[CANCER RESEARCH 59, 1225–1230, March 15, 1999]

Lower Prostate Cancer Risk in Men with Elevated Plasma Lycopene Levels: Results of a Prospective Analysis[1]

Peter H. Gann,[2] Jing Ma, Edward Giovannucci, Walter Willett, Frank M. Sacks, Charles H. Hennekens, and Meir J. Stampfer

Division of Preventive Medicine [C. H. H.] and Channing Laboratory [J. M., E. G., M. J. S.], Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts 02115; Departments of Epidemiology [C. H. H., M. J. S.] and Nutrition [E. G., W. W., F. M. S., M. J. S.], Harvard School of Public Health, Boston, Massachusetts 02115; and Department of Preventive Medicine, Northwestern University Medical School, Chicago, Illinois 60611 [P. H. G.]

ABSTRACT

Dietary consumption of the carotenoid lycopene (mostly from tomato products) has been associated with a lower risk of prostate cancer. Evidence relating other carotenoids, tocopherols, and retinol to prostate cancer risk has been equivocal. This prospective study was designed to examine the relationship between plasma concentrations of several major antioxidants and risk of prostate cancer.

We conducted a nested case-control study using plasma samples obtained in 1982 from healthy men enrolled in the Physicians' Health Study, a randomized, placebo-controlled trial of aspirin and β-carotene. Subjects included 578 men who developed prostate cancer within 13 years of follow-up and 1294 age- and smoking status-matched controls. We quantified the five major plasma carotenoid peaks (α- and β-carotene, β-cryptoxanthin, lutein, and lycopene) plus α- and γ-tocopherol and retinol using high-performance liquid chromatography. Results for plasma β-carotene are reported separately. Odds ratios (ORs), 95% confidence intervals (CIs), and *P*s for trend were calculated for each quintile of plasma antioxidant using logistic regression models that allowed for adjustment of potential confounders and estimation of effect modification by assignment to either active β-carotene or placebo in the trial.

Lycopene was the only antioxidant found at significantly lower mean levels in cases than in matched controls ($P = 0.04$ for all cases). The ORs for all prostate cancers declined slightly with increasing quintile of plasma lycopene (5th quintile OR = 0.75, 95% CI = 0.54–1.06; *P*, trend = 0.12); there was a stronger inverse association for aggressive prostate cancers (5th quintile OR = 0.56, 95% CI = 0.34–0.91; *P*, trend = 0.05). In the placebo group, plasma lycopene was very strongly related to lower prostate cancer risk (5th quintile OR = 0.40; *P*, trend = 0.006 for aggressive cancer), whereas there was no evidence for a trend among those assigned to β-carotene supplements. However, in the β-carotene group, prostate cancer risk was reduced in each lycopene quintile relative to men with low lycopene and placebo. The only other notable association was a reduced risk of aggressive cancer with higher α-tocopherol levels that was not statistically significant. None of the associations for lycopene were confounded by age, smoking, body mass index, exercise, alcohol, multivitamin use, or plasma total cholesterol level.

These results concur with a recent prospective dietary analysis, which identified lycopene as the carotenoid with the clearest inverse relation to the development of prostate cancer. The inverse association was particularly apparent for aggressive cancer and for men not consuming β-carotene supplements. For men with low lycopene, β-carotene supplements were associated with risk reductions comparable to those observed with high lycopene. These data provide further evidence that increased consumption of tomato products and other lycopene-containing foods might reduce the occurrence or progression of prostate cancer.

INTRODUCTION

The remarkable variation in prostate cancer incidence and mortality across geographic and ethnic groups and the changes in risk observed among migrants have accelerated the search for dietary factors that affect prostate cancer development. Micronutrients such as antioxidants and retinol are logical candidates for study because of their ability to inhibit carcinogenesis in a variety of experimental systems. Previous epidemiological studies, however, have not shown a substantial association between prostate cancer risk and total intake of fruits and vegetables (1). Prior observational studies and randomized trials of β-carotene supplements have also failed to reveal consistent evidence for an overall protective effect against prostate cancer (2). Recently, however, in a large prospective cohort study, we found that consumption of lycopene, a non-provitamin A carotenoid, was inversely related to prostate cancer risk, especially for aggressive disease (3). Because tomato-based foods provide nearly all of the lycopene in the United States diet, this finding is consistent with an earlier cohort study that identified tomatoes as one of several specific food items related to lower prostate cancer risk (4). A previous study of circulating lycopene levels in relation to prostate cancer reported a 50% lower risk for the highest *versus* the lowest quartile of serum lycopene, but this nonsignificant result was based on only 103 case-control pairs and could have been attributed to chance (5).

Lycopene is the predominant circulating carotenoid in most Americans and ranks highest among major natural carotenoids in its capacity for quenching singlet oxygen and scavenging free radicals (6). Lipid-soluble antioxidants such as the carotenoids and tocopherols could, in theory, reduce cancer risk by protecting targets such as DNA and membrane lipids from oxidation. However, recent evidence suggests that other mechanisms, such as modulation of intercellular communication via gap junctions or alterations in intracellular signaling pathways, could also contribute to their anticarcinogenic potential (7).

Here, we describe the results of a nested case-control analysis of plasma antioxidant concentrations and subsequent development of prostate cancer in a cohort of United States physicians participating in a long-term randomized trial of aspirin and β-carotene. We specifically examined plasma concentrations, prior to randomization, of all five major types of carotenoid (including lycopene), two major types of tocopherol (α- and γ-tocopherol), and retinol. Because the randomized trial intervention included β-carotene, results concerning baseline β-carotene levels are reported elsewhere, along with results on the effects of randomized assignment to active β-carotene supplements.[3]

Received 10/6/98; accepted 1/15/99.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported by Public Health Service Grants HL26490 and HL34595 (National Heart, Lung, and Blood Institute), CA34944, CA40360, CA42182, and CA58684 (National Cancer Institute), NIH, Department of Health and Human Services, and American Cancer Society Grant SIG-15.

