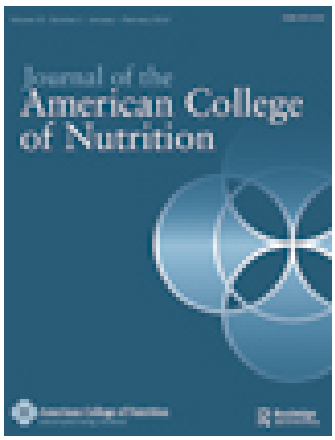

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Low Plasma Lycopene Concentration is Associated with Increased Mortality in a Cohort of Patients with Prior Oral, Pharynx or Larynx Cancers

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Original Research

Low Plasma Lycopene Concentration is Associated with Increased Mortality in a Cohort of Patients with Prior Oral, Pharynx or Larynx Cancers

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Key words: lycopene, carotenoids, mortality, prospective study, beta-carotene, human

Objective: This analysis was conducted to evaluate the association between plasma beta-carotene, alpha-carotene, lycopene, lutein/zeaxanthin, total carotenoids, retinol, alpha-tocopherol and subsequent mortality.

Methods: Blood samples collected longitudinally from 259 participants in a chemoprevention trial aimed at the prevention of second cancers of the oral cavity, pharynx, or larynx were analyzed by high performance liquid chromatography for selected micronutrients. All-cause mortality (primary outcome) and cause-specific mortality (secondary outcomes) were evaluated in relation to plasma micronutrient concentrations at baseline and longitudinally.

Results: A total of 61 deaths occurred over a follow-up time of up to 90 months. Cox proportional hazards models with time-dependent covariates were used for data analyses. In models adjusted for age, plasma cholesterol, time-dependent smoking, treatment arm, study site and gender, only plasma lycopene was significantly inversely associated with total mortality [hazard ratio (HR) above *versus* below median = 0.53, 95% confidence interval (CI) 0.30–0.93]. Plasma alpha-carotene was inversely associated (HR 0.24, 95% CI 0.08–0.75) while plasma retinol was positively associated (HR 5.12, 95% CI 1.54–17.05) with cardiovascular death. Smoking status modified plasma nutrient associations with total mortality. Lycopene (HR 0.08, 95% CI 0.02–0.36), alpha-carotene (HR 0.25, 95% CI 0.09–0.73) and total carotenoids (HR 0.22, 95% CI 0.07–0.70) were inversely associated with mortality in non-smokers, while plasma retinol (HR = 3.56, 95% CI 1.40–9.09) and alpha-tocopherol (HR = 2.47, 95% CI 1.02–5.98) were positively associated with mortality in smokers.

Conclusions: Only plasma lycopene was significantly associated (inversely) with total mortality in the full study population. Smoking modifies associations between nutrients and mortality.

Carotenoids are naturally occurring plant pigments. Humans ingest many different carotenoids in the diet, with the primary dietary sources being fruits and vegetables. Carotenoids are absorbed following dietary ingestion, and plasma concentrations of carotenoids can be readily measured. Such analyses have indicated that plasma carotenoids are correlated with consumption of fruits and vegetables, with plasma carotenoid concentrations considered one of the best biomarkers of fruit and vegetable intake currently available [1,2].

A large number of observational studies, including both case-control studies and cohort studies, of carotenoids and chronic disease risk have been conducted as reviewed elsewhere [2,3]. Many of the studies consider estimated dietary intake of individual carotenoids, or carotenoid concentrations in the blood. Estimated consumption of carotenoids for chronic disease epidemiology is usually based on food frequency questionnaires, with the result that intake estimates are only semi-quantitative, at best. In contrast, blood concentration data are more quantitative and generally more comparable across studies. Given this, the use of blood-based measures of carotenoids for chronic disease epidemiology is attractive, especially in the setting of prospectively collected blood samples [2]. A potential limitation to blood-based

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measures of nutrients is that concentrations may be more reflective of recent intake and thus not representative for use in cohort studies; however, Comstock *et al*. demonstrated that ranked concentrations of antioxidant nutrients from a single blood sample were sufficiently representative to be used as predictors of subsequent concentrations and for assessment as risk factors for subsequent disease outcomes [4].

