Research Article

Cancer Prevention Research

Plasma Tocopherols and Risk of Prostate Cancer in the Selenium and Vitamin E Cancer Prevention Trial (SELECT)

Demetrius Albanes[1], Cathee Till[2], Eric A. Klein[3], Phyllis J. Goodman[2], Alison M. Mondul[1], Stephanie J. Weinstein[1], Philip R. Taylor[1], Howard L. Parnes[4], J. Michael Gaziano[5], Xiaoling Song[6], Neil E. Fleshner[7], Powel H. Brown[8], Frank L. Meyskens Jr[9], and Ian M. Thompson[10]

Abstract

The Selenium and Vitamin E Cancer Prevention Trial (SELECT) showed higher prostate cancer incidence in men supplemented with high-dose α-tocopherol. We, therefore, examined whether presupplementation plasma α-tocopherol or γ-tocopherol was associated with overall or high-grade prostate cancer. A stratified case–cohort sample that included 1,746 incident prostate cancer cases diagnosed through June 2009 and a subcohort of 3,211 men was derived from the SELECT trial of 35,533 men. Plasma was collected at entry from 2001 to 2004, and median follow-up was 5.5 years (range, 0–7.9 years). Incidence of prostate cancer as a function of plasma α-tocopherol, γ-tocopherol, and supplementation with α-tocopherol or selenomethionine was estimated by the hazard ratio (HR). Plasma γ-tocopherol was not associated with prostate cancer. Men with higher α-tocopherol concentrations seemed to have risk similar to that of men with lower concentrations [overall HR for fifth (Q5) vs. first quintile (Q1), 1.21; 95 % confidence interval (CI), 0.88–1.66; *P*-trend = 0.24; in the trial placebo arm, Q5 HR, 0.85; 95% CI, 0.44–1.62; *P*-trend = 0.66]. We found a strong positive plasma α-tocopherol association among men receiving the trial selenomethionine supplement [Q5 HR, 2.04; 95% CI, 1.29–3.22; *P*-trend = 0.005]. A positive plasma α-tocopherol–prostate cancer association also seemed limited to high-grade disease (Gleason grade, 7–10; overall Q5 HR, 1.59; 95% CI, 1.13–2.24; *P*-trend = 0.001; among men receiving selenomethionine, Q5 HR, 2.12; 95% CI, 1.32–3.40; *P*-trend = 0.0002). Our findings indicate that higher plasma α-tocopherol concentrations may interact with selenomethionine supplements to increase high-grade prostate cancer risk, suggesting a biologic interaction between α-tocopherol and selenium itself or selenomethionine. *Cancer Prev Res; 7(9); 886–95. ©2014 AACR.*

Introduction

How vitamin E influences prostate carcinogenesis and cancer risk is not fully understood. Laboratory studies point to anti–prostate-tumorigenic properties for most of the eight vitamin E compounds (including four tocopherols and four tocotrienols), but findings from large controlled trials have been contradictory. Whereas the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study found a 32% and 41% reduction in prostate cancer incidence and mortality, respectively, in male smokers taking α-tocopheryl–acetate 50 IU daily (1), the Selenium and Vitamin E Cancer Prevention Trial (SELECT) showed 17% higher prostate cancer incidence rates among men supplemented with 400 IU daily compared with those receiving placebo (2, 3), and the contemporaneous Physicians' Health Study (PHS) II found no effect using a 400 IU dose every other day (4). At the same time, observational studies indicate that higher vitamin E status (e.g., plasma α-tocopherol) is associated with reduced risk of prostate cancer, particularly among smokers, and possibly only for aggressive disease (5–13). It is, therefore, possible that physiologic vitamin E status and high-dose vitamin E supplementation have independent, synergistic, or antagonistic effects on prostate carcinogenesis.

To address this timely issue, we examined prerandomization plasma concentrations of two key vitamin E compounds for humans, α-tocopherol and γ-tocopherol, in relation to risk of developing prostate cancer, overall and within each of the four intervention arms, in a case–cohort study of 4,754 men within SELECT. The plasma tocopherol-

[1]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland. [2]SWOG Statistical Center, Fred Hutchinson Cancer Research Center, Seattle, Washington. [3]Department of Urology, Glickman Urological and Kidney Institute, Cleveland Clinic, Cleveland, Ohio. [4]Division of Cancer Prevention, National Cancer Institute, Bethesda, Maryland. [5]Massachusetts Veteran's Epidemiology, Research and Information Center, VA Boston Healthcare System, Boston, Massachusetts. [6]Biomarker Laboratory, Fred Hutchinson Cancer Research Center, Seattle, Washington. [7]Princess Margaret Hospital, University of Toronto, Toronto, Ontario, Canada. [8]The University of Texas MD Anderson Cancer Center, Houston, Texas. [9]University of California at Irvine, Department of Medicine, Orange, California. [10]Cancer Therapy and Research Center, University of Texas Health Science Center, San Antonio, Texas.

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Corresponding Author: Demetrius Albanes, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, 9609 Medical Center Drive, Room 6E342, Bethesda, MD 20892-9704. E-mail: daa@nih.gov

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risk association was also evaluated according to biologically relevant factors, including Gleason grade, time from randomization to prostate cancer diagnosis, and smoking status. The impact of the trial's α-tocopherol and selenomethionine supplementation on prostate cancer risk was also examined within categories of the prerandomization plasma concentrations.