[2] To whom requests for reprints should be addressed, at Department of Preventive Medicine, Northwestern University Medical School, 680 North Lake Shore Drive, Suite 1102, Chicago, IL 60611. Phone: (312) 908-8432; Fax: (312) 908-9588; E-mail: pgann@nwu.edu. Until August 1999, P. H. G. may be reached at the following address: Unit of Nutrition and Cancer, International Agency for Cancer Research, 150 Cours Albert-Thomas, 69372 Lyon Cedex 08, France. Phone: 33.4.72.73.85.82; Fax: 33.4.72.73.83.61.

[3] N. R. Cook, M. J. Stampfer, and C. H. Hennekens. Effects of β carotene supplementation on total and prostate cancer incidence among randomized participants by baseline plasma levels in the Physicians' Health Study, submitted for publication.

MATERIALS AND METHODS

Study Population. The Physicians' Health Study was a randomized, double-blind, placebo-controlled trial of aspirin and β-carotene among 22,071 United States male physicians, ages 40–84 years, that began in 1982. The Human Subjects Committee at Brigham and Women's Hospital approved the study in accordance with assurances filed with the Department of Health and Human Services; all participants provided informed consent. Men were excluded if they reported a prior history of myocardial infarction, stroke, transient ischemic attacks, unstable angina, cancer (except for nonmelanoma skin cancer), current renal or liver disease, peptic ulcer or gout, contraindications to the use of aspirin, or current use of other platelet-active agents or vitamin A supplements. The aspirin component of the trial was terminated in January 1988 due to a 44% reduction in myocardial infarction in the aspirin group, and the β-carotene component of the study, which involved assignment to either 50 mg of naturally derived β-carotene every other day or placebo, was terminated in December 1995 (8).

Study participants completed two mailed questionnaires before being assigned to exposure groups. Additional questionnaires were mailed at 6 months, 12 months, and annually thereafter. Before randomization, we sent kits to all participants with instructions to have their blood drawn into vacutainer tubes containing EDTA. The participants fractionated the blood by centrifugation and returned the samples (by overnight courier) in plastic cryopreservation vials. No special precautions were taken to shield samples from light during collection or processing. Each kit included a cold pack to keep specimens cool until receipt at our laboratory the following morning, when they were divided into aliquots and stored at −82°C. Degradation of carotenoids, retinol, and tocopherols was reported to be nondetectable in plasma stored at −70°C for up to 51.5 months (9). During storage, precautions were taken so that no specimens thawed or warmed substantially. We received specimens from 14,916 (68%) of the randomly assigned physicians; >70% of the specimens were received between September and November 1982.

Selection of Case Patients and Controls. When participants reported a diagnosis of cancer, we requested medical records (including pathology reports), which were reviewed by study physicians from the End Points Committee. Of the confirmed prostate cancer cases, 578 had samples sufficient for analysis. The absence of plasma samples for some study participants is unlikely to have introduced selection bias because it is implausible that physicians who did or did not provide an adequate sample would differ in the relation between baseline plasma antioxidant levels and subsequent diagnosis of prostate cancer. Case patients with and without adequate plasma did not differ materially in terms of baseline demographic or lifestyle characteristics. For each case patient, one to four control participants were selected who had plasma available, had not had a previous prostatectomy, and had not reported a diagnosis of prostate cancer up to the date the case patient was diagnosed. Controls were matched on smoking status and age within 1 year, except for two case patients over age 80, for whom age was matched within 2 years. Of the 578 cases, 176 had one matched control, 95 had two, 300 had three, and 7 had four. After 13 years of follow-up, >99% of surviving participants were still reporting morbidity events; vital status was ascertained for 100%.

Laboratory Assays. Frozen plasma samples were delivered to the Micro-Nutrient Analysis Laboratory in the Department of Nutrition at the Harvard School of Public Health. Each case and matching control samples were assayed in the same batch to minimize interassay variability and aliquots from a pool of quality control plasma were inserted randomly. Laboratory personnel were unable to distinguish case, control, or quality control samples. Thawed samples were treated with ethanol to precipitate proteins, internal standards were added, and multiple hexane extractions were performed to remove lipid extractable analytes. The extracted samples were then dried and reconstituted with a 3:1:1 mixture of acetonitrile:ethanol:dioxane to a total volume of 250 μl. Each group of 20 unknown samples was combined with 1 blank sample, 1 internal quality control serum pool sample, 2 method blanks, and 2 internal standard blanks. The system is validated against serum samples from the National Institute of Standards and Technology two to three times annually. Measurement of carotenoids, retinols, and tocopherols was achieved by high-performance liquid chromatography. Mean intra-assay coefficients of variation based on the blinded quality control samples ranged from 7.6% for α-carotene to 11.9% for α-tocopherol.

Medical Record Review. Because aggressive prostate cancer has different epidemiological features than less aggressive disease and because previous findings for lycopene suggested a specific relationship to aggressive disease, we classified each case according to its demonstrated aggressiveness at the diagnosis. Physician members of the study staff, unaware of the antioxidant assay results, reviewed the medical records for each confirmed case to determine the tumor stage at diagnosis, tumor grade, and Gleason score. Stage was recorded according to the modified Whitmore-Jewett classification scheme (10). If multiple tissue samples were examined, the highest reported grade and Gleason score were recorded. Cases without pathological staging were classified as indeterminate stage unless there was clinical evidence of distant metastases. Aggressive cases were defined as those diagnosed either at stage C or D (extraprostatic) plus those diagnosed at stage A, stage B, or indeterminate stage with either poor histological grade or Gleason score of ≥7. Among 578 total cases analyzed, 259 were classified as having aggressive disease. Patients with localized prostate cancers having poor histological features experience increased mortality; thus, categorization of these cancers as aggressive is appropriate.

Table 1 *Selected baseline characteristics of prostate cancer cases and controls: the Physicians' Health Study*

	Cases (n = 578)	Controls (n = 1294)
Mean age (yr)[a]	60.7	61.5
Stage at diagnosis (%)		
Localized	60.9	
Regional	18.4	
Distant	12.0	
Unknown	8.7	
Cigarette smokers (%)[a]		
Current	8.3	9.1
Former	45.9	46.4
Alcohol use once or more than once a day (%)	32.9	32.0
Exercise more than once a week (%)	57.0	53.2
Mean body mass index	24.9	24.7

[a] Controls were matched to cases on age and smoking history.