A few recent studies have prospectively evaluated the association between plasma beta-carotene concentrations or total carotenoid concentrations and various outcomes, including mortality. For example, in a cohort study of subjects enrolled in a skin cancer prevention trial, persons in the highest baseline quartile of plasma beta-carotene (compared to the lowest) had a relative risk for death of 0.52 (95% CI 0.44–0.87) [5]. In another prospective cohort study, persons with high as compared to low total plasma carotenoids (quintile 5 *versus* 1) had a lower risk of death (RR = 0.56, 95% CI 0.32–0.97 following adjustment for age, gender, and serum cholesterol; with additional adjustment for other covariates the RR was 0.78, 95% CI 0.44–1.38) [6].

Of the individual carotenoids, beta-carotene has received the most research attention, but other carotenoids are equally or more concentrated in human blood samples, depending on individual food consumption habits. In the U.S. Third National Health and Nutrition Examination Survey (NHANES III) for example, mean serum carotenoid concentrations overall were as follows: lycopene (234 (μg/L), lutein + zeaxanthin (209 (μg/L), beta-carotene (189 (μg/L), and alpha-carotene (46 (μg/L) [2]. No data are currently available regarding the association of plasma concentrations of carotenoids other than beta-carotene and mortality. We had a unique opportunity to analyze plasma carotenoid concentrations both at baseline and longitudinally in a cohort of persons enrolled in a cancer prevention trial. While the a priori emphasis of our analyses was plasma carotenoids, our carotenoid assay also allows for the measurement of plasma retinol and alpha-tocopherol; therefore, these nutrients are considered as well.

MATERIALS AND METHODS

Subjects

Participants in this study were part of a randomized, double-blind, placebo-controlled trial to determine whether supplemental beta-carotene reduces the incidence of treatment failure due to second primary tumors and local recurrences in patients curatively treated for early stage cancers of the oral cavity, pharynx, or larynx. Efficacy results of the chemoprevention trial have been published, with full details of the trial's methodology [7]. The study methods will be briefly described here, with particular emphasis on the exposures (plasma micronutrients) and outcomes (ascertainment of mortality) of interest for this analysis.

Subjects were recruited from two recruitment sites, one based at Yale University and recruiting from the state of Connecticut (population-based identification and recruitment), and the second based at the University of Miami and recruiting from 14 hospitals in South Florida. In order to be eligible, subjects had to have completed treatment (surgery and/or radiation) for a recently diagnosed Stage I or Stage II squamous cell carcinoma of one of the following sites: tongue, gum or mouth, oropharynx, hypopharynx, pharynx or larynx. Patients with carcinoma in situ at the above sites were also eligible. Subjects also had to be between 20 and 79 years of age, be considered free of cancer at any site at entry into the trial, have no significant co-morbidities, and not have taken supplements of retinol, beta-carotene, vitamin E or selenium within the past year (multivitamin use allowed).

The following procedures were approved by the Connecticut Department of Public Health Human Investigations Committee and the Institutional Review Boards at all hospitals from which subjects were recruited (49 total hospitals). Certain data used in this study were also obtained from the Connecticut Tumor Registry located in the Connecticut Department of Public Health. The author(s) assume full responsibility for analyses and interpretation of these data. First, physician consent was obtained prior to contacting potential participants, both for consent purposes as well as to determine that patients had completed treatment with curative intent. Participants were approached for participation by letter and then by phone; those who agreed were subsequently visited in-person by trained nurse or physician-interviewers, who obtained signed consent prior to proceeding. Participants were interviewed in depth using a structured questionnaire to obtain information about demographics, smoking and alcohol habits, diet, and other possible risk factors, and those who agreed to participate in the trial underwent a placebo run-in period of one month. Subjects who consumed >75% of the placebo capsules during the run-in were randomized to receive either supplemental beta-carotene or placebo. The intervention consisted of a 50 mg dose of beta-carotene/day, packaged into one capsule (Lurotin, BASF, Parsippany, NJ) or a corresponding placebo. Details of the randomization procedure and assessment of compliance are described elsewhere [7].

Biochemical Analyses

At the in-person visits, the interviewer/phlebotomist obtained a blood sample by venipuncture. Two blood samples were taken before the intervention (before and after the placebo run-in), then samples were obtained at 3, 12, 24 months and yearly thereafter for up to 60 months. Blood was collected into two 10 ml heparinized (green top) vacutainer tubes. Bloods were kept cold in the dark until the plasma could be separated. Plasma was aliquotted and stored at −70°C pending analysis. Samples from the Miami recruitment site were stored temporarily at −70°C, and then shipped frozen to the clinical trial

laboratory at Yale, where all samples were analyzed. Samples were analyzed on an ongoing basis, usually within six months of collection.

