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Kennadiid Ali Abdulla Date

Investigation of factors associated with circulating γ-tocopherol concentrations

By

Kennadiid Ali Abdulla
Degree to be awarded: MPH

Epidemiology

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Roberd M. Bostick, MD, MPH
Committee Chair

# Investigation of factors associated with circulating γ-tocopherol concentrations

By

Kennadiid Ali Abdulla

Bachelor of Arts

State University of New York At Buffalo

2013

Faculty Thesis Advisor: Roberd M. Bostick, MD, MPH

An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
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in Epidemiology
2017

## Abstract

Investigation of factors associated with circulating γ-tocopherol concentrations

By Kennadiid Ali Abdulla

**Background:** Although α- and γ-tocopherol are co-consumed antioxidants, circulating γ-tocopherol concentrations were paradoxically found to be inversely associated with total vitamin E intake and circulating α-tocopherol concentrations. There are limited data on this apparent paradox or on determinants of circulating γ-tocopherol concentrations.
**Methods:** We pooled data from two outpatient, elective colonoscopy populations (pooled n=419) on whom extensive dietary, lifestyle, and medical information was collected, and the following circulating concentrations were measured: α- and γ-tocopherol (via high-performance liquid chromatography), $F_2$-isoprostanes (FiP; via gas chromatography-mass spectrometry, and high sensitivity C-reactive protein (hsCRP; via latex-enhanced immunonephelometry). Multivariable general linear models were used to assess mean γ-tocopherol differences across quantiles of FiP, hsCRP, and multiple dietary and lifestyle factors.
**Results:** Adjusted for serum total cholesterol, mean γ-tocopherol concentrations among those in the highest relative to the lowest tertiles of circulating α-tocopherol and β-carotene, a composite dietary/lifestyle oxidative balance score (higher score indicates higher antioxidant relative to pro-oxidant exposures), and total calcium and dietary fiber intakes, were -31.0% ($p<0.0001$), -29.0% ($p<0.0001$), -27.6% ($p=0.0001$), -29.7% ($p<0.0001$), and -18.6% ($p=0.008$) lower, respectively. For those in the highest relative to the lowest tertiles of circulating FiP and hsCRP, mean γ-tocopherol concentrations were +50% ($p<0.0001$) and +39.0% ($p<0.0001$) higher, respectively.
**Conclusions:** These findings suggest that circulating γ-tocopherol concentrations may be inversely associated with antioxidant exposures and directly associated with systemic oxidative stress and inflammation. Additional research on possible mechanisms underlying these findings and on whether circulating γ-tocopherol may serve as a biomarker of oxidative stress and/or inflammation is needed.

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## Introduction

Oxidative stress, which refers to a harmful imbalance of pro-oxidant to antioxidant exposures and endogenous factors (1), has been implicated in the etiology of various chronic diseases (2-9). Vitamin E, which collectively refers to a group of eight fat soluble compounds, including four tocopherols and four tocotrienols, is a particularly important antioxidant exposure for maintaining an optimal oxidative balance (10). Although α-tocopherol is the most studied form of vitamin E, the main form used in supplements (11), and the predominant form found in human tissues (10, 11), γ-tocopherol is the major form found in the U.S diet (11). Although α- and γ-tocopherol are co-consumed antioxidants, circulating γ-tocopherol concentrations were found to be inversely associated with total vitamin E intake and/or circulating α-tocopherol concentrations in human observational studies, while α-tocopherol supplementation was found to decrease circulating γ-tocopherol concentrations in intervention studies (12-23). However, many of the human studies in support of this seeming paradoxical association had very small sample sizes, non-representative populations, minimal assessment of potential confounding, study design issues, and/or measured few relevant biomarkers.

To address this, as reported herein, we investigated cross-sectional associations of circulating γ-tocopherol concentrations with circulating concentrations of α-tocopherol and β-carotene and biomarkers of systemic oxidative stress and inflammation, and multiple antioxidant, pro-oxidant, and other dietary and lifestyle exposures in adult humans.

## Methods

**Study population**

We pooled data from two cross-sectional studies conducted in outpatient elective colonoscopy populations. The first study, the Markers of Adenomatous Polyps study (MAP I), was conducted in Winston-Salem and Charlotte, North Carolina from 1994 to 1997. The second study, MAP II, was conducted in Columbia, South Carolina in 2002. Both studies were conducted by the same principal investigator (author RMB) and utilized the same study protocols and questionnaires.

Details on the study protocols were previously published (24, 25). Participants for the studies were recruited from patients scheduled for an elective outpatient colonoscopy at several large local gastroenterology practices. Eligibility for the studies included age 30 –74 years, English speaking, and capable of providing written informed consent. Exclusion criteria included a history of inflammatory bowel disease, a personal history of any cancer other than non-melanoma skin cancer, or previous colorectal adenomatous polyps. Of those who met the eligibility criteria, the consent rates were 67% and 76%, respectively, for the MAP I and MAP II studies, yielding sample sizes of 420 and 204, respectively.

All participants provided written informed consent, and the studies were approved by the institutional review boards of the institutions where these studies were conducted (Wake Forest University for MAP I and the University of South Carolina for MAP II).