Data Analysis. To compare antioxidant levels in cases *versus* matched controls, we computed paired t test statistics using log-transformed values. Correlations between antioxidant levels were evaluated using both Spearman and Pearson correlation coefficients. We used the cutoff points from the control subjects to assign study participants to a quintile for each antioxidant. To estimate relative risks by level of plasma antioxidant, we computed ORs[4] and 95% CIs using conditional logistic regression models with the lowest quintile as the referent category (11). Tests for trend were performed by testing model coefficients for antioxidant level coded as a continuous variable with values equal to the median for each quintile. Additional covariates such as baseline body mass index, exercise frequency, alcohol consumption, plasma total cholesterol concentration, and multivitamin use were added to the models to test for confounding of the antioxidant associations. We refit models within subgroups defined by tumor aggressiveness and follow-up length. We divided follow-up into two roughly equal segments, with the cutoff point at 6 years. Detailed comparison of cases *versus* matched controls for each follow-up year, plus testing of other follow-up cutoff points in the models, revealed no meaningful differences in results according to selection of the cutoff point. To assess effect modification, we fit regression models within subgroups of case-control sets defined by age and smoking status. We also evaluated whether any of the associations we observed for baseline antioxidant levels were affected by assignment to either active β-carotene or placebo. Effect modification by β-carotene assignment was assessed by comparing ORs determined separately by unconditional logistic regression within the active supplement and placebo groups and by fitting conditional logistic regression models with multiplicative interaction terms involving β-carotene assignment and baseline antioxidant level.

RESULTS

Selected characteristics of the 578 prostate cancer cases and 1294 controls at baseline are compared in Table 1. The relatively young age of the cases, compared to prostate cancer cases in the general population, reflects the younger age distribution of the Physicians' Health Study cohort. Fifty-nine % of the cases were described as confined to

[4] The abbreviations used are: OR, odds ratio; CI, confidence interval.

Table 2 *Matched set comparison of plasma antioxidant levels in prostate cancer cases versus controls: all cases and aggressive cases only*

	Median plasma level[a] (ng/ml)					
	All cases (n = 578 sets)			Aggressive cancer (n = 259 sets)		
	Cases	Controls	P[b]	Cases	Controls	P
α-Carotene	55.8	57.4	0.46	57.9	57.4	0.89
β-Cryptoxanthin	60.7	65.4	0.07	60.1	66.0	0.15
Lutein	102.4	103.0	0.80	98.0	97.0	0.74
Lycopene	369.2	388.0	0.04	356.3	385.0	0.05
α-Tocopherol	10,809	11,068	0.56	10,020	11,160	0.17
γ-Tocopherol	1,659	1,703	0.50	1,570	1,700	0.26
Retinol	580.3	565.6	0.02	570.4	565.1	0.66

[a] Antioxidant concentrations were log-transformed for parametric statistical testing.
[b] P are based on paired t test for nonindependent samples.

the prostate at diagnosis, based on surgical exploration. Among the controls, we found a moderate degree of correlation between plasma antioxidant concentrations. For example, correlations (Spearman r) with β-carotene ranged from 0.70 for α-carotene to -0.01 for γ-tocopherol; for lycopene, correlations ranged from 0.43 with β-carotene to 0.17 with γ-tocopherol. Body mass index was inversely correlated (weakly) with each carotenoid except lycopene. Lycopene concentrations were lower among older participants: the geometric mean concentration was 424 ng/ml in control men under age 60 and 358 ng/ml in men more than 60 years old. Plasma levels of carotenoids were generally lower in men who reported more frequent alcohol intake and little or no exercise; these relationships were less striking for lycopene than other carotenoids. Carotenoid levels had significant but modest associations with plasma total cholesterol (r = 0.23 and 0.15 for lycopene and β-carotene, respectively), presumably because carotenoids are primarily transported by lipoproteins in blood.

Table 2 shows the median levels of 8 plasma antioxidants in cases and controls, for all cancers and aggressive cancers only. Considering all cases, geometric mean lycopene levels were 4.8% lower in cases than controls (P = 0.04). Among aggressive cases, the case-control difference in geometric mean lycopene was greater (7.5%, P = 0.05). The only other notable association we observed in this matched set analysis was a higher geometric mean plasma retinol for all cases compared to controls (P = 0.02). However, this association was entirely confined to nonaggressive cases.

Table 3 presents the ORs and 95% CIs by quintile of plasma antioxidant concentration. For all cases, we found slight inverse associations with higher levels of plasma lycopene and α-carotene. We observed a positive association with higher levels of plasma retinol. When only aggressive cancers were considered, however, the inverse association became stronger for lycopene (for the highest *versus* lowest quintile OR = 0.56; 95% CI = 0.34–0.91) and essen-

Table 3 *Unadjusted ORs[a] for prostate cancer by control quintile[b] of plasma antioxidant concentration: all cases and aggressive cases only*

	OR (95% CI)											
	All cases, by quintile (n = 578 sets)						Aggressive cases, by quintile (n = 259 sets)					
	1	2	3	4	5	P, trend	1	2	3	4	5	P, trend
α-Carotene	1.00	1.11 (0.81–1.54)	0.97 (0.70–1.35)	1.14 (0.82–1.58)	0.77 (0.54–1.10)	0.09	1.00	1.31 (0.80–2.15)	1.02 (0.62–1.70)	1.25 (0.77–2.04)	1.02 (0.61–1.72)	0.82
β-Cryptoxanthin	1.00	0.85 (0.61–1.18)	0.87 (0.62–1.21)	0.88 (0.64–1.21)	0.80 (0.57–1.11)	0.29	1.00	0.68 (0.41–1.14)	0.63 (0.38–1.03)	0.86 (0.53–1.39)	0.70 (0.42–1.16)	0.54
Lutein	1.00	1.01 (0.72–1.42)	1.08 (0.77–1.51)	1.09 (0.78–1.52)	1.10 (0.73–1.65)	0.63	1.00	1.36 (0.82–2.26)	1.29 (0.77–2.18)	1.39 (0.85–2.28)	1.28 (0.66–2.47)	0.60
Lycopene	1.00	0.89 (0.64–1.23)	0.90 (0.65–1.24)	0.87 (0.63–1.19)	0.75 (0.54–1.06)	0.12	1.00	0.64 (0.40–1.03)	0.71 (0.44–1.15)	0.70 (0.44–1.10)	0.56[c] (0.34–0.92)	0.05
α-Tocopherol	1.00	1.04 (0.75–1.43)	1.09 (0.78–1.51)	1.17 (0.85–1.62)	1.06 (0.76–1.48)	0.70	1.00	0.97 (0.61–1.54)	0.81 (0.50–1.32)	1.02 (0.63–1.66)	0.64 (0.38–1.07)	0.11
α-Tocopherol	1.00	0.91 (0.67–1.23)	0.90 (0.65–1.24)	0.87 (0.64–1.19)	0.98 (0.71–1.35)	0.89	1.00	0.91 (0.58–1.42)	0.83 (0.51–1.33)	0.79 (0.49–1.29)	1.00 (0.62–1.60)	0.96
Retinol	1.00	1.08 (0.76–1.53)	1.37 (0.97–1.92)	1.21 (0.84–1.74)	1.56 (1.07–2.27)	0.02	1.00	1.38 (0.84–2.27)	1.44 (0.86–2.40)	1.26 (0.74–2.15)	1.27 (0.73–2.23)	0.64