Materials and Methods

Study population

SELECT was a randomized, double-blind, placebo-controlled trial of daily supplementation of selenium (200 μg elemental selenium as L-selenomethionine), vitamin E (400 IU of *all-rac*-α-tocopheryl acetate), both, or placebo (14). African American men ≥50 years and non–African American men ≥55 years (total $n = 35{,}533$) were recruited from 427 sites in the United States, Canada, and Puerto Rico between August 2001 and June 2004. Men with a prostate-specific antigen level >4 ng/mL or a digital rectal examination suspicious for prostate cancer were ineligible. Study supplementation continued through October 2008 (2), after which approximately 18,000 men were enrolled into the observational cohort follow-up study (15). Sociodemographic and risk factor information was collected at baseline, including age, race, diet, vitamin supplement use, smoking status, medical history, and family history of cancer (14, 16). Participants were monitored twice annually for blood pressure, weight, smoking status, and new medical events. Information about the standard-of-care monitoring and biopsy cores for SELECT has been reported previously (2, 14). The study was approved by the local institutional review boards of each study site, and all participants provided written informed consent (Clinicaltrials.gov Identifier: NCT00006392).

Incident prostate cancer cases were self-reported and confirmed by central review. Medical records were abstracted for diagnostic method and clinical stage, and prostate tissue with corresponding pathology reports were sent for central pathology review, diagnostic confirmation, and Gleason score determination (2). A total of 1,856 prostate cancer cases serving as the primary endpoints were identified through June 2009, including 1,746 with plasma available for tocopherol, phospholipid, and other analyses (17, 18). A subcohort, representative of the SELECT participants, was then created *a priori* as the comparison group for all biomarker studies of prostate cancer and other endpoints. Men were sampled from nine race/age categories of (i) African American versus not and (ii) ages <55 (African Americans only), 55–59, 60–64, 65–70, and ≥70 years for both African Americans and others. For each case, men were selected for the subcohort at random from each race/age category using a ratio of 1 case to 3 controls for the African American categories, and 1 to 1.5 for others. The higher proportion of controls for African Americans was aimed at boosting study power for that subgroup. Annually, beginning in 2005, the number of prostate cancer cases in each of the nine race/age strata was determined, and subcohort members were randomly selected within each stratum, based on the sampling ratio. The subcohort comprised 3,211 individuals (including some of the cases) for this analysis, with a total combined prostate cancer-subcohort sample of 4,754 men whose follow-up time ranged from 0 to 7.9 years (median, 5.5 years). Supplementary Table S1 shows the age and race groups of the subcohort.

Plasma tocopherols

At randomization, participants provided a blood sample (EDTA), collected at least 3 hours after a meal, processed for plasma, buffy coat, and red blood cells, and stored at −70°C (available for 84% of the SELECT participants; ref. 16). All cases and a representative fraction of the cohort were assayed annually to minimize batch and storage time effects. Plasma α-tocopherol and γ-tocopherol concentrations were determined by reverse-phase high-performance liquid chromatography as described (18). Cases and subcohort participant plasma samples randomly were ordered within individual batches along with 381 blinded pooled quality control (QC) duplicate and triplicate samples. The weighted mean coefficients of variation (CV) were 3.1% and 4.5% for α-tocopherol and γ-tocopherol, respectively. Tocopherol concentrations did not differ on the basis of year of plasma collection, with median α-tocopherol values of 12.9, 12.7, 12.5, 12.3, and 12.6 mg/L for 2005, 2006, 2007, 2008, and 2009, respectively, and 1.7, 1.7, 1.8, 1.7, and 1.9 mg/L, respectively, for γ-tocopherol. Total cholesterol was measured using a Roche Cobas Mira Plus Chemistry Analyzer and Roche Multi-Analyte Serum Calibrator. Samples were run in duplicate and every 10 study samples were bracketed by QC duplicate samples (Bio-Rad Liquid Assayed Multiqual 1, 2, 3) with a CV of 2.5%.

Statistical analysis

Spearman rank order correlation coefficients were determined within the subcohort. Proportional hazards modeling was used to test the association between plasma tocopherols and the risk of total and high-grade (Gleason ≥ 7) prostate cancer. Because the sampling scheme used in creating the subcohort was stratified, all analyses were stratified by the nine race–age groups. The proportional hazards assumptions were applied to each race–age group, and the resulting summary parameters are a weighted mean across the strata, with sampling weights derived as the inverse of the probability of being selected for the cohort within each stratum (19, 20). A second type of weighting was used in constructing the pseudo-likelihood function. Non-cases within the subcohort are weighted equally, from baseline until censor date. Cases outside the subcohort are weighted only at the time of diagnosis. Cases within the selected cohort carry a weight of 1, but are considered non-cases up until the time of diagnosis. A SAS macro that computes the weighted estimates and the robust covariance matrix was used (http://lib.stat.cmu.edu/general/robphreg). Proportional hazards assumptions were tested and met using the methods of Lin and colleagues (21).

The primary analysis examines the plasma tocopherol associations overall, within the four intervention arms of the trial, and by any selenomethionine and any α-tocopherol supplementation. Secondary analyses of key *a priori* hypotheses included examination of the plasma tocopherol associations within the four intervention arms and any selenomethionine and any α-tocopherol by disease grade (Gleason ≤6 vs. 7–10), smoking status (never vs. current and former), and follow-up time (<3 years post randomization vs. ≥3 years). In addition, the effects of the intervention supplementation versus placebo within tertiles of tocopherol concentrations were examined (tertiles rather than quintiles being used to maintain adequate sample sizes). Quintiles of α-tocopherol and γ-tocopherol based on the entire subcohort sample were modeled as indicator variables, with the lowest used as the referent category. Multivariable models were adjusted for baseline prostate-specific antigen (PSA), family history of prostate cancer, body mass index (BMI; continuous), diabetes, cholesterol (continuous), smoking status (current, former, and never), and mutually adjusted for the other tocopherol. Results are also age- and race-adjusted as a result of all models being stratified by age/race groups before being weighted and combined to generate summary statistics. Tests for linear trend were obtained by treating the quantile ordering as a continuous variable. All subgroup associations were examined using subgroup-specific sampling weights. Statistical interaction was evaluated by comparing models with and without a cross-product interaction term using the log-likelihood ratio test. Statistical analyses were performed by the SELECT/SWOG Statistical Center using SAS software version 9.2 (SAS Institute, Inc.). All statistical tests were two-sided.