For the nutrient assays, all operations were carried out under dimmed light in the clinical trial laboratory. A plasma sample was thawed and an aliquot removed and transferred into a screw cap test tube. Tocol (Kodak, Rochester, NY) in 100% ethanol was added as an internal standard, and the sample was extracted twice using hexane. The hexane phase was collected into a new test tube and evaporated under nitrogen gas. The residue was reconstituted in the mobile phase for HPLC injection. Plasma beta-carotene, alpha-carotene, lutein + zeaxanthin, lycopene, retinol, and alpha-tocopherol were analyzed by reverse-phase high performance liquid chromatography according to modifications of established methods [8]. The high performance liquid chromatography system consisted of two Rainin Rabbit-HP pumps with a Rainin Dynamax Dual Chamber mixer, a Gilson Model 401 dilutor, a Gilson Model 231 autosampler, a Rheodyne model 7010 injection valve with a 20 μl loop, a Rainin column temperature controller, a Hewlett Packard model 1050 multiple wavelength detector, a Beckman DABS Ultrasphere 250 $\times$ 4.6mm, 5μ column, and the Rainin Dynamax HPLC Method Manager 1.2 interfaced with a dedicated Macintosh SE Computer.

The HPLC assay was performed via a gradient system beginning with 100% methanol and changing to methanol/acetonitrile/tetrahydrofuran (60/22/18, v/v, all HPLC grade) with 0.01% ammonium acetate over a period of 25 minutes. The flow rate was 1.3 mL/minute, and the column was thermostatically controlled at 30°C. Retinol and alpha-tocopherol were monitored on one channel at 300 nm and carotenoids were monitored simultaneously on a second channel at 450 nm. Peaks were identified by comparison of retention times with those of external standards. This method was not designed to separate all-trans lycopene from its cis-isomers, so lycopene as reported herein refers to total lycopene.

The analytical laboratory was a participating member of the National Bureau of Standards/National Cancer Institute micronutrient measurement proficiency testing program. Certified reference materials from the National Bureau of Standards and plasma pools analyzed over time were used to ensure quality control. Coefficients of variation averaged less than 10% for all micronutrients. Plasma cholesterol was analyzed in the same laboratory in duplicate by enzymatic assays (Sigma diagnostics, method #352, Sigma, St. Louis, MO).

Follow-Up of Cohort

As described previously, in-person visits were made at 3 months post-randomization, 12 months, and then annually thereafter for all active participants (those who did not drop out or experience a study endpoint). At these visits, an annual update questionnaire was administered, which included questions on smoking. In-person contacts were supplemented with phone calls; active participants were contacted every three months by phone (or visit). Inactive participants were contacted by phone every six months, so most deaths were reported to us by next-of-kin. Eleven subjects, all from south Florida, were lost to follow-up. Searches of the Florida Cancer Data System, which links with Florida's Bureau of Vital Statistics, were performed to ascertain vital status for these subjects. Death certificates were obtained for all deceased subjects and coded by a trained nosologist. The primary outcome of interest for this analysis was all-cause mortality, with secondary outcomes being cancer mortality or cardiovascular mortality.

Statistical Analysis

All of the analyses were performed with SAS (SAS Institute, Cary, NC). For initial analyses, the population was stratified by vital status at the end of follow-up and differences between the two groups in demographic characteristics and plasma nutrient concentrations were evaluated using Student's *t* test or Chi-square analyses as appropriate. If subjects had nutrient and cholesterol data available on both of the baseline blood samples (before and after one-month placebo run-in), then the average of the two values was used for the baseline concentration. Survival analyses were generated according to the method of Kaplan and Meier, and the log-rank test was used to compare survival distributions between the two groups as a function of baseline nutrient status. All of the *p* values were two-tailed, with $p < 0.05$ considered statistically significant. Cox proportional hazards models were used to calculate hazard ratios (HR) based on the nutrient concentrations (baseline and time-dependent), allowing for adjustment of other covariates. Time-dependent models included both baseline nutrient concentrations and nutrient concentrations at various times during the follow-up, along with covariates.