[a] Cases and controls were matched on age, follow-up time at risk, and smoking status. ORs adjusted for exercise frequency, body mass index, plasma total cholesterol, alcohol, and multivitamin supplement use were similar to the unadjusted estimates.
[b] Quintile cutoff points (in ng/ml) were as follows: α-carotene, 34.6, 50.4, 67.8, and 103.3; β-cryptoxanthin, 36.9, 53.5, 75.2, and 111.4; lutein, 60.8, 79.3, 101.8, and 147.0; lycopene, 261.7, 353.6, 442.9, and 580.1; α-tocopherol, 8,564, 10,204, 11,887, and 14,441; γ-tocopherol, 1,254, 1,627, 1,990, and 2,530; retinol, 450.0, 523.5, 587.1, and 679.9.
[c] P = 0.02.

Table 4 *ORs and 95% CIs for prostate cancer according to plasma lycopene at baseline and random assignment to active β-carotene supplements or placebo*

Plasma lycopene quintile	All cases (n = 578 sets)				Aggressive cases (n = 259 sets)			
	Placebo		β-Carotene		Placebo		β-Carotene	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Q1 (low)	1.00		0.57	0.37–0.88	1.00		0.69	0.37–1.27
Q2	0.72	0.46–1.12	0.61	0.39–0.97	0.72	0.38–1.36	0.32	0.15–0.68
Q3	0.70	0.45–1.09	0.65	0.42–1.02	0.59	0.29–1.16	0.56	0.29–1.09
Q4	0.58	0.37–0.89	0.75	0.49–1.15	0.45	0.23–0.86	0.72	0.38–1.37
Q5	0.59	0.37–0.94	0.55	0.35–0.87	0.40	0.19–0.84	0.48	0.24–0.94
P, trend	0.01				0.006			
P, interaction	0.04				0.05			

tially disappeared for both α-carotene and retinol. There was also some evidence for reduced risk of aggressive prostate cancer associated with the highest quintile of α-tocopherol (OR = 0.64; 95% CI = 0.38–1.07). Simultaneous adjustment for body mass index, exercise frequency, alcohol intake, plasma total cholesterol, and use of multivitamin supplements had no effect on the OR estimates; therefore, these variables were omitted from the final models.

Using both multiplicative interaction terms and subgroup analyses, we explored the possibility that the associations we observed between baseline antioxidant levels and prostate cancer risk could have varied according to assignment to either the β-carotene or placebo group. The results for lycopene, as shown in Table 4, indicate distinct inverse trends in risk for all prostate cancers ($P = 0.01$) and aggressive cancers ($P = 0.006$) in the placebo group, and no evidence for a reduction in risk with higher lycopene in the group randomly assigned to β-carotene. However, compared to men with the lowest lycopene in the placebo group, assignment to β-carotene was associated with a reduction in risk at all lycopene levels. Results were the same when adjusted for potential confounders. For compounds other than lycopene, the relationship of baseline plasma levels to prostate cancer risk did not vary in a substantial or consistent manner between groups according to randomized assignment. When subjects were stratified by β-carotene assignment, the significant inverse association with plasma lycopene in the placebo group was apparent during both the early (≤6 years) and late (>6 years) follow-up periods. In the active β-carotene group, there was no trend with plasma lycopene during either follow-up period.

Plasma lycopene tended to be lower in older men, but we found no evidence that the reduced risk associated with plasma lycopene level varied across age groups. Variation in the risk by smoking status was difficult to assess due to the small number of subjects who were current smokers at baseline. Formal tests for interaction were not significant; however, among the small subgroup of current smokers ($n = 48$ sets for all prostate cancers), the ORs for successive quintiles of lycopene were 1.0, 0.8, 0.5, 0.4, and 0.4; P, trend = 0.03. We found no significant effect modification for other antioxidants according to age or smoking, although for plasma α-tocopherol, the previously mentioned inverse association with aggressive cancer was slightly stronger among current/ex-smokers (5th quintile OR = 0.51; 95% CI = 0.26–0.98) than among never smokers (5th quintile OR = 0.84; 95% CI = 0.36–1.94).

DISCUSSION

In this prospective analysis of plasma antioxidant levels, lycopene was the only compound that appeared to have a significant and internally consistent association with development of prostate cancer. It is important to note that our study was conducted among participants in a randomized trial involving β-carotene supplements and that the relationship of plasma lycopene level to prostate cancer risk clearly appeared to depend on whether β-carotene supplements were consumed. The inverse association between lycopene and risk was confined to men not randomly assigned to take β-carotene supplements. Moreover, although lycopene might be the most important dietary carotenoid in this context, our results indicate that for men in the highest risk category due to low lycopene levels, a risk reduction similar to that obtained with high lycopene level might be achieved with a high-dose β-carotene supplement. In fact, the average risk reduction (relative to the group with lowest lycopene and placebo) for all prostate cancers across all lycopene quintiles in the β-carotene group was 37.3%, very close to the 41% risk reduction observed for placebo group men with the highest lycopene. Taken together, these less-than-additive joint effects are consistent with the hypothesis that there is a ceiling on the benefit gained by consumption of these carotenoids and that this ceiling might be reached either through high dietary lycopene intake or regular use of β-carotene supplements that raise plasma β-carotene to very high levels. This interpretation lends indirect support for antioxidant activity as the mechanism of action because of evidence cited earlier that lycopene shares antioxidant properties with β-carotene but has higher antioxidant potency and capacity based on *in vitro* and *in vivo* studies. The potential importance of diverse antioxidants in prostate cancer development is further supported by recent results indicating decreased prostate cancer incidence among men with increased exposure to selenium and vitamin E supplements (12–14).