Results

Baseline characteristics of case–cohort participants

Median follow-up was 3.4 years and 6.2 years for prostate cancer cases and subcohort non-cases, respectively. Cases were more likely to be college graduates, nonsmokers, have a family history of prostate cancer and higher PSA, and less likely to be diabetic (Table 1). Men with higher baseline plasma α-tocopherol were more likely to be Caucasian, college graduates, former smokers, had lower energy and fat intake, higher calcium and vitamin E intake (diet only), and lower plasma γ-tocopherol and higher cholesterol concentrations (Supplementary Table S2). Men with higher plasma γ-tocopherol had lower α-tocopherol but higher cholesterol and were more likely to be African American, diabetic, and to have a higher BMI, and less likely to have a college degree, a history of benign prostatic hyperplasia, or to smoke. These men also had higher energy and fat intake, and lower calcium, vitamin D, and vitamin E intake (diet only; Supplementary Table S2). Baseline plasma α-tocopherol and γ-tocopherol among the subcohort non-cases (means, 13.6 and 2.0 mg/L, respectively) were consistent with nationally representative values for men in the United States (i.e., 11.6 and 2.1 mg/L, respectively; ref. 22), and the two tocopherols were inversely correlated in our study sample ($r = -0.21$; $P < 0.0001$).

Plasma tocopherol–prostate cancer association

Prerandomization concentrations of plasma α-tocopherol were not significantly associated with the risk of prostate cancer overall or in men receiving the trial placebo or α-tocopherol supplement alone (Table 2). Men with higher baseline α-tocopherol who received the trial selenomethionine supplement alone or in combination with the α-tocopherol supplement, however, had substantially increased prostate cancer risk [all men receiving selenomethionine, hazard ratio (HR) for the fifth (Q5) vs. first quintile (Q1), 2.04 (95% CI 1.29–3.22); *P*-trend = 0.005]. HR estimates from a joint effects model with men in the first plasma α-tocopherol quintile who received the trial placebo as the referent category yielded similar results overall (Supplementary Table S3).

Plasma γ-tocopherol was not associated with prostate cancer overall or within the trial intervention arms, although concentrations seemed inversely related to risk in the placebo arm and possibly positively associated with risk among men receiving α-tocopherol alone; neither test for trend was statistically significant, however (Table 2). The primary findings for plasma α-tocopherol and γ-tocopherol did not differ by race/ethnicity (data not shown).

Secondary analyses revealed that a positive association between plasma α-tocopherol and prostate cancer was evident only for high-grade disease (Gleason grade, 7–10; Q5 HR, 1.59; 95% CI, 1.13–2.24; *P*-trend = 0.001), particularly among men who received the selenomethionine supplement (alone or with α-tocopherol; Table 3). The positive α-tocopherol risk association and interaction with selenomethionine was similar among never and current/former smokers, but was only observed within the first 3 years of the trial. The γ-tocopherol–prostate cancer relation did not differ by Gleason grade, smoking status, or follow-up time, although the interaction with follow-up time was statistically significant ($P = 0.009$; Supplementary Table S4).

The previously reported higher prostate cancer incidence in men receiving the SELECT α-tocopherol supplement, alone or with selenomethionine (versus placebo; refs. 2, 3), seemed restricted to those in the second and third tertiles of baseline plasma α-tocopherol (HR, 1.11 and 1.25, respectively; Table 4), with an HR for any α-tocopherol versus placebo of 1.15 (95% CI, 0.91–1.47) for the two tertiles combined. Risk was also elevated for men receiving selenomethionine if they were in the highest plasma α-tocopherol tertile. All 95% CIs included 1.0, however, reflecting diminished power for testing the intervention effects in these subgroups, and trend tests were not significant. Median (range) on-study plasma α-tocopherol concentrations 6 months into the trial in the first, second, and third tertiles of baseline α-tocopherol were 9.6 (5.7–15.0), 11.3 (7.4–16.2), and 15.0 (9.0–22.2) mg/L among men not supplemented with α-tocopherol, and

Table 1. Baseline demographic and health-related characteristics for prostate cancer cases and subcohort members, SELECT case–cohort study