The intervention for this study consisted of supplemental beta-carotene, and plasma beta-carotene and alpha-carotene concentrations (but not other micronutrient concentrations) increased as expected in response to supplementation, as we have reported in detail elsewhere [9]. Therefore, the association of plasma concentrations of alpha-carotene and beta-carotene with mortality was only evaluated at baseline, and in time-dependent analyses restricted to those in the placebo group. All other micronutrients were modeled both at baseline and as time-dependent variables in the full study population.

A large number of potentially confounding variables were examined in the multivariate models. Age (continuous), gender, study site (Connecticut *versus* Florida), and treatment arm (beta-carotene *versus* placebo) were selected a priori for inclusion in all models (note that the intervention had no significant impact on mortality; see [7]). Plasma cholesterol (continuous) was also included, since plasma cholesterol concentrations have been previously associated with both plasma carotenoid [10,11] and alpha-tocopherol [12] concentrations and with mortality [13]. Models of baseline nutrient concentrations included

baseline cholesterol concentrations, and models with time-dependent nutrient concentrations included time-dependent cholesterol concentrations for adjustment. Alcohol consumption (lifetime and during the follow-up period) was evaluated as a potential confounder; risk estimates were not materially altered so final models did not include alcohol. Smoking is another important potential confounder, since plasma micronutrient concentrations might be impacted by the smoking status at the time of the blood collection. To adjust for this, we modeled smoking as a time-dependent binary indicator variable (smoking = 1 if smoking at that time point and 0 otherwise; referred to as "time-dependent smoking"). To evaluate interactions between the nutrients and smoking, we created a 3-level categorical variable referred to as "smoking status" as follows: non-smoker (non-smoker at diagnosis and throughout study period); transient smoker (smoked for part but not all of the study period, or smoked at the time of diagnosis but quit post-diagnosis); and smoker (smoked at diagnosis and consistently throughout the study period). Cross-product terms between nutrient quantile and the smoking status variable were evaluated in Cox proportional hazards models for mortality. To interpret possible interactions, we performed stratified Cox proportional hazards models, stratifying on smoking status. We also evaluated interactions between nutrient concentrations and the time-dependent smoking variables. Results were similar to those obtained from the categorical smoking status variable, so we elected to report the results based on the simpler smoking status variable in models involving interactions.

Lifetime smoking history could also be associated with mortality, so we ran additional models to control for both smoking during the longitudinal follow-up period and for total pack-years of smoking. While pack-years of smoking was significantly associated with mortality in multivariate models, it did not confound the relationship between plasma nutrient concentrations and mortality (results for all nutrients nearly identical). Thus, we show final models controlling for smoking during the follow-up period as described above.

RESULTS

A total of 264 persons were randomized in the chemoprevention trial, and at least one blood sample was successfully obtained from 259 of those subjects (98%); therefore, the sample size for this analysis was 259 subjects. Subjects were enrolled beginning in January 1991 and followed through June 1998. The median follow-up in the study population was 51 months, up to a maximum of 90 months.

A total of 61 deaths occurred during the follow-up period. This consisted of 34 deaths due to cancer (most of which were head and neck cancer, esophageal cancer, or lung cancer), 18 deaths due to cardiovascular disease, and 9 deaths due to other causes. Subjects who died during the follow-up period were older at baseline (64.0 *vs*. 61.1 years, $p = 0.01$) and more likely to be smoking throughout the study period (49% *versus* 14%, $p < 0.0001$) as compared to subjects who remained alive during the follow-up period as shown in Table 1. Mean baseline plasma nutrient concentrations were similar in the two groups of subjects (see Table 2), with the exception of plasma lycopene, which was significantly lower at baseline in persons who subsequently died during the follow-up period (270 *versus* 340 (μg/L, $p < 0.01$) Total carotenoids were nonsignificantly lower ($p = 0.09$) in persons who died during the follow-up period.