**Data collection and Laboratory analysis**

Questionnaires were mailed to study participants 1 – 2 weeks prior to colonoscopy asking them to provide detailed demographic, medical, family history, anthropometric (self-measured height, weight, and waist and hip circumferences), lifestyle, and dietary information. Diet and

vitamin/mineral supplement use was assessed using Willett food frequency questionnaires, and physical activity was assessed using modified Paffenbarger questionnaires. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared ($kg/m^2$) and used as an indicator of adiposity. Participants brought their completed questionnaires to their colonoscopy visit at which time fasting peripheral venous blood samples were drawn into pre-chilled, red-coated Vacutainer tubes prior to their colonoscopy procedure. The blood samples were then placed in ice and protected from light to minimize degradation, and immediately taken to the laboratory. In the laboratory, the samples were immediately fractionated via centrifugation in a refrigerated centrifuge, aliquoted into amber-colored cryopreservation tubes, the air in the aliquots was displaced with nitrogen in MAP I and argon in MAP II, and then the aliquot tubes were capped with O-ring screw caps and immediately placed in a $^{-}70°$ C freezer until analysis. All biomarker assays for the present study were conducted at the Molecular Epidemiology and Biomarker Research Laboratory at the University of Minnesota, as follows.

Plasma α-tocopherol, γ-tocopherol, β-cryptoxanthin, α-carotene, β-carotene, lycopene, and lutein concentrations were measured via high-performance liquid chromatography (HPLC)-based assays. Details of the original method (26), calibration (27), sample handling (28), and modifications to the original method (29) were previously reported. Calibration for the analysis was performed with pure compounds (Hoffman-La Roche; Sigma Chemical Co.). Quality control of control pools revealed coefficients of variation (CVs) of <11% for all analytes.

$F_2$-isoprostanes (FiP) were measured via a highly specific and quantitative gas chromatography-mass spectrometry-based (GCMS) method (30). This method, considered the gold standard for measuring FiP, measures a well-defined set of FiP isomers. These were extracted from participants' samples using deuterium (4)-labeled 8-iso-prostaglandin $F_2\alpha$ as an

internal standard. Quality control procedures included the analysis of two control pools that had varying concentration ranges of FiP (CVs 9.5% and 11%, respectively).

High sensitivity C-reactive protein (hsCRP) was measured via latex-enhanced immunonephelometry on a Behring nephelometer II (BN-II) analyzer (inter-assay CV 4%; Behering Diagnostics, San Jose, CA). Serum 25-OH-vitamin $D_3$ (25[OH]$D_3$) concentrations were measured using a liquid chromatography/tandem mass spectrometry (LC/MS/MS) method as previously described (31); the average intra-assay CV was 3%.

Serum cholesterol was measured via an enzymatic, timed end point method on a SYNCHRON CX5 system (Beckman Instruments, Inc., Brea, Calif) (32, 33). Cholesterol tests on SYNCHRON CX5 systems have been certified by the National Cholesterol Education Program. The CV for the total cholesterol measurements was 6%.

**Statistical Analysis**

For the present analysis, we excluded participants with serum cholesterol concentrations <100 mg/dl or >400 mg/dl (n = 146), those who did not answer ≥10% of the FFQ items or reported implausibly high or low total energy intakes (<500 or >6,000 kcal/day) (n = 1), and those who were missing plasma samples for measurements of antioxidant micronutrients (n = 58), leaving a final sample size of 419. Of these, sufficient plasma samples were available for FiP on 76.9% of participants (n = 322) and hsCRP on 99.8% of participants (n = 418).

The characteristics of the study participants were summarized and compared across tertiles of plasma γ-tocopherol concentrations using general linear models for continuous variables (normalized by the natural logarithm when indicated) and extended chi-square tests for categorical variables.

An equal-weight, 15-component oxidative balance score (OBS) was calculated for each participant using previously described methods (34). Briefly, the 15 components were chosen *a priori* based on their expected anti- or pro-oxidant effects, and included dietary and supplemental antioxidants (pro-vitamin A carotenoids, lutein, lycopene, vitamin C, vitamin E, omega-3 fatty acids, flavonoids, and glucosinolates), dietary pro-oxidants (iron, omega-6 fatty acids, and saturated fats), and lifestyle factors, including physical activity (considered to have predominantly antioxidant effects) and adiposity (BMI), smoking, and alcohol intake (considered to have predominantly pro-oxidant effects). Antioxidant exposures were assigned a weight of +1, and pro-oxidants a weight of -1. The component values were then summed, with a higher score representing a higher balance of anti-oxidant to pro-oxidant exposures.