Our results for plasma lycopene levels are strikingly similar to those we obtained in a recent analysis of dietary intake and prostate cancer occurrence during 6 years of follow-up in the Health Professionals Follow-up Study (3). In that study, the relative risk of prostate cancer (excluding stage A1) for men in the highest quintile of total lycopene intake compared to the lowest was 0.79 (95% CI = 0.64–0.99). The relative risk of advanced prostate cancer (stage C or D) was 0.57, close to that obtained here, for men with the highest lycopene score, which was based on plasma levels predicted by dietary intake. The strongest inverse associations were observed in men who frequently consumed cooked tomato products, such as tomato sauce; heating tomatoes in oil enhances the bioavailability of lycopene (15).

An anticarcinogenic role for lycopene in the prostate is biologically plausible for several reasons. In cell-free systems, lycopene is more efficient at quenching singlet oxygen and scavenging free radicals than any other commonly consumed carotenoid and ranks higher than other carotenoids tested in prevention of singlet-oxygen induced damage in cultured human lymphoid cells (16–18). Oxidative damage, either to DNA or membranes, might play a role in the development of prostate and other cancers (19, 20). However, carotenoids and lycopene in particular have biological effects apart from antioxidant activity that could be relevant. In cultured cells, these effects include an increase in intercellular communication via gap junctions (21), increased differentiation (22), and altered phosphorylation of regulatory proteins (23). Whatever the mechanism, it is apparent that lycopene is capable of suppressing the growth of human cancer cells *in vitro* and of inhibiting both spontaneous and induced tumor development in animal models. Levy *et al.* (24) reported that lycopene was far more efficient than either α- or β-carotene at inhibiting both the basal and insulin-like growth factor type I-induced proliferation of human endometrial, breast, and lung cancer cell lines. Lycopene significantly reduced the occurrence of spontaneous mammary tumors in mice fed a lycopene-enriched diet (25) and, in contrast to β-carotene, reduced mammary tumor formation in DMBA-treated mice when it was injected i.p. (26).

The effects of lycopene and similar compounds on cancer development are likely to depend to some degree on factors that control local tissue concentrations. Unlike most other carotenoids, lycopene cannot be converted to vitamin A; other metabolic pathways notwithstanding, this could leave more of it available for action at the tissue level. Lycopene concentrations vary greatly among tissues, with the highest concentrations observed in the adrenal gland and testes (27). Lycopene is also highly concentrated in the prostate and, in some men, is present at levels comparable to those that are biologically active *in vitro* (28). The possible functional significance of lycopene's affinity for hormonally regulated tissues is intriguing but has not yet been explored.

Apart from the cohort analysis by Giovannucci *et al.* (3), several other epidemiological studies suggest that lycopene could have an important effect on prostate cancer risk. Lycopene has been inversely associated with risk for several cancers of the digestive tract (29–31), and lycopene intake is high among Southern Europeans, whose prostate cancer risk is relatively low, and low among African-Americans,

who are at relatively high risk (3). A prospective cohort study of elderly Massachusetts residents found that regular tomato consumption was associated with a 50% reduction in mortality from cancer at all sites, with no association for other carotenoid-rich foods (32). Most studies of diet and prostate cancer have not addressed lycopene intake, but tomatoes were one of only four specific food items associated with significantly reduced prostate cancer risk in a prospective study of Seventh-Day Adventist men (4). A nonsignificant inverse association for tomato intake was observed in a Minnesota case-control study (33), whereas another, in Hawaii, failed to find any such association (34). Two previous studies have assessed plasma lycopene in relation to prostate cancer risk; both used prediagnostic samples (5, 35). The first study found a 6.2% lower median lycopene level in cases compared to controls, and OR = 0.50 (95% CI = 0.20–1.29) for the highest *versus* lowest lycopene quartile. The inverse association for lycopene in this study by Hsing *et al.* (5) was stronger for men under age 70 at diagnosis (4th quartile OR = 0.35), a subgroup roughly comparable to the cases in our study, who had a mean age at diagnosis of ~67 years. The second plasma study, conducted in a Japanese-American population in Hawaii, failed to detect any association between lycopene concentrations and prostate cancer (35). This study was relatively small (142 cases), but more importantly, the plasma lycopene levels were very low: the median plasma concentration among controls was only 134 ng/ml, compared to 320 ng/ml in the population studied by Hsing *et al.* (5) and 392 ng/ml in our study population. Moreover, 28% of the cases were diagnosed incidentally during surgery for benign prostatic hyperplasia, and only 14 occurred within the first 5 years of follow-up, when risk estimates would be less attenuated.

Intake of pro-vitamin A compounds, particularly β-carotene, has been studied extensively in relation to prostate cancer risk. Overall, the results from case-control and cohort studies have been inconclusive, with a fairly equal number of studies indicating positive and inverse associations (2). Moreover, neither the Physicians' Health Study[3] nor the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (13) found an overall lower incidence of prostate cancer among men randomly assigned to a β-carotene supplement compared to those assigned to placebo. In preliminary results from the Physician's Health Study, among men in the lowest quartile for plasma β-carotene at baseline, those assigned to active β-carotene had a significantly lower incidence of prostate cancer. However, among those with high baseline plasma β-carotene, those assigned to β-carotene had a comparable but nonsignificant increase in incidence.[3] Hsing *et al.* (5) and Nomura *et al.* (35) found no relationship between serum β-carotene levels and subsequent prostate cancer.