Table 3 shows the results of the Cox proportional hazards models, estimating the hazard ratio for persons with above *versus* below the median concentrations for each of the micronutrients at baseline. The only hazard ratio that was significantly different from one was that for lycopene. Participants with above *versus* below the median concentration of plasma lycopene at baseline had a 47% reduction in the mortality hazard ratio (HR = 0.53, 95% CI 0.30–0.93). Higher plasma alpha-carotene was also associated with a lower risk of mortality, although this association was of borderline statistical significance (HR = 0.59, 95% CI 0.35–1.01). Higher plasma retinol concentrations were associated with a non-significantly higher risk of mortality (HR = 1.65, 95% CI 0.95–2.87). We also ran models using the time-dependent plasma nutrient concentrations (continuous variables) as predictors of mortality. Only plasma lycopene was significantly inversely associated with mortality in these time-dependent models.

Fig. 1 shows the cumulative probability of survival according to baseline plasma lycopene status (Kaplan Meier plot) in the full population. The log-rank test statistic for lycopene (above *versus* below the median) was 0.006. The Kaplan Meier plot for above *versus* below the median lycopene concentration was also done restricting the analysis to the placebo group only (Fig. 2), confirming the result in the full population. Of note, plasma lycopene concentrations did not vary by treatment arm [9,10].

While the primary outcome of these analyses was all-cause

Table 1. Demographic Characteristics of Study Population, by Final Mortality Status (Carotene Prevention Trial)

Variable	Deceased N = 61	Alive n = 198
Age (baseline)	64.0	61.1[a]
Percent male	84%	81%
Beta-carotene treatment arm	47%	53%
Smoking status during study period[a]		
Non-smoker	30%	50%
Transient smoker[1]	21%	36%
Continuous smoker[2]	49%	14%

[a] $p < 0.05$.

[1] Smoked at diagnosis and quit post-diagnosis, or smoked part but not all of study period.

[2] Smoked at diagnosis and consistently throughout study period.

Table 2. Mean (± SD) Baseline Plasma Nutrient Concentrations, by Final Mortality Status (Carotene Prevention Trial)

Micronutrient Concentration (μg/L)[1]	Deceased n = 61	Alive n = 198
Beta-carotene	180 ± 210	180 ± 200
Alpha-carotene	32 ± 30	35 ± 31
Lycopene	270 ± 180	340 ± 210[a]
Lutein + zeaxanthin	150 ± 80	170 ± 90
Alpha-tocopherol	13,500 ± 4,600	13,300 ± 6,500
Retinol	540 ± 180	500 ± 170
Total carotenoids (nmol/L)	1150 ± 690	1330 ± 720

[1] To convert to SI units of μmol/L apply the following conversion factors: beta-carotene, alpha-carotene, and lycopene = 0.001863; lutein + zeaxanthin = 0.001758; alpha-tocopherol = 0.00232; retinol = 0.00349.
[a] $p < 0.05$.

Table 3. Adjusted Hazard Ratios for Total Mortality, Comparing above *versus* below Median Baseline Plasma Nutrient Concentrations (Carotene Prevention Trial)

Micronutrient (median cutpoint)	Hazard Ratio[1]	95% CI
Beta-carotene (120 μg/L)[2]	1.04	0.60–1.81
Alpha-carotene (26 μg/L)	0.59	0.35–1.01
Lycopene (280 μg/L)	0.53[a]	0.30–0.93
Lutein + zeaxanthin (141 μg/L)	0.80	0.48–1.34
Alpha-tocopherol (12,400 μg/L)	1.42	0.79–2.54
Retinol (482 μg/L)	1.65	0.95–2.87
Total carotenoids (1096 nmol/L)	0.66	0.38–1.15

[1] Adjusted for age, gender, treatment arm, time-dependent smoking, baseline plasma cholesterol, study site.
[2] To convert to SI units of μmol/L apply the following conversion factors: beta-carotene, alpha-carotene, and lycopene = 0.001863; lutein + zeaxanthin = 0.001758; alpha-tocopherol = 0.00232; retinol = 0.00349.
[a] $p < 0.05$.

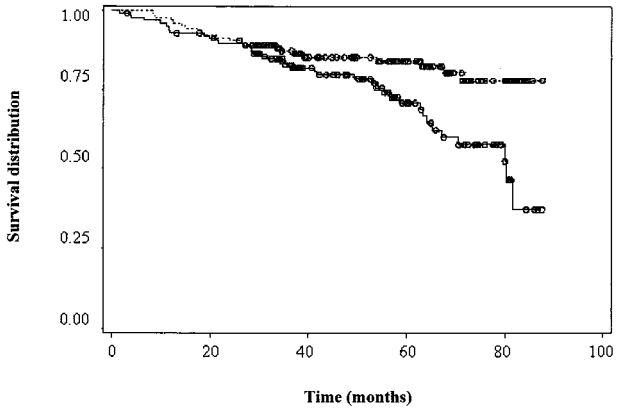

Fig. 1. Kaplan Meier plot showing cumulative probability of survival by plasma lycopene concentration in all study participants at baseline. Dotted line shows above the median plasma lycopene concentration [>280 μg/L (>0.492 μmol/L)] while solid line shows below the median plasma lycopene concentrations. The two curves are significantly different ($p = 0.006$ log-rank test).