Mean adjusted plasma γ-tocopherol concentrations according to tertiles of multiple dietary and lifestyle exposures (including the OBS) and the circulating antioxidant micronutrients and biomarkers oxidative stress (FiP) and inflammation (hsCRP) described above were calculated and compared using general linear models. The multiple exposures were chosen based on literature review and biological plausibility, and included macronutrients (e.g., intakes of fats, protein, carbohydrates, sucrose), micronutrients (e.g., intakes of calcium, dietary fiber, all the anti- and pro-oxidants included in the OBS described above, riboflavin, niacin, magnesium, manganese, zinc), waist-hip ratio, and circulating 25[OH]$D_3$ concentrations. When indicated, continuous variables were transformed by the natural logarithm to improve normality prior to hypothesis testing. Because plasma γ-tocopherol concentrations were log transformed, geometric means and their 95% confidence intervals (CI) were calculated and reported. Because γ-tocopherol and fat are absorbed by the intestine and secreted in chylomicron particles along with cholesterol (11, 35), as is customary in the literature, serum cholesterol was included in all

final models. All potential covariates listed above were considered as potential confounding variables. The criteria for inclusion in the final models were biological plausibility, previous literature, and whether inclusion/exclusion of the variable from the model changed the estimated proportional differences in mean γ-tocopherol concentrations between the upper and lower categories of the primary exposure variable by ≥10%. Only serum cholesterol was retained as a covariate in the final models. In addition to these primary analyses, the analyses were repeated after exclusion of vitamin E supplement users.

All analyses were conducted using SAS statistical software, version 9.4. A 2-sided P-value ≤0.05 was considered statistically significant.

## Results

Selected characteristics of the participants according to tertiles of plasma γ-tocopherol concentrations are summarized in Table 1. The mean age of the participants was 56.3 years, and the total serum cholesterol-adjusted circulating γ-tocopherol concentrations ranged from 0.04 to 0.61 mg/dL. Participants in the upper relative to the lower γ-tocopherol tertile were more likely to be white; less likely to be male, have higher than a high school education, and more likely to currently drink alcohol; and, on average, more likely to be more physically active and have a higher BMI and circulating hsCRP and FiP concentrations, and lower intakes of red and processed meats and total calcium, and lower circulating concentrations of 25[OH]$D_3$, α- and β-carotene, and α-tocopherol.

The total serum cholesterol-adjusted mean circulating γ-tocopherol concentrations, by tertiles of factors hypothesized to be associated with circulating γ-tocopherol concentrations, are

shown in Table 2. Mean circulating γ-tocopherol concentrations were statistically significantly lower among those in the upper relative to the lower tertiles of circulating α-tocopherol (31.0% lower), β-carotene (29.0% lower), α-carotene (23.4% lower), 25[OH]$D_3$ (18.2% lower); the oxidative balance score (27.6% lower); and intakes of total calcium (29.7% lower) and dietary fiber (18.6% lower). In contrast, mean plasma γ-tocopherol concentrations were statistically significantly higher among those who were obese relative to those who were less than overweight (15.1% higher) and in those in the upper relative to the lower tertiles of FiP (50% higher) and hsCRP (39.0% higher) concentrations. The strengths of the findings for the individual OBS components were weaker than those for the overall OBS (data not shown); in addition to BMI, the other OBS pro-oxidant dietary and lifestyle exposure components tended to be modestly associated with higher γ-tocopherol concentrations, and the other OBS antioxidant exposures tended to be modestly associated with lower γ-tocopherol concentrations. In addition, other dietary micronutrients that are commonly found in vitamin/mineral supplements along with α-tocopherol (e.g., niacin, vitamin B-12, riboflavin, magnesium, and manganese) tended to be modestly inversely associated with circulating γ-tocopherol and directly associated with circulating α-tocopherol (data not shown). Finally, none of the macronutrients or major food groups were associated with γ-tocopherol concentrations (data not shown).

The analyses for Table 2 were repeated after excluding those who took vitamin E supplements (Table 3). After excluding those who took vitamin E supplements, circulating γ-tocopherol concentrations ranged from 0.05 to 0.61 mg/dL, and the median γ-tocopherol concentrations for those in the middle and, especially, the upper tertiles of factors hypothesized to be associated with circulating γ-tocopherol concentrations, were less than those noted in the

analyses for the full study population. The results were similar to those found in Table 2 except that, for the most part, the proportional differences were of lower magnitudes.

## Discussion

Our findings suggest that circulating γ-tocopherol concentrations are 1) inversely associated with tocopherol intakes, 2) inversely associated with other antioxidant exposures, and 3) directly associated with systemic oxidative stress and inflammation. Given the more usual expectation of an increased ingestion of a dietary constituent increasing circulating concentrations of it (except when circulating concentration are tightly regulated [e.g., calcium, sodium, potassium]), and that γ-tocopherol is a known antioxidant (36) and anti-inflammatory agent (37, 38), our findings at first glance may seem paradoxical. However, as discussed below, there are several lines of evidence to support our findings.

An apparent mechanism for the inverse association of tocopherol intakes with circulating γ-tocopherol concentrations, despite γ-tocopherol being the major form of vitamin E in the human diet (11, 39), involves the hepatic α-tocopherol transfer protein (α-TTP). The affinity of α-TTP, which is responsible for reincorporating tocopherols in nascent very-low-density lipoprotein (VLDL) and maintaining plasma tocopherol concentrations (11, 40), for α-tocopherol is 100% but only 9% for γ-tocopherol (41). Since the two tocopherols competitively bind to α-TTP, and α-tocopherol is preferred, more α- than γ-tocopherol is transferred from the liver into plasma (11, 35). This leaves more γ-tocopherol to be catabolized by cytochrome P450 into the hydrophilic metabolite γ-carboxyethyl-hydroxychromanol (γ–CEHC), which is excreted primarily in urine (39, 42).