	Prostate cancer cases $n = 1,746$ (%)	Subcohort non-cases $n = 3,008$ (%)
Age, y, mean (SD)	63.5 (6.1)	63.3 (6.6)
Race/ethnicity, *n* (%)		
African American	251 (14.4)	735 (24.4)
Hispanic	58 (3.3)	139 (4.6)
Caucasian	1,406 (80.5)	2,083 (69.2)
Other	31 (1.8)	51 (1.7)
College graduate, *n* (%)	948 (54.3)	1,428 (47.5)
History of benign prostatic hyperplasia, *n* (%)		
Yes	292 (16.7)	470 (15.6)
Missing/unknown	142 (8.1)	190 (6.3)
Family history of prostate cancer, *n* (%)	506 (29.0)	423 (14.1)
History of diabetes, *n* (%)	127 (7.3)	386 (12.8)
Height, cm, mean (SD)	176.8 (7.4)	176.8 (7.5)
BMI, kg/m^2, mean (SD)	28.5 (4.4)	28.8 (4.6)
Smoking status, *n* (%)		
Lifetime nonsmoker	829 (47.5)	1,250 (41.6)
Former smoker	821 (47.0)	1,497 (49.8)
Current smoker	96 (5.5)	261 (8.7)
Trial supplementation arm, *n* (%)		
α-Tocopherol only	479 (27.4)	765 (25.4)
Selenomethionine only	437 (25.0)	749 (24.9)
α-Tocopherol and selenomethionine	424 (24.3)	753 (25.0)
Placebo	406 (23.3)	741 (24.6)
Dietary intake/day, mean (SD)		
Energy, kcal	2,352 (1,081)	2,312 (1,185)
Total fat, g	94.2 (52.1)	93.6 (56.4)
Calcium, mg	1,059 (597)	1,012 (604)
Vitamin D (calciferol), μg	7.1 (5.0)	6.8 (5.0)
Vitamin E, IU	18.1 (12)	17.9 (14.4)
Supplemental vitamin E use before trial, *n* (%)	501 (28.7)	860 (28.6)
Plasma biochemical measures, mean (SD)		
Alpha-tocopherol (AT), mg/L	14.0 (5.3)	13.6 (5.6)
Gamma-tocopherol (GT), mg/L	1.9 (1.1)	2.0 (1.2)
AT:GT molar ratio	12.5 (17.0)	11.6 (16.0)
Cholesterol, mg/dL	203 (37)	200 (38)
PSA (μg/mL), *n* (%)		
<1.00	130 (7.4)	1,330 (44.2)
1.00–1.99	464 (26.6)	1,039 (34.5)
2.00–2.99	587 (33.6)	420 (14.0)
≥3.00	564 (32.3)	219 (7.3)
PSA (μg/mL), median (inter-quartile range)	2.4 (1.7–3.2)	1.1 (0.6–1.8)

15.2 (7.3–33.2), 18.0 (10.6–28.6), and 17.9 (9.8–55.4) mg/L among men receiving α-tocopherol. With respect to plasma γ-tocopherol, only men in the highest baseline tertile seemed to experience increased prostate cancer risk in response to the α-tocopherol supplementation (versus placebo), although these findings also were not statistically significant (Table 4).

Discussion

Within the SELECT trial that recently showed higher prostate cancer incidence resulting from supplementation with 400 IU α-tocopherol acetate daily (3), we found that men with the highest prerandomization plasma α-tocopherol concentrations were twice as likely to be diagnosed with prostate cancer if they received the trial selenomethionine

Table 2. Association between quintiles of plasma α-tocopherol, γ-tocopherol, and prostate cancer risk overall and by intervention arm, SELECT case–cohort study

	Plasma tocopherol quintiles					
	1	2	3	4	5	P_{trend}
α-Tocopherol	≤9.83	>9.83–≤11.71	>11.71–≤13.70	>13.70–≤16.80	>16.80	
Median, mg/L	8.64	10.75	12.65	15.01	20.09	
Cases/subcohort, *n*[a]	299/681	343/656	361/629	375/616	362/624	
Overall HR[b] (95% CI)	1.00	1.07 (0.82–1.40)	1.06 (0.81–1.41)	1.12 (0.83–1.49)	1.21 (0.88–1.66)	0.24
HR[b] (95% CI) within intervention arms						
Placebo	1.00	0.97 (0.56–1.68)	0.91 (0.49–1.68)	0.96 (0.53–1.74)	0.85 (0.44–1.62)	0.66
α-Tocopherol only	1.00	0.60 (0.35–1.03)	0.72 (0.43–1.21)	0.67 (0.38–1.19)	0.59 (0.33–1.07)	0.24
Selenomethionine only	1.00	1.21 (0.72–2.03)	1.42 (0.79–2.55)	1.69 (0.96–2.99)	1.83 (0.95–3.54)	0.04
α-Tocopherol and selenomethionine	1.00	1.72 (1.03–2.89)	1.34 (0.78–2.30)	1.53 (0.86–2.73)	2.05 (1.15–3.67)	0.06
Any selenomethionine	1.00	1.47 (1.02–2.13)	1.44 (0.97–2.14)	1.62 (1.07–2.43)	2.04 (1.29–3.22)	0.005
Any α-tocopherol	1.00	1.00 (0.69–1.46)	0.96 (0.66–1.39)	0.99 (0.66–1.49)	1.12 (0.74–1.70)	0.64
γ-Tocopherol	≤0.96	>0.96–≤1.47	>1.47–≤2.01	>2.01–≤2.76	>2.76	
Median, mg/L	0.66	1.24	1.73	2.35	3.41	
Cases/subcohort, *n*[a]	355/633	361/628	360/628	363/629	301/688	
Overall HR[b] (95% CI)	1.00	1.02 (0.78–1.33)	1.11 (0.84–1.45)	1.13 (0.84–1.50)	0.93 (0.69–1.24)	0.90
HR (95% CI) within intervention arms						
Placebo	1.00	0.84 (0.50–1.43)	0.98 (0.56–1.72)	0.71 (0.39–1.30)	0.68 (0.37–1.25)	0.17
α-Tocopherol only	1.00	1.24 (0.74–2.07)	1.52 (0.91–2.53)	1.32 (0.75–2.31)	1.52 (0.88–2.60)	0.16
Selenomethionine only	1.00	0.90 (0.52–1.55)	0.97 (0.56–1.68)	1.38 (0.79–2.39)	0.75 (0.42–1.36)	0.82
α-Tocopherol and selenomethionine	1.00	0.96 (0.56–1.62)	0.94 (0.55–1.60)	1.05 (0.62–1.76)	0.77 (0.44–1.36)	0.58
Any selenomethionine	1.00	0.93 (0.63–1.37)	0.94 (0.63–1.39)	1.20 (0.80–1.79)	0.77 (0.50–1.16)	0.64
Any α-tocopherol	1.00	1.15 (0.79–1.65)	1.22 (0.85–1.77)	1.21 (0.82–1.79)	1.14 (0.77–1.69)	0.48

