correlated positively with vitamin C in our study.

γ-Tocopherol's antioxidant activity is also supported by the increases in two common measures of total antioxidant capacity, FRAP and ORAC, following supplementation. We did not estimate the precise contribution of γ-tocopherol to FRAP and ORAC values. However, it is known that α-tocopherol only explains ~6% of FRAP and ~1% of ORAC [48], and γ-tocopherol concentrations remained much lower than α-tocopherol concentrations even after supplementation in our study. Nonetheless, greater plasma ORAC and FRAP following supplementation support γ-tocopherol–mediated sparing of other antioxidants from oxidation, consistent with our observed increases in vitamin C and GSH.

Our findings support that γ-tocopherol may prevent the adverse effects of postprandial hyperglycemia through its antioxidant activity. This pilot study was relatively small and limited to healthy, college-aged men, which may preclude our ability to extrapolate the findings to women, other age groups or individuals with established hyperglycemic conditions. However, our approach allowed us to define γ-tocopherol–mediated effects on postprandial MGO without the potential confounding effects of underlying disease, age and gender. We observed considerable overlap in absolute plasma MGO between the two trials during the first 60 min as a result of slightly higher (~11%) baseline concentrations in the γ-tocopherol trial, but this difference was not significant (P=.50). Few data are currently available regarding daily fluctuations in MGO concentrations. Thus, this slight difference could represent an effect of short-term γ-tocopherol supplementation or adherence to the study protocol over time, but most likely is a result of natural variation between trials. Although our supplement contained small amounts of other tocopherols, pure γ-tocopherol supplements are not commercially available. The use of a γ-tocopherol supplement containing small amounts of mixed tocopherols therefore better reflects the sources of γ-tocopherol consumers will encounter in food and supplements. The use of an oral glucose tolerance test provided clear evidence that these postprandial MGO responses are mediated by hyperglycemia, and our investigative approach allowed us to identify a specific dietary antioxidant that regulates these responses in normoglycemic individuals. Our analysis of 3-day dietary records also enabled us to rule out dietary changes that may affect MGO responses, particularly zinc and carbohydrate. Indeed, zinc is a required cofactor for glyoxalase-I [30], which is transcriptionally regulated by zinc and insulin [49]. Significant questions raised by the present study include the threshold at which MGO becomes pathologic, how chronically elevated or acute postprandial spikes of MGO differentially contribute to pathologic conditions and whether α-tocopherol would be also be effective in regulating MGO. Longer-term studies examining the effects of γ-tocopherol on the accumulation of tissue AGE adducts and clinical outcomes and comparing its efficacy to that of α-tocopherol are clearly warranted.

In conclusion, our findings suggest that a significant proportion of individuals may potentially benefit from improved γ-tocopherol status. This is of significance because ~75% of Americans have plasma γ-tocopherol concentrations lower than those achieved following our 5-day supplementation regimen [50]. Supplementation with α-tocopherol, however, decreases γ-tocopherol concentrations [17], suggesting that vitamin E supplements should contain both vitamers to obtain the benefits unique to each. Long-term studies examining the effects γ-tocopherol on the generation of AGE and related clinical endpoints are needed to more comprehensively define the role of this understudied and important vitamin E form in hyperglycemic conditions and CVD.

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