A proposed mechanism for the inverse association of γ-tocopherol with other antioxidant exposures, and the direct association with systemic oxidative stress and inflammation, is that oxidative stress and its resulting inflammation can inhibit cytochrome P450's catabolism of γ-tocopherol (43, 44), leaving more of it to be reincorporated into the plasma (11). This is particularly important to note because cytochrome P450 activity is a strong determinant of plasma γ-tocopherol concentrations (11). In support of this is that in several studies, pro-inflammatory cytokines and interleukins were found to inhibit the metabolic function of cytochrome P450 (45-48).

There is also support for our findings of inverse associations of circulating γ-tocopherol concentrations with tocopherol intakes and circulating α-tocopherol concentrations from previous human observational and interventional studies. Three small cross-sectional studies reported inverse associations of α-tocopherol intakes or plasma concentrations with plasma γ-tocopherol concentrations. In a study of 86 elderly adults (49 women and 37 men), there was a statistically significant, moderate negative correlation of plasma α- and γ-tocopherol concentrations ($r = -0.45$; $P < 0.0001$) (17). In a cross-sectional analysis of 65 men, there was a statistically significant, moderate negative correlation of α-tocopherol intake with plasma γ-tocopherol concentrations ($r = -0.33$; $p=0.0007$), but no correlation between plasma γ- and α-tocopherol concentrations ($r = -0.02$; $p=0.86$) (22). Finally, in a cross-sectional analysis of 110 male participants from the Health Professionals Follow-up Study and 162 female participants from the Nurse's Health Study, there were negative correlations between plasma γ- and α-tocopherol concentrations ($r = -0.34$ [$p<0.05$] and $r = -0.49$ [$p<0.005$], for men and women, respectively) (12). Although the results of these studies support our findings, the studies were

limited by their small sample sizes and not being conducted in representative population samples.

In a cross-sectional analysis of the baseline measurements of serum micronutrients from a 6% sample of participants (n = 5,450) from the Women's Health Initiative, there was a negative correlation between serum α-tocopherol concentrations and serum γ-tocopherol concentrations ($r = -0.38$; $p < 0.0001$). In another cross-sectional analysis of a subset of 657 men in the Multi-Ethnic Prospective Cohort study, plasma γ- and α-tocopherol concentrations were moderately negatively correlated ($r = -0.40$; $p < 0.0001$) (43). Limitations of these studies were that there was no control for potential confounding variables and the study populations were each limited to one sex.

Seven small (sample sizes of 4 – 24) intervention studies reported that persons supplemented daily with approximately 30, 100, 500 or 800 mg of α-tocopherol from 4 weeks to 1 year had concurrent increases in plasma or tissue α-tocopherol concentrations and decreases in γ-tocopherol concentrations (13, 14, 16, 17, 19, 21, 23). In addition to the small sample sizes, only two of the six intervention studies had a placebo group (14, 19).

However, in a randomized, placebo-controlled trial of 184 nonsmokers, 91 participants who were given 296 mg of RRR-α-tocopheryl acetate had a median 57% increase in their serum α-tocopherol concentrations ($p<0.0001$), and a 58% decrease in their serum γ-tocopherol concentrations ($p<0.0001$) relative to the 93 participants in the placebo group (18). In another randomized, placebo-controlled, crossover trial, 12 participants were given 200 IU, 400 IU, and 800 IU of RRR-α-tocopherol combined with 250 mg, 500 mg and 1,000 mg of vitamin C, respectively. When the participants were given these dose combinations, relative to the placebo, there were increases of approximately 30%, 45%, and 55% in plasma α-tocopherol

concentrations, respectively ($p<0.0001$), but an approximate 50% decrease in plasma γ-tocopherol after administration of each of the three dose combinations ($p<0.0001$) (15).

In an intervention study involving metabolic profiling, 200 participants were randomly selected from white male smokers participating in a cancer prevention trial. The active intervention arm included 100 participants, 50 of whom received 50 mg of all-rac-α-tocopherol acetate daily, while the other 50 received the same dosage plus 20 mg of β-carotene daily over 5 – 8 years. The control arm included another 100 participants, 50 of whom received a placebo alone while the other 50 received placebo plus 20 mg of β-carotene daily. There was an increase in serum α-tocopherol and a decrease in γ-tocopherol concentration among those in the active arms relative to the control arms (p-values <0.0001; however, the findings were assessed using linear regression, and only beta coefficients were reported for effect sizes) (20).