[a]The subcohort contains both cases and non-cases, with overlap between case and subcohort numbers.

[b]HRs based on proportional hazard regression, adjusted for PSA (continuous), family history of prostate cancer, BMI (continuous), diabetes at baseline, cholesterol (continuous), and smoking status (current smoker, former smoker, and never smoker) and mutually adjusted for γ-tocopherol or α-tocopherol (continuous). Results are also age- and race-adjusted as a result of all models being stratified by age/race groups before being weighted and combined to generate summary statistics. Overall HR also adjusted for intervention arm. The first plasma tocopherol quintiles serve as referent categories. Interaction between treatment arm and tocopherol quintiles was performed using a linear trend for quintiles in a 3 degrees-of-freedom χ^2 test, with the *P* value for α-tocopherol being 0.20 and for γ-tocopherol being 0.27.

supplement. Plasma α-tocopherol was unrelated to risk among men in the α-tocopherol only and placebo trial arms. In contrast with recent data from transgenic rat for adenocarcinoma of prostate (TRAP) model experiments (23), there was no prostate cancer–plasma γ-tocopherol association overall, although a modest inverse association was suggested in the placebo arm.

Higher plasma concentrations of α-tocopherol result from supplementation and increased dietary intake, higher cholesterol and triglyceride levels, and some genetic variants related to vitamin E transport and metabolism (22, 24, 25). Experimental and other human data show a wide range of effects relevant to prostate tumorigenesis for vitamin E compounds, including inhibition of membrane-associated lipid peroxidation, inflammation, angiogenesis, and cell proliferation, enhanced apoptosis, and reduced circulating androgen levels (25–31). Protective associations have been observed between higher serum α-tocopherol and prostate cancer risk in several (5–13), but not all (32–38), studies, and one large controlled trial found a one-third reduction in prostate cancer incidence in men taking a relatively low, 50 IU dose of α-tocopheryl acetate daily (1).

How elevated plasma α-tocopherol concentrations resulted in higher prostate cancer risk among men supplemented with selenium is unknown and, to our knowledge, has not been previously observed. The fact that selenomethionine was the compound administered in SELECT leaves open the possibility that either elemental selenium or the amino acid moiety (or both) was responsible for the observed harmful interaction with plasma α-tocopherol. Selenium and vitamin E have previously been shown to enhance each other's antioxidant functions in various model systems (39, 40), yet our findings suggest the possibility of an alternative biologic interaction when both micronutrients are elevated. It is noteworthy in this regard that metabolites of methionine as well as the related one-carbon metabolic pathway have been associated with prostate cancer recurrence. In one study, patients with prostate

Table 3. Association between quintiles of plasma α-tocopherol and prostate cancer risk stratified by Gleason grade, smoking status, follow-up time, and by trial supplementation, SELECT case–cohort study