An elevated risk of prostate cancer in men with higher intake of retinol, such as we observed for plasma retinol and all prostate cancers, was observed previously (3, 36–39). However, in our data, the association was entirely confined to nonaggressive tumors. Furthermore, other epidemiological studies of retinol intake report either no association or an inverse association in older men only (40–43), whereas the positive association in our data for retinol was only apparent in younger men (<62 years of age at baseline). Finally, our null results for plasma retinol agree with those of Nomura *et al.* (35) but are at odds with earlier studies of serum retinol, which found an inverse association with prostate cancer risk (5, 44, 45). The possible degradation of retinol on exposure to light and the lack of a strong correlation between dietary and blood levels of retinol further complicate interpretation of these inconsistencies in the retinol-prostate cancer literature.

We found weak evidence for a lower risk of aggressive prostate cancer among men in the highest quintile for α-tocopherol (OR = 0.64; 95% CI = 0.38–1.07). Three prospective studies have examined the relation of serum tocopherol levels to prostate cancer risk, and all reported null associations (5, 13, 46). However, in the Alpha-Tocopherol, Beta-Carotene trial, conducted in male Finnish smokers, investigators reported a 32% lower incidence of prostate cancer over a median follow-up of 6.1 years among men randomly assigned to a daily supplement of 50 mg of α-tocopherol (13). This dose was ~5 times greater than mean dietary intake in the cohort. Our results, which suggest a stronger inverse association for α-tocopherol level and aggressive cancer among current and ex-smokers, offer some support for a possible interaction between smoking and vitamin E at higher dose levels. The effects of tocopherols on prostate cancer growth, particularly at dosages produced by diet supplementation, clearly require further investigation. Although some studies have reported a reduction in plasma α-tocopherol in humans and animals receiving β-carotene supplements (47, 48), we found no evidence for this effect in this analysis or an earlier substudy (49).

The strengths of this study include a large study size, collection of blood samples before diagnosis, and unbiased selection of control subjects. In addition, we were able to adjust associations for several potential confounders and found no evidence that the observed results were due to the effects of age, body mass index, exercise frequency, alcohol intake, smoking, multivitamin use, or plasma cholesterol level. The ability to evaluate the influence of plasma cholesterol was important because lycopene is transported by lipoproteins in the blood, which could affect its bioavailability. A difference in lycopene levels between cases and controls in the earlier, as opposed to later, follow-up years could have occurred if the presence of an undiagnosed prostate cancer caused a reduction in plasma lycopene, perhaps by altering intake or lycopene metabolism. However, among men not assigned to β-carotene supplements, reduced risk of prostate cancer was associated with elevated plasma lycopene during each segment of follow-up time.

The chief limitation of this study lies in the availability of a single baseline plasma sample to characterize long-term levels of circulating lycopene. Presumably, the baseline lycopene value gives an increasingly poor indication of true lycopene level as follow-up length increases and individual lycopene intakes change. Because this misclassification of true lycopene status is independent of disease status, estimates of an association between lycopene and prostate cancer risk will attenuate as follow-up length increases. In a small substudy of Physicians' Health Study participants, we found that the correlation between two plasma lycopene levels, measured an average of 10 years apart, was substantial but imperfect ($r = 0.58$). We do not have sufficient data for estimating dietary lycopene in this cohort. From controlled feeding studies, we know that plasma lycopene levels rise within 1 day following a lycopene-rich meal, peak within 24–48 h in the lipoprotein fraction and then decline with a plasma half-life of 2–3 days (7). Therefore, to the extent that a single plasma measure reflects short-term rather than usual lycopene level, our results could underestimate the actual association between lycopene and prostate cancer.

Our results are consistent with a strong prior hypothesis regarding lycopene and agree closely with our earlier findings, but nevertheless, we believe these results should be interpreted cautiously. Chance cannot be eliminated as an explanation, nor can any observational study demonstrate that lycopene itself, rather than some other compound or factor related to tomato consumption, is responsible for a reduction in prostate cancer risk. Apart from the need for confirmatory epidemiological analyses, many questions regarding the link between lycopene and prostate cancer remain ripe for investigation. The absorption and bioavailability of lycopene appear to be complex processes involving food processing, concurrent dietary lipid intake, cooking method, and, perhaps, levels of lipoproteins (50). Therefore, the relation of diet to blood levels has not been clarified, nor has the relation of blood levels to those in the prostate itself. The presence of

both *cis* and *trans* isomers of lycopene in the prostate has been established, but the biological significance of the various isomers is still unknown (28). If our findings are confirmed in other observational studies, randomized trials should be considered. Meanwhile, these results provide new evidence that increased consumption of tomato products, as part of a diet generally rich in fruits and vegetables, might reduce prostate cancer risk.

ACKNOWLEDGMENTS

We thank Stefanie Parker, Rachel Meyer, and Kathryn Starzyk for their administrative support and management of the plasma samples; Xiaoyang Liu, Dr. Umed Ajani, and Dr. Nancy Cook for assistance with computer programming and statistical analysis; and James Anderson for assistance with the high-performance liquid chromatography assays. We are also grateful to all of the staff as well as the 22,071 participants in the Physicians' Health Study whose dedication and commitment made this work possible.