Finally, in the randomized, double-blind, placebo-controlled, 2x2 factorial Selenium and Vitamin E Cancer Prevention Trial (SELECT), participants were given supplemental selenium (200 µg/day) and/or α-tocopherol (400 IU/day as rac-α-tocopheryl acetate) to test their efficacy in reducing prostate cancer incidence. A subset of participants (n=575) had their serum selenium and cholesterol-adjusted γ- and α-tocopherol concentrations measured periodically over four years to assess bioadherence. In the vitamin E supplemented group (n=290) relative to the placebo group (n=285), after 6 months, 1 year, 2 years, and 4 years, serum γ-tocopherol concentrations decreased by 0.84, 0.90, 0.95, and 1.01 µg/ml, respectively, whereas the α-tocopherol concentrations increased by 6.12, 6.45, 5.88, and 4.14 µg/ml, respectively (49).

There are also some reported studies in which animals or humans were administered γ- rather than α-tocopherol. In a randomized, controlled trial in Japanese men (50), seven participants received 372.8 mg of γ-tocopherol plus 10 mg of RRR-α-tocopherol, and six

received 5 mg of RRR-α-tocopherol daily over 28 days. After 28 days, in the γ- plus α-tocopherol group relative to the low-dose α-tocopherol comparison group, there was a mean plasma γ-tocopherol increase of 13 μmol/L and a mean plasma α-tocopherol decrease of 6 μmol/L ($p<0.01$) (50). In two rodent studies, α-tocopherol supplementation increased serum α-tocopherol concentrations, and γ-tocopherol supplementation increased serum γ-tocopherol concentrations (51, 52). However in the mouse study, γ-tocopherol supplementation decreased serum α-tocopherol concentrations (52), and in the vitamin E-deficient rat model study, increasing doses of γ-tocopherol combined with a fixed dose of α-tocopherol slightly increased serum α-tocopherol concentrations (51). The difference in the response of serum α-tocopherol concentrations to γ-tocopherol supplementation in the rat study compared to the human and mouse studies may be attributable to inter-species metabolism differences and the use of a vitamin E-deficient model.

Some investigators suggested that an inverse correlation of α-tocopherol intake/circulating concentrations with circulating γ-tocopherol concentrations may be limited to persons who take α-tocopherol supplements (22). In the cross-sectional analysis of 65 men noted above in which there was a negative correlation between total vitamin E intake and circulating γ-tocopherol concentrations ($r = -0.33$; $p=0.0007$), there was no correlation when vitamin E supplement users were excluded from the analysis ($r = 0.008$; $p=0.57$) (22). However, in a cross-sectional analysis of 482 adults (347 men and 111 women), of whom only approximately 5% took a vitamin E supplement, dietary α-tocopherol was positively correlated with plasma γ-tocopherol concentrations ($r = 0.24$; 95% CI 0.15, 0.32) (53). In our study, whereas the association of plasma γ- with α-tocopherol concentrations among participants who did not take vitamin E supplements (vitamin E supplement non-users) was not statistically

significant (an estimated modest direct association), the directions of the associations of all the other factors with γ-tocopherol concentrations were the same as among all participants combined, but generally weaker. We attribute any differences between our findings limited to vitamin E supplement non-users and those in which all participants were included to the relative lack of heterogeneity in vitamin E intakes and plasma γ-tocopherol concentrations among vitamin E supplement non-users, and suggest that this is the likely explanation for the findings in the previous study (22) that addressed this issue.

There is also support from previous studies in humans for our findings of inverse associations of γ-tocopherol concentrations with other antioxidant exposures, and direct associations of γ-tocopherol concentrations with systemic oxidative stress and inflammation. In the above-mentioned cross-sectional analysis of 657 men in the Multi-Ethnic Prospective Cohort study, plasma γ-tocopherol concentrations were modestly positively correlated with plasma concentrations of hsCRP ($r = 0.14$; $p < 0.0001$) and FiP ($r = 0.13$, $p< 0.0001$), and modestly negatively correlated with plasma 25[OH]$D_3$ concentrations ($r = -0.24$, $p< 0.0001$) (43). In a cross-sectional analysis of participants in a trial of the effect of antioxidant supplements on oxidative damage in smokers (n=298; 121 men and 177 women) there was a pattern of stepwise higher mean plasma FiP concentrations across increasing plasma γ-tocopherol concentration quartiles, culminating in an 88.2% higher mean concentration in the $4^{th}$ relative to the $1^{st}$ quartile ($p<0.0001$) (54). There was no difference in plasma FiP concentrations across plasma α-tocopherol concentration quartiles ($p=0.57$) (54). In a cross-sectional analysis of questionnaire data and stored serum samples from 207 adolescent girls and 183 premenopausal women in the FAM3 (Female Adolescent Maturation) Cohort, serum γ-tocopherol concentrations were

modestly positively correlated with BMI in the girls and the women (r = 0.17 [p=0.02] and r = 0.25 [p=0.0008], respectively) (55).

Our study has several limitations and strengths. Limitations include the cross-sectional design, which prohibits assessing the temporality of the associations, and the known limitations of assessing diet with food frequency questionnaires, such as recall error and limited food choices (56-58). However, it would be expected that error related to assessing diet would be non-differential, which would most likely attenuate the estimated diet-biomarker associations. Another limitation was that the study population was mostly white and, rather than being randomly selected and recruited from a general population, was limited to persons scheduled for colonoscopies, both of which may limit the generalizability of our findings. On the other hand, strengths of the study included the relatively large sample size, especially for a biomarker-heavy study; the inclusion of both men and women; the high-quality measurement of multiple biomarkers of exposure and outcome; and the collection of data on, and investigation of, an extensive number of potential confounding variables.