	Plasma α-tocopherol quintiles					
	1	2	3	4	5	P_{trend}
Histologic grade						
Gleason 2–6						
Cases/subcohort, *n*[a]	171/681	201/656	209/629	209/616	203/624	
Overall HR[b] (95% CI)	1.00	1.14 (0.89–1.45)	1.13 (0.88–1.44)	1.12 (0.88–1.44)	1.06 (0.82–1.36)	0.80
Placebo	1.00	1.04 (0.64–1.69)	1.16 (0.71–1.89)	0.94 (0.57–1.53)	1.04 (0.63–1.73)	0.93
α-Tocopherol only	1.00	0.83 (0.52–1.32)	0.95 (0.60–1.52)	0.82 (0.51–1.32)	0.56 (0.34–0.93)	0.04
Selenomethionine only	1.00	1.12 (0.69–1.81)	1.33 (0.82–2.16)	1.22 (0.75–1.99)	1.10 (0.67–1.79)	0.65
α-Tocopherol and selenomethionine	1.00	1.72 (1.04–2.85)	0.94 (0.56–1.58)	1.62 (0.98–2.68)	1.75 (1.06–2.91)	0.08
Any selenomethionine	1.00	1.37 (0.96–1.94)	1.15 (0.81–1.63)	1.41 (0.99–2.00)	1.39 (0.98–1.98)	0.10
Any α-tocopherol	1.00	1.17 (0.83–1.64)	0.96 (0.68–1.35)	1.12 (0.80–1.59)	0.98 (0.69–1.40)	0.82
Gleason 7–10						
Cases/subcohort, *n*[a]	72/681	89/656	103/629	117/616	117/624	
Overall HR[b] (95% CI)	1.00	1.15 (0.81–1.64)	1.45 (1.03–2.04)	1.58 (1.13–2.21)	1.59 (1.13–2.24)	0.001
Placebo	1.00	1.64 (0.75–3.60)	2.50 (1.18–5.30)	1.81 (0.85–3.86)	1.73 (0.78–3.82)	0.28
α-Tocopherol only	1.00	0.89 (0.47–1.69)	0.89 (0.47–1.68)	1.24 (0.67–2.32)	0.93 (0.48–1.80)	0.77
Selenomethionine only	1.00	1.13 (0.52–2.45)	2.10 (1.01–4.36)	2.41 (1.19–4.88)	2.58 (1.27–5.26)	0.001
α-Tocopherol and selenomethionine	1.00	1.27 (0.65–2.50)	1.24 (0.64–2.41)	1.51 (0.78–2.92)	1.75 (0.92–3.32)	0.08
Any selenomethionine	1.00	1.21 (0.73–2.01)	1.62 (1.00–2.64)	1.92 (1.18–3.10)	2.12 (1.32–3.40)	0.0002
Any α-tocopherol	1.00	1.08 (0.68–1.71)	1.06 (0.67–1.68)	1.35 (0.86–2.13)	1.29 (0.81–2.04)	0.17
Smoking status						
Never smokers						
Cases/subcohort, *n*[a]	146/289	163/276	168/268	172/264	177/259	
Overall HR[b] (95% CI)	1.00	0.96 (0.72–1.30)	1.05 (0.78–1.41)	1.03 (0.76–1.40)	1.10 (0.81–1.49)	0.44
Placebo	1.00	1.00 (0.54–1.85)	1.21 (0.65–2.24)	0.81 (0.42–1.57)	0.99 (0.51–1.92)	0.78
α-Tocopherol only	1.00	0.66 (0.37–1.19)	0.66 (0.37–1.19)	0.67 (0.37–1.21)	0.58 (0.32–1.05)	0.11
Selenomethionine only	1.00	1.29 (0.71–2.35)	1.64 (0.90–2.97)	1.47 (0.80–2.69)	1.84 (1.00–3.39)	0.05
α-Tocopherol and selenomethionine	1.00	1.19 (0.65–2.18)	0.89 (0.49–1.63)	1.08 (0.58–2.01)	1.66 (0.90–3.06)	0.18
Any selenomethionine	1.00	1.19 (0.78–1.82)	1.16 (0.76–1.77)	1.35 (0.88–2.08)	1.70 (1.11–2.61)	0.01
Any α-tocopherol	1.00	0.90 (0.59–1.36)	0.79 (0.52–1.20)	0.84 (0.55–1.29)	0.91 (0.59–1.39)	0.62
Current and former smokers						
Cases/subcohort, *n*[a]	146/402	187/369	193/363	202/352	186/364	
Overall HR[b] (95% CI)	1.00	1.40 (1.06–1.84)	1.35 (1.03–1.78)	1.43 (1.09–1.88)	1.26 (0.95–1.67)	0.18
Placebo	1.00	1.18 (0.67–2.10)	1.18 (0.67–2.06)	1.14 (0.65–1.99)	1.01 (0.57–1.80)	0.93
α-Tocopherol only	1.00	1.05 (0.62–1.77)	1.27 (0.75–2.16)	0.99 (0.58–1.67)	0.85 (0.48–1.50)	0.51
Selenomethionine only	1.00	1.17 (0.63–2.16)	1.48 (0.82–2.66)	1.64 (0.91–2.93)	1.52 (0.84–2.75)	0.09
α-Tocopherol and selenomethionine	1.00	1.68 (0.98–2.89)	1.11 (0.64–1.92)	1.86 (1.09–3.16)	1.53 (0.88–2.64)	0.15
Any selenomethionine	1.00	1.33 (0.87–2.03)	1.47 (0.98–2.21)	1.60 (1.06–2.41)	1.44 (0.95–2.20)	0.06
Any α-tocopherol	1.00	1.33 (0.92–1.93)	1.16 (0.80–1.70)	1.32 (0.90–1.92)	1.11 (0.75–1.63)	0.72
Follow-up time to diagnosis						
<3 years						
Cases/subcohort, *n*[a]	113/681	124/656	158/629	149/616	154/624	
Overall HR[b] (95% CI)	1.00	1.08 (0.80–1.45)	1.41 (1.06–1.87)	1.29 (0.97–1.72)	1.30 (0.98–1.74)	0.03
Placebo	1.00	0.96 (0.55–1.69)	1.27 (0.74–2.19)	0.74 (0.41–1.34)	0.88 (0.48–1.59)	0.42
α-Tocopherol only	1.00	0.89 (0.51–1.55)	1.05 (0.61–1.80)	1.22 (0.71–2.08)	0.73 (0.41–1.27)	0.61
Selenomethionine only	1.00	1.14 (0.59–2.19)	2.40 (1.31–4.37)	1.94 (1.06–3.54)	2.09 (1.15–3.79)	0.003
α-Tocopherol and selenomethionine	1.00	1.31 (0.73–2.36)	1.24 (0.69–2.25)	1.67 (0.93–2.99)	2.04 (1.16–3.61)	0.01
Any selenomethionine	1.00	1.27 (0.82–1.96)	1.78 (1.17–2.70)	1.78 (1.17–2.71)	2.08 (1.38–3.12)	0.0001
Any α-tocopherol	1.00	1.09 (0.73–1.64)	1.14 (0.76–1.70)	1.39 (0.93–2.06)	1.22 (0.81–1.83)	0.17

(Continued on the following page)

Table 3. Association between quintiles of plasma α-tocopherol and prostate cancer risk stratified by Gleason grade, smoking status, follow-up time, and by trial supplementation, SELECT case–cohort study (Cont'd)