REFERENCES

1. Steinmetz, K. A., and Potter, J. D. Vegetables, fruit, and cancer. II. Mechanisms. Cancer Causes Control, *2:* 427–442, 1991.
2. Kolonel, L. N. Nutrition and prostate cancer. Cancer Causes Control, *7:* 83–94, 1996.
3. Giovannucci, E., Ascherio, A., Rimm, E. B., Stampfer, M. J., Colditz, G. A., and Willett, W. C. Intake of carotenoids and retinol in relation to risk of prostate cancer. J. Natl. Cancer Inst. (Bethesda), *87:* 1767–1776, 1995.
4. Mills, P. K., Beeson, W. L., Phillips, R. L., and Fraser, G. E. Cohort study of diet, lifestyle, and prostate cancer in Adventist men. Cancer (Phila.), *64:* 598–604, 1989.
5. Hsing, A. W., Comstock, G. W., Abbey, H., and Polk, B. F. Serologic precursors of cancer: retinol, carotenoids, and tocopherol and risk of prostate cancer. J. Natl. Cancer Inst. (Bethesda), *82:* 941–946, 1990.
6. Gerster, H. The potential role of lycopene for human health. J. Am. Coll. Nutr., *16:* 109–126, 1997.
7. Stahl, W., and Sies, H. Lycopene: a biologically important carotenoid for humans? Arch. Biochem. Biophys., *336:* 1–9, 1996.
8. Hennekens, C. H., Buring, J. E., Manson, J. E., Stampfer, M., Rosner, B., Cook, N. R., Belanger, C., LaMotte, F., Gaziano, J. M., Ridker, P. M., Willett, W., and Peto, R. Lack of effect of long-term supplementation with β carotene on the incidence of malignant neoplasms and cardiovascular disease. N. Engl. J. Med., *334:* 1145–1149, 1996.
9. Comstock, G. W., Norkus, E. P., Hoffman, S. C., Xu, M. W., and Helzlsouer, K. J. Stability of ascorbic acid, carotenoids, retinol, and tocopherols in plasma stored at −70 degrees C for 4 years. Cancer Epidemiol. Biomark. Prev., *4:* 505–507, 1995.
10. Catalona, W. J., and Avioli, L. V. Diagnosis, staging, and surgical management of prostatic carcinoma. Arch. Intern. Med., *147:* 361–363, 1987.
11. Rothman, K. J. Modern Epidemiology, pp. 285–310. Boston: Little, Brown, 1986.
12. Clark, L. C., Dalkin, B., Krongrad, A., Combs, G. F., Jr., Turnbull, B. W., Slate, E. H., Witherington, R., Herlong, J. H., Janosko, E., Carpenter, D., Borosso, C., Folk, S., and Rounder, J. Decreased incidence of prostate cancer with selenium supplementation: results of a double-blind cancer prevention trial. Br. J. Urol., *81:* 730–734, 1998.
13. Yoshizawa, K., Willett, W. C., Morris, S. J., Stampfer, M. J., Spiegelman, D., Rimm, E. B., and Giovannucci, E. Study of prediagnostic selenium level in toenails and the risk of advanced prostate cancer. J. Natl. Cancer Inst., *90:* 1219–1224, 1998.
14. Heinonen, O. P., Albanes, D., Virtamo, J., Taylor, P. R., Huttunen, J. K., Hartman, A. M., Haapakoski, J., Malila, N., Rautalahti, M., Ripatti, S., Maenpaa, H., Teerenhori, L., Kos, L., Virolainen, M., and Edwards, B. K. Prostate cancer and supplementation with α-tocopherol and β-carotene: incidence and mortality in a controlled trial. J. Natl. Cancer Inst. (Bethesda), *90:* 440–446, 1998.
15. Stahl, W., and Sies, H. Uptake of lycopene and its geometrical isomers is greater from heat-processed than from unprocessed tomato juice in humans. J. Nutr., *122:* 2161–2166, 1992.
16. Di Mascio, P., Kaiser, S., and Sies, H. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. Arch. Biochem. Biophys., *274:* 532–538, 1989.
17. Miller, N. J., Sampson, J., Candeias, L. P., Bramley, P. M., and Rice-Evans, C. A. Antioxidant activities of carotenes and xanthophylls. FEBS Lett., *384:* 240–242, 1996.
18. Trinkler, J. H., Bohm, F., Schalch, W., and Truscott, T. G. Dietary carotenoids protect human cells from damage. J. Photochem. Photobiol., *26:* 283–285, 1994.
19. Ripple, M. O., Henry, W. F., Rago, R. P., and Wilding, G. Pro-oxidant-antioxidant shift induced by androgen treatment of human prostate carcinoma cells. J. Natl. Cancer Inst. (Bethesda), *89:* 40–48, 1997.
20. Malins, D. C., Polissar, N. L., and Gunselman, S. J. Models of DNA structure achieve almost perfect discrimination between normal prostate, benign prostatic hyperplasia (BPH), and adenocarcinoma and have a high potential for predicting BPH and prostate cancer. Proc. Natl. Acad. Sci. USA, *94:* 259–264, 1997.
21. Zhang, L. X., Cooney, R. V., and Bertram, J. S. Carotenoids up-regulate *connexin43* gene expression independent of their provitamin A or antioxidant properties. Cancer Res., *52:* 5707–5712, 1992.
22. Bankson, D. D., Countryman, C. J., and Collins, S. J. Potentiation of the retinoic acid-induced differentiation of HL-60 cells by lycopene. Am. J. Clin. Nutr., *53* (Suppl.)*:* P-13, 1991.
23. Matsushima-Nishiwaki, R., Shidoji, Y., Nishiwaki, S., Yamada, T., Moriwaki, H., and Muto, Y. Suppression by carotenoids of microcystin-induced morphological changes in mouse hepatocytes. Lipids, *30:* 1029–1034, 1995.
24. Levy, J., Bosin, E., Feldman, B., Giat, Y., Miinster, A., Danilenko, M., and Sharoni, Y. Lycopene is a more potent inhibitor of human cancer cell proliferation than either α-carotene or β-carotene. Nutr. Cancer, *24:* 257–266, 1995.
25. Nagasawa, H., Mitamura, T., Sakamoto, S., and Yamamoto, K. Effects of lycopene on spontaneous mammary tumour development in SHN virgin mice. Anticancer Res., *15:* 1173–1178, 1995.
26. Sharoni, Y., Giron, E., Rise, M., and Levy, J. Effects of lycopene-enriched oleoresin on 7,12-dimethyl-benz[*a*]anthracene-induced rat mammary tumors. Cancer Detect. Prev., *21:* 118–123, 1997.
27. Stahl, W., Schwarz, W., Sundquist, A. R., and Sies H. *cis-trans* isomers of lycopene and β-carotene in human serum and tissues. Arch. Biochem. Biophys., *294:* 173–177, 1992.