In conclusion, our results, taken together with previous basic science and human studies, strongly support that circulating γ-tocopherol concentrations are 1) inversely associated with α-tocopherol intake, 2) inversely associated with other antioxidant exposures, and 3) directly associated with systemic oxidative stress and inflammation. Further investigations into the exact mechanisms behind these associations, as well as circulating γ-tocopherol concentrations as a potential biomarker of oxidative stress are warranted.

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**Table 1**. Characteristics of participants (n = 419) by tertiles of plasma γ-tocopherol concentrations, in the pooled Markers of Adenomatous Polyps I & II cross-sectional studies

| **Selected characteristics**[a] | **Tertiles of $\gamma$-tocopherol**[b] | | | **P-value**[c] |
|---|---|---|---|---|
| | **< 0.156** | **0.156 - 0.247** | **> 0.247** | |
| Age, y | 56.8 (9.6) | 55.6 (9.0) | 56.5 (8.5) | 0.58 |
| Male, % | 48.6 | 52.9 | 38.3 | 0.04 |
| White, % | 59.2 | 61.2 | 75.2 | 0.06 |
| > High school education, % | 95.0 | 89.6 | 80.6 | 0.0001 |
| Current smoker, % | 17.7 | 23.7 | 23.1 | 0.61 |
| Current drinker, % | 19.9 | 22.2 | 38.0 | 0.008 |
| Physical activity,[d] MET-hrs/wk | 534 (399) | 569 (416) | 588 (376) | 0.08 |
| Body mass index, kg/m$^2$ | 26.5 (5.4) | 27.3 (5.3) | 30.3 (7.2) | 0.0001 |
| Oxidative balance score (OBS)[e] | 1.6 (4.6) | -0.3 (4.2) | -1.1 (4.2) | 0.0001 |
| Dietary intakes | | | | |
| Total energy, kcal/d | 1,730 (597) | 1,775 (580) | 1,818 (651) | 0.49 |
| Red & processed meats, servings/d | 2.4 (3.7) | 2.3 (2.8) | 1.5 (1.9) | 0.04 |
| Total vegetables & fruit, servings/d | 5.1 (3.2) | 5.0 (2.9) | 5.1 (3.5) | 0.94 |
| Total[f] calcium intake, mg/1,000 kcal/d | 607 (401) | 453 (272) | 407 (249) | 0.0001 |
| Dietary fiber intake, g/1,000 kcal/d | 11.7 (3.6) | 11.4 (4.4) | 10.7 (3.4) | 0.13 |
| Serum 25-OH-vitamin $D_3$, ng/mL | 27.8 (12.7) | 28.7 (12.6) | 22.9 (9.9) | 0.08 |
| Serum cholesterol, mg/dL | 216.2 (75.4) | 237.9 (77.9) | 236.7 (81.3) | 0.04 |
| Plasma C-reactive protein, µg/mL | 3.5 (4.2) | 6.7 (21.6) | 7.3 (6.9) | 0.0001 |
| Plasma $F_2$-isoprostanes, pg/mL | 70.3 (23.7) | 80.3 (29.9) | 116.3 (54.8) | 0.0001 |
| Plasma antioxidants | | | | |
| α-carotene, µg/dL | 4.1 (3.6) | 3.5 (4.2) | 2.6 (2.7) | 0.0002 |
| β-carotene, µg/dL | 20.6 (17.6) | 14.9 (12.3) | 11.1 (8.2) | 0.0001 |
| Zeaxanthin, µg/dL | 16.8 (7.0) | 16.6 (7.9) | 17.0 (8.8) | 0.86 |
| β-cryptoxanthin, µg/dL | 6.9 (4.6) | 7.3 (6.9) | 6.6 (5.9) | 0.32 |
| α-tocopherol, mg/dL | 1.5 (0.7) | 1.1 (0.5) | 1.0 (0.3) | 0.0001 |

Abbreviations: MET, metabolic equivalents of task; SD, standard deviation

[a] Mean (SD) unless otherwise indicated

[b] Plasma $\gamma$-tocopherol concentrations in mg/dL

[c] Based on chi-square test for categorical variables and general linear models for continuous variables; for continuous variables, p-values based on analyses using natural log-transformed values

[d] Moderate + vigorous activity

[e] A composite of anti- and pro-oxidant dietary and lifestyle exposures (see text); a higher score represents higher anti-oxidant relative to pro-oxidant dietary and lifestyle exposures; study population range: -13.3 to 15.6