	Plasma α-tocopherol quintiles					
	1	2	3	4	5	P_{trend}
≥3 years						
Cases/subcohort, *n*[a]	186/631	219/614	203/594	226/575	208/577	
Overall HR[b] (95% CI)	1.00	1.10 (0.87–1.40)	1.00 (0.79–1.28)	1.08 (0.85–1.38)	1.01 (0.78–1.29)	0.94
Placebo	1.00	1.18 (0.70–1.98)	1.33 (0.79–2.25)	1.15 (0.69–1.91)	1.04 (0.60–1.81)	0.99
α-Tocopherol only	1.00	0.78 (0.49–1.23)	0.79 (0.49–1.26)	0.68 (0.42–1.10)	0.58 (0.35–0.96)	0.03
Selenomethionine only	1.00	1.05 (0.65–1.70)	1.00 (0.61–1.65)	1.29 (0.80–2.09)	1.14 (0.69–1.86)	0.40
α-Tocopherol and selenomethionine	1.00	1.56 (0.95–2.55)	0.94 (0.57–1.53)	1.37 (0.84–2.25)	1.36 (0.82–2.24)	0.44
Any selenomethionine	1.00	1.27 (0.90–1.79)	0.99 (0.70–1.40)	1.33 (0.94–1.88)	1.28 (0.90–1.82)	0.19
Any α-tocopherol	1.00	1.09 (0.78–1.52)	0.87 (0.62–1.21)	0.95 (0.67–1.35)	0.90 (0.63–1.27)	0.36

[a]The subcohort contains both cases and non-cases, with overlap between case and subcohort numbers.

[b]HRs and 95% CIs based on proportional hazards regression, mutually adjusted for γ-tocopherol or α-tocopherol (continuous), with PSA, family history of prostate cancer, BMI, diabetes, cholesterol, and smoking status included in the models. Results are also age- and race-adjusted as a result of all models being stratified by age/race groups before being weighted and combined to generate summary statistics. The first plasma tocopherol quintile serves as referent category. Interaction *P* values for plasma α-tocopherol × smoking status are 0.95, 0.75, and 0.94 for the four intervention arm, any selenium, and any α-tocopherol strata (respectively). Interaction *P* values for plasma α-tocopherol × follow-up time to diagnosis are 0.21, 0.35, and 0.30 for the four intervention arm, any selenium, and any α-tocopherol strata (respectively).

cancer whose malignancies recurred within 2 years were more likely to have higher methionine, cysteine, and sarcosine metabolite concentrations at the time of prostatectomy than were patients who remained in remission for at least 5 years (41). The fact that higher prerandomization plasma α-tocopherol was primarily related to increased risk of high-grade prostate cancer and within the first 3 years of SELECT is consistent with these clinical observations of increased tumor progression and recurrence; i.e., selenomethionine in the setting of higher α-tocopherol concentrations may have promoted the growth of subclinical cancers (42). Also relevant in this regard are the higher overall prostate cancer risk in the selenomethionine arms in the highest plasma α-tocopherol tertile (Table 4), and the somewhat increased incidence of high-grade prostate cancers in men receiving selenomethionine in SELECT (Table 3 in reference # 3); HR, 1.21 ($P = 0.11$) for selenomethionine alone, and HR, 1.23 ($P = 0.08$) for selenomethionine plus α-tocopherol, compared with the placebo arm). How increased plasma α-tocopherol under conditions of methionine or selenium supplementation might lead to higher prostate cancer risk requires further study.

Given the increased prostate cancer incidence in SELECT participants randomized to a high, commonly used dose of α-tocopherol, it is possible that men with higher prerandomization plasma levels were taking α-tocopherol supplements before enrolment and, as a result, remained at elevated risk for the first 3 years of the trial. Prestudy "vitamin E" supplement use (i.e., use of specific tocopherols or formulations was not queried) was comparable with patterns for U.S. men (i.e., 29% in SELECT vs. 27%; ref. 43), but was in fact substantially higher among men in the two highest plasma α-tocopherol quintiles (46%) compared with men in the two lowest quintiles (14%). Such a continuing effect in the earliest trial years is consistent with data reported from the ATBC Study showing a 2 to 3 year posttrial lag for return from lower to baseline prostate cancer risk (44). We found no material association between plasma PSA and α-tocopherol ($r = -0.004$) or γ-tocopherol ($r = -0.04$), and no intervention arm differences in PSA or digital rectal exam (DRE) testing in SELECT were found (3). Therefore, increased risk of high-grade disease during the first 3 years of intervention is not likely due to detection bias.

The present findings also indicate that the impact of the daily 400 IU α-tocopherol supplement on prostate cancer in SELECT may have been modified by prerandomization α-tocopherol status, with elevated risk suggested primarily among men with higher plasma concentrations. This could reflect a stronger adverse impact of high-dose α-tocopherol among men who had higher baseline biochemical status who may have experienced a heightened response to supplementation. Men in the α-tocopherol arms with higher baseline plasma α-tocopherol (e.g., >11 mg/L) did achieve 18% to 24% higher follow-up concentrations as compared with men of lower baseline status (i.e., medians of 18 mg/L and 15 mg/L, respectively, and means of 20.0 mg/L and 16.1 mg/L, respectively), suggesting the possibility of an α-tocopherol threshold above which a deleterious impact on the development of prostate cancer may occur, and adding to the growing debate about the risks and benefits of high-dose vitamin supplementation. Our

Table 4. Supplementation effects on prostate cancer HRs within tertiles of plasma α-tocopherol and γ-tocopherol, SELECT case–cohort study