28. Clinton, S. K., Emenhiser, C., Schwartz, S. J., Bostwick, D. G., Williams, A. W., Moore, B. J., and Erdman, J. W. *cis-trans* lycopene isomers, carotenoids, and retinol in the human prostate. Cancer Epidemiol. Biomark. Prev., *5:* 823–833, 1996.
29. Buiatti, E., Palli, D., Decarli, A., Amadori, D., Avellini, C., Bianchi, S., Biserni, R., Cipriani, F., Cocco, P., Giacosa, A., Marubini, E., Puntoni, R., Vindigni, C., Fraumeni, J., and Blot, W. A case-control study of gastric cancer and diet in Italy. Int. J. Cancer, *44:* 611–616, 1989.
30. Franceschi, S., Bidoli, E., La Vecchia, C., Talamini, R., D'Avanzo, B., and Negri, E. Tomatoes and risk of digestive-tract cancers. Int. J. Cancer, *59:* 181–184, 1994.
31. Bueno de Mesquita, H. B., Maisonneuve, P., Runia, S., and Moerman, C. J. Intake of foods and nutrients and cancer of the exocrine pancreas: a population-based case-control study in the Netherlands. Int. J. Cancer, *48:* 540–549, 1991.
32. Colditz, G. A., Branch, L. G., and Lipnick, RJ. Increased green and yellow vegetable intake and lowered cancer deaths in an elderly population. Am. J. Clin. Nutr., *41:* 32–36, 1985.
33. Schuman, L. M., Mandel, J. S., Radke, A., Seal, U., and Halberg, F. Some selected features of the epidemiology of prostatic cancer: Minneapolis-St. Paul, Minnesota, case-control study, 1976–1979. *In:* Magnus, K. (ed.), Trends in Cancer Incidence: Causes and Practical Implications, pp. 345–354. Washington, DC: Hemisphere, 1982.
34. Le Marchand, L., Hankin, J. H., Kolonel, L. N., and Wilkens, L. R. Vegetable and fruit consumption in relation to prostate cancer risk in Hawaii: a reevaluation of the effect of dietary β-carotene. Am. J. Epidemiol., *133:* 215–219, 1991.
35. Nomura, A. M. Y., Stemmermann, G. N., Lee, J., and Craft, N. E. Serum micronutrients and prostate cancer in Japanese Americans in Hawaii. Cancer Epidemiol. Biomark. Prev., *6:* 487–491, 1997.
36. Graham, S., Haughey, B., Marshall, J., Priore, R., Byers, T., Rzepka, T., Mettlin, C., and Pontes, J. E. Diet in the epidemiology of carcinoma of the prostate gland. J. Natl. Cancer Inst. (Bethesda), *70:* 687–692, 1983.
37. West, D. W., Slattery, M. L., Robinson, L. M., French, T. K., and Mahoney, A. W. Adult dietary intake and prostate cancer risk in Utah: a case-control study with special emphasis on aggressive tumors. Cancer Causes Control, *2:* 85–94, 1991.
38. Kolonel, L. N., Yoshizawa, C. N., and Hankin, J. H. Diet and prostatic cancer: a case-control study in Hawaii. Am. J. Epidemiol., *127:* 999–1011, 1988.
39. Talamini, R., Franceschi, S., La Vecchia, C., Serraino, D., Barra, S., and Negri, E. Diet and prostatic cancer: a case-control study in Northern Italy. Nutr. Cancer, *18:* 277–286, 1992.
40. Ross, R. K., Shimizu, H., Paganini-Hill, A., Honda, G., and Henderson B. E. Case-control studies of prostate cancer in blacks and whites in Southern California. J. Natl. Cancer Inst. (Bethesda), *78:* 869–874, 1987.
41. Hsing, A. W., McLaughlin, J. K., Schuman, L. M., Bjelke, E., Gridley, G., Wacholder, S., Chien, H. T., and Blot, W. J. Diet, tobacco use, and fatal prostate cancer: results from the Lutheran Brotherhood Cohort Study. Cancer Res., *50:* 6838–6840, 1990.
42. Middleton, B., Byers, T., Marshall, J., and Graham, S. Dietary vitamin A and cancer: a multisite case-control study. Nutr. Cancer, *8:* 107–116, 1986.
43. Ohno, Y., Yoshida, O., Oishi, K., Okada, K., Yamabe, H., and Schroeder, F. H. Dietary β-carotene and cancer of the prostate: a case-control study in Kyoto, Japan. Cancer Res., *48:* 1331–1336, 1988.
44. Hayes, R. B., Bogdanovica, J. F. A. T., Schroeder, F. H., De Bruijn, A., Raatgever, J. W., Van Der Maas, P. J., Oishi, K., and Yoshida, O. Serum retinol and prostate cancer. Cancer (Phila.), *62:* 2021–2026, 1988.
45. Reichman, M. E., Hayes, R. B., Ziegler, R. G., Schatzkin, A., Taylor, P. R., Kahle, L. L., and Fraumeni, J. F., Jr. Serum vitamin A and subsequent development of prostate cancer in the First National Health and Nutrition Examination Survey Epidemiologic Follow-up Study. Cancer Res., *50:* 2311–2315, 1990.
46. Knekt, P., Aromaa, A., Maatela, J., Aaran, R., Nikkari, T., Hakama, M., Hakulinen, T., Peto, R., Saxen, E., and Teppo, L. Serum vitamin E and risk of cancer among Finnish men during a 10-year follow-up. Am. J. Epidemiol., *127:* 28–41, 1988.
47. Bendich, A., and Shapiro, S. S. Effect of β-carotene and canthaxanthin on the immune response of rats. J. Nutr., *116:* 2254–2262, 1986.
48. Xu, M. J., Plezia, P. M., Alberts, D. S., Emerson, S. S., Peng, Y. M., Sayers, S. M., Liu, Y., Ritenbaugh, C., and Gensler, H. L. Reduction in plasma or skin alpha-tocopherol concentration with long-term oral administration of beta-carotene in humans and mice. J. Natl. Cancer Inst. (Bethesda), *84:* 1559–1565, 1992.
49. Fotouhi, N., Meydani, M., Santos, M. S., Meydani, S. N., Hennekens, C. H., and Gaziano, J. M. Carotenoid and tocopherol concentrations in plasma, peripheral blood mononuclear cells, and red blood cells after long-term β-carotene supplementation in men. Am. J. Clin. Nutr., *63:* 553–558, 1996.
50. Gärtner, C., Stahl, W., and Sies, H. Lycopene is more bioavailable from tomato paste than from fresh tomatoes. Am. J. Clin. Nutr., *66:* 116–122, 1997.

Lower Prostate Cancer Risk in Men with Elevated Plasma Lycopene Levels: Results of a Prospective Analysis

Peter H. Gann, Jing Ma, Edward Giovannucci, et al.

Cancer Res 1999;59:1225-1230.