[f] Total = dietary + supplemental

**Table 2.** Mean plasma γ-tocopherol concentrations by tertiles of selected participant characteristics; pooled Markers of Adenomatous Polyps I & II cross-sectional studies (n = 419)

| **Characteristics (tertile medians)** | **Mean γ-tocopherol[a] (mg/dL)** | **95% CI** | **Proportional difference[b] (%)** | **P-value[c]** |
|---|---|---|---|---|
| Plasma α-tocopherol, mg/dL | | | | |
| 1 (0.75) | 0.213 | (0.196, 0.232) | - | - |
| 2 (1.05) | 0.208 | (0.192, 0.227) | - 2.3 | - |
| 3 (1.61) | 0.147 | (0.135, 0.160) | - 31.0 | < 0.0001 |
| Plasma β-carotene, µg/dL | | | | |
| 1 (5.5) | 0.217 | (0.199, 0.236) | - | - |
| 2 (11.0) | 0.196 | (0.180, 0.213) | - 9.7 | - |
| 3 (25.3) | 0.154 | (0.141, 0.167) | - 29.0 | < 0.0001 |
| Plasma α-carotene, µg/dL | | | | |
| 1 (0) | 0.209 | (0.192, 0.231) | - | - |
| 2 (2.6) | 0.195 | (0.178, 0.212) | - 6.7 | - |
| 3 (5.3) | 0.160 | (0.150, 0.177) | - 23.4 | 0.0002 |
| Plasma cryptoxanthin, µg/dL | | | | |
| 1 (2.8) | 0.202 | (0.185, 0.221) | - | - |
| 2 (5.4) | 0.183 | (0.168, 0.200) | - 9.4 | - |
| 3 (10.4) | 0.176 | (0.161, 0.192) | - 12.9 | 0.14 |
| Plasma zeaxanthin, µg/dL | | | | |
| 1 (10.0) | 0.187 | (0.171, 0.204) | - | - |
| 2 (15.4) | 0.195 | (0.179, 0.213) | + 4.3 | - |
| 3 (23.8) | 0.178 | (0.164, 0.195) | - 4.8 | 0.46 |
| Serum 25-OH-vitamin-$D_3$, ng/mL | | | | |
| 1 (15.5) | 0.203 | (0.190, 0.218) | | |
| 2 (25.0) | 0.176 | (0.159, 0.195) | - 13.3 | |
| 3 (38.0) | 0.166 | (0.150, 0.183) | - 18.2 | 0.005 |
| Oxidative balance score[d] | | | | |
| 1 (-4.4) | 0.214 | (0.196, 0.233) | - | - |
| 2 (-0.2) | 0.196 | (0.180, 0.214) | - 8.4 | - |
| 3 (4.3) | 0.155 | (0.142, 0.169) | - 27.6 | 0.0001 |
| Body mass index, $kg/m^2$ [e] | | | | |
| < Overweight | 0.169 | (0.128, 0.222) | - | - |
| Overweight | 0.168 | (0.154, 0.184) | + 0.6 | - |
| Obese | 0.199 | (0.186, 0.212) | + 15.1 | 0.001 |

| | | | | |
|---|---|---|---|---|
| Total[f] calcium intake, mg/1,000 kcal/d | | | | |
| 1 (256.5) | 0.219 | (0.200, 0.240) | - | - |
| 2 (382.1) | 0.193 | (0.178, 0.211) | - 11.9 | - |
| 3 (741.0) | 0.154 | (0.141, 0.168) | - 29.7 | < 0.0001 |
| Dietary fiber intake, g/1,000 kcal/d | | | | |
| 1 (8.0) | 0.204 | (0.187, 0.222) | - | - |
| 2 (10.9) | 0.193 | (0.177, 0.211) | - 5.4 | - |
| 3 (14.4) | 0.166 | (0.152, 0.181) | - 18.6 | 0.008 |
| Plasma $F_2$-isoprostanes, pg/mL | | | | |
| 1 (56.3) | 0.164 | (0.153, 0.175) | - | - |
| 2 (78.0) | 0.183 | (0.166, 0.201) | + 11.6 | - |
| 3 (118.8) | 0.246 | (0.224, 0.271) | + 50.0 | < 0.0001 |
| Plasma hsCRP, µg/mL | | | | |
| 1 (0.9) | 0.164 | (0.150, 0.178) | - | - |
| 2 (2.7) | 0.175 | (0.160, 0.190) | + 6.7 | - |
| 3 (10.0) | 0.228 | (0.209, 0.248) | + 39.0 | < 0.0001 |

Abbreviations: CI, confidence interval; hsCRP, high sensitivity C-reactive protein

[a] Adjusted for serum total cholesterol concentrations

[b] Proportional difference, in percent, between mean value in the corresponding tertile and mean value in the first tertile; e.g.: ([tertile 3 mean – tertile 1 mean] / tertile 1 mean) x 100%

[c] From general linear model, adjusted for serum total cholesterol concentration; non-transformed means (shown) transformed by natural logarithm to improve normality prior to hypothesis testing

[d] A composite of 15 anti- and pro-oxidant dietary and lifestyle exposures (see text); a higher score represents higher anti-oxidant relative to pro-oxidant dietary and lifestyle exposures; study population range: -13.3 to 15.6

[e] Categories for body mass index based on WHO guidelines for underweight-normal weight, overweight, and obese