	Plasma tocopherol tertiles			
	1	**2**	**3**	
α-Tocopherol	≤11.05	>11.05–≤14.56	>14.56	
Median, mg/L	9.50	12.65	17.61	
Cases/subcohort, *n*[a]	529/1,108	602/1,059	609/1,039	P_{trend}
Intervention HR[b] (95% CI)				
α-Tocopherol vs. placebo	1.03 (0.68–1.58)	1.24 (0.84–1.84)	1.10 (0.76–1.59)	0.54
Selenomethionine vs. placebo	0.84 (0.55–1.27)	1.06 (0.72–1.57)	1.26 (0.85–1.88)	0.31
α-Tocopherol and selenomethionine vs. placebo	0.97 (0.65–1.45)	0.92 (0.62–1.37)	1.34 (0.90–2.01)	0.39
Any selenomethionine vs. placebo	0.90 (0.63–1.29)	1.05 (0.74–1.49)	1.38 (0.98–1.95)	
Any α-tocopherol vs. placebo	1.03 (0.72–1.48)	1.11 (0.78–1.57)	1.25 (0.90–1.74)	
γ-Tocopherol	≤1.32	>1.32–≤ 2.23	>2.23	
Median, mg/L	0.86	1.73	2.96	
Cases/subcohort, *n*[a]	597/1,051	613/1,031	530/1,124	
Intervention HR[b] (95% CI)				
α-Tocopherol vs. placebo	0.98 (0.66–1.45)	1.14 (0.77–1.68)	1.37 (0.93–2.03)	0.42
Selenomethionine vs. placebo	1.08 (0.73–1.58)	1.03 (0.70–1.52)	1.22 (0.80–1.86)	0.74
α-Tocopherol and selenomethionine vs. placebo	1.19 (0.80–1.78)	1.02 (0.69–1.50)	1.22 (0.81–1.83)	0.48
Any selenomethionine vs. placebo	1.17 (0.84–1.64)	1.02 (0.73–1.43)	1.25 (0.86–1.82)	
Any α-tocopherol vs. placebo	1.09 (0.77–1.54)	1.03 (0.73–1.44)	1.29 (0.90–1.83)	

[a]The subcohort contains both cases and non-cases, with overlap between case and subcohort numbers.

[b]HRs and 95% CIs based on proportional hazard regression, stratified by tocopherol tertile and adjusted for PSA (continuous), family history of prostate cancer, BMI (continuous), diabetes at baseline, cholesterol (continuous), and smoking status (current smoker, former smoker, and never smoker) and mutually adjusted for γ-tocopherol or α-tocopherol (continuous). Results are also age- and race-adjusted as a result of all models being stratified by age/race groups before being weighted and combined to generate summary statistics. For each HR, the referent category is placebo arm within that category of plasma tocopherol concentration.

finding of excess risk of high-grade, Gleason 7–10 cancers is of particular concern in this regard.

Strengths of the present study include the ability to examine prerandomization vitamin E biochemical status (i.e., plasma α-tocopherol and γ-tocopherol) within the setting of a large controlled trial of α-tocopherol and selenium supplementation, and to detect strong interactions that may have biologic significance. With the exception of being oversampled for African Americans, the subcohort was representative of the SELECT population and likely unbiased. Laboratory assays of the plasma tocopherols demonstrated high QC, and all prostate cancer diagnoses were reviewed at a central pathology laboratory. We were, however, limited by the relatively short follow-up, most of which occurred during the trial supplementation period, and by the requirement for PSA and DRE results to be negative for prostate cancer at baseline, resulting in an event rate that reflected men at average risk. The latter eligibility criterion led to the preponderance of early, small incident tumors with very little advanced disease being diagnosed. Our study was also somewhat underpowered for evaluating interactions between the trial intervention arms and plasma tocopherol concentrations.

Ambiguous human data for the role of vitamin E in prostate cancer risk and prevention have resulted in uncertainty about the potential benefit of dietary supplementation with this essential nutrient. High-dose α-tocopherol supplementation was shown in SELECT to increase prostate cancer incidence, and the present study suggests that men with higher prerandomization plasma α-tocopherol status experienced that adverse effect disproportionately, possibly as a result of pre-trial self-supplementation or an accentuated response to the trial supplementation. This strengthens the argument that attaining supraphysiologic concentrations of vitamin E has deleterious consequences, and suggests that some persons having lower vitamin E "set-points" may not be susceptible to those effects. Whether (and how) higher α-tocopherol status interacts with selenomethionine supplementation to increase prostate cancer risk, and high-grade disease in particular, requires further investigation.

Disclosure of Potential Conflicts of Interest

P.H. Brown is a consultant/advisory board member for The Susan G. Komen Foundation. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: D. Albanes, E.A. Klein, P.R. Taylor, J.M.Gaziano, N.E. Fleshner, I.M. Thompson

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): E.A. Klein, J.M.Gaziano, X. Song, N.E. Fleshner, I.M. Thompson

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): D. Albanes, C. Till, P.J. Goodman, A.M. Mondul, P.R. Taylor, F.L. Meyskens Jr
Writing, review, and/or revision of the manuscript: D. Albanes, C. Till, E.A. Klein, P.J. Goodman, A.M. Mondul, S.J. Weinstein, P.R. Taylor, H.L. Parnes, J.M.Gaziano, X. Song, N.E. Fleshner, P.H. Brown, F.L. Meyskens Jr, I.M. Thompson
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): E.A. Klein, S.J. Weinstein, I.M. Thompson
Study supervision: D. Albanes, P.J. Goodman, X. Song, N.E. Fleshner, I.M. Thompson

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