[f] Total = dietary + supplemental

**Table 3.** Mean plasma γ-tocopherol concentrations by tertiles of selected participant characteristics (vitamin E supplement users excluded); pooled Markers of Adenomatous Polyps I & II cross-sectional studies (n = 244)

| Characteristics (tertile medians) | Mean γ-tocopherol[a] (mg/dL) | 95% CI | Proportional difference[b] (%) | P-value[c] |
|---|---|---|---|---|
| Plasma α-tocopherol, mg/dL | | | | |
| 1 (0.7) | 0.210 | (0.192, 0.231) | - | - |
| 2 (0.9) | 0.245 | (0.223, 0.270) | + 16.7 | - |
| 3 (1.2) | 0.231 | (0.210, 0.253) | + 10.0 | 0.14 |
| Plasma β-carotene, µg/dL | | | | |
| 1 (5.2) | 0.235 | (0.214, 0.258) | - | - |
| 2 (9.3) | 0.245 | (0.223, 0.269) | + 4.3 | - |
| 3 (19.7) | 0.207 | (0.189, 0.228) | - 12.0 | 0.08 |
| Plasma α-carotene, µg/dL | | | | |
| 1 (0) | 0.235 | (0.215, 0.258) | - | - |
| 2 (2.6) | 0.241 | (0.222, 0.261) | + 2.6 | - |
| 3 (5.9) | 0.199 | (0.179, 0.223) | - 15.3 | 0.047 |
| Plasma cryptoxanthin, µg/dL | | | | |
| 1 (2.8) | 0.231 | (0.210, 0.253) | - | - |
| 2 (5.1) | 0.234 | (0.213, 0.258) | + 1.3 | - |
| 3 (9.7) | 0.220 | (0.200, 0.242) | - 4.8 | 0.76 |
| Plasma zeaxanthin, µg/dL | | | | |
| 1 (9.8) | 0.223 | (0.203, 0.245) | - | - |
| 2 (14.9) | 0.243 | (0.221, 0.267) | + 9.0 | - |
| 3 (23.5) | 0.220 | (0.200, 0.242) | - 1.3 | 0.46 |
| Serum 25-OH-vitamin $D_3$, ng/mL | | | | |
| 1 (16) | 0.252 | (0.234, 0.270) | | |
| 2 (24.5) | 0.217 | (0.193, 0.245) | - 13.8 | |
| 3 (36) | 0.193 | (0.174, 0.214) | - 23.4 | 0.0005 |
| Oxidative balance score[d] | | | | |
| 1 (-4.7) | 0.237 | (0.215, 0.260) | - | - |
| 2 (-1.3) | 0.234 | (0.213, 0.258) | - 1.3 | - |
| 3 ( 3.2) | 0.215 | (0.195, 0.236) | - 9.3 | 0.46 |
| Body mass index, $kg/m^2$ [e] | | | | |
| < Overweight | 0.202 | (0.184, 0.222) | - | - |
| Overweight | 0.241 | (0.219, 0.265) | + 19.3 | - |
| Obese | 0.243 | (0.222, 0.266) | + 20.3 | 0.02 |

| | | | | |
|---|---|---|---|---|
| Total[f] calcium intake, mg/1,000 kcal/d | | | | |
| 1 (244.2) | 0.258 | (0.235, 0.283) | - | - |
| 2 (345.4) | 0.219 | (0.199, 0.240) | - 15.1 | - |
| 3 (510.2) | 0.211 | (0.192, 0.232) | - 18.2 | 0.02 |
| Dietary fiber intake, g/1,000 kcal/d | | | | |
| 1 (7.6) | 0.240 | (0.219, 0.264) | - | - |
| 2 (10.4) | 0.242 | (0.221, 0.265) | + 0.8 | - |
| 3 (13.9) | 0.205 | (0.187, 0.225) | - 14.6 | 0.05 |
| Plasma $F_2$-isoprostanes, pg/mL | | | | |
| 1 (59.9) | 0.206 | (0.191, 0.222) | - | - |
| 2 (84.2) | 0.232 | (0.209, 0.257) | + 12.6 | - |
| 3 (130.2) | 0.272 | (0.246, 0.302) | + 32.0 | 0.0004 |
| Plasma hsCRP, µg/mL | | | | |
| 1 (0.9) | 0.198 | (0.181, 0.216) | - | - |
| 2 (2.9) | 0.222 | (0.203, 0.243) | + 12.1 | - |
| 3 (10.0) | 0.271 | (0.247, 0.296) | + 36.9 | < 0.0001 |

Abbreviations: CI, confidence interval; hsCRP, high sensitivity C-reactive protein

[a] Adjusted for serum total cholesterol concentrations

[b] Proportional difference, in percent, between mean value in the corresponding tertile and mean value in the first tertile; e.g.: ([tertile 3 mean – tertile 1 mean] / tertile 1 mean) x 100%

[c] From general linear model, adjusted for serum total cholesterol concentration; non-transformed means (shown) transformed by natural logarithm to improve normality prior to hypothesis testing

[d] A composite of 15 anti- and pro-oxidant dietary and lifestyle exposures (see text); a higher score represents higher anti-oxidant relative to pro-oxidant dietary and lifestyle exposures; study population range: -13.3 to 15.6

[e] Categories for body mass index based on WHO guidelines for underweight-normal weight, overweight, and obese

[f] Total = dietary + supplemental