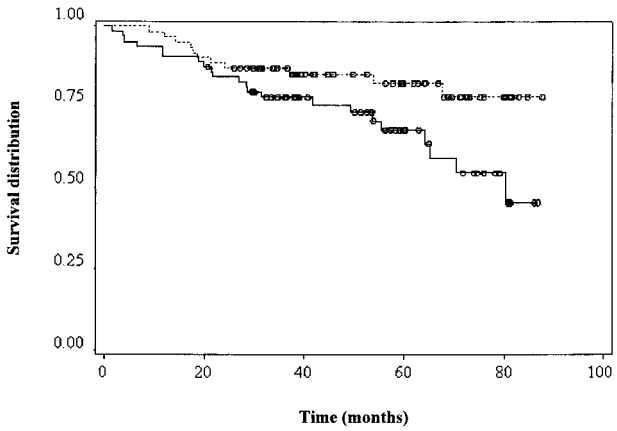

Fig. 2. Kaplan Meier plot showing cumulative probability of survival by plasma lycopene concentration in placebo group only at baseline. Dotted line shows above the median plasma lycopene concentration [>280 μg/L (>0.492 μmol/L)] while solid line shows below the median plasma lycopene concentrations. The two curves are significantly different ($p = 0.037$ log-rank test).

mortality, we examined the association between plasma nutrient concentrations and cause-specific mortality on an exploratory basis (see Table 4). Higher plasma lycopene concentrations were inversely associated with both cancer mortality (HR = 0.63, 95% CI 0.30–1.32) and cardiovascular mortality (HR = 0.42, 95% CI 0.14–1.30), although the confidence intervals were no longer significant given the reduced sample size for these stratified analyses. The only nutrient that was significantly inversely associated with cause-specific mortality was alpha-carotene, which was inversely associated with cardiovascular death (HR 0.24, 95% CI 0.08–0.75). Higher plasma retinol was significantly positively associated with risk of cardiovascular death (HR 5.12, 95% CI 1.54–17.05).

Smoking is associated with both higher mortality and lower plasma concentrations of some carotenoids so we explored possible interactions between micronutrient concentrations and smoking via the use of interaction terms and stratified analyses (stratifying by smoking status). For current smoking status, all of the nutrients examined were found to significantly interact ($p < 0.05$) in the multivariate models. For transient smoking status, only retinol was found to significantly interact ($p < 0.05$) in the multivariate models. To further explore the basis for this effect modification by smoking, we did stratified analyses, comparing nutrient associations in non-smokers, transient smokers, and continuous smokers (Table 5). In non-smokers, mortality hazard ratios for all of the micronutrients examined were less than one, and non-smokers with higher plasma concentrations of alpha-carotene, lycopene and total carotenoids had a significantly lower risk of mortality. In contrast, in continuous smokers, mortality hazard ratios for 5 of 7 micronutrients examined were greater than one, and continuous smokers with higher plasma concentrations of retinol (HR 3.56, 95% CI 1.40–9.09) and alpha-tocopherol (HR 2.47, 95% CI

Table 4. Adjusted Hazard Ratios for Cause-Specific Mortality, Comparing above *versus* below Median Baseline Plasma Nutrient Concentrations (Carotene Prevention Trial)

	Hazard Ratio[1]	95% CI
Cancer Mortality		
Beta-carotene	2.00	0.94–4.28
Alpha-carotene	0.92	0.46–1.86
Lycopene	0.63	0.30–1.32
Lutein + zeaxanthin	0.85	0.43–1.71
Alpha-tocopherol	1.42	0.65–3.10
Retinol	0.95	0.47–1.93
Total carotenoids	0.86	0.41–1.78
CHD Mortality		
Beta-carotene	0.48	0.16–1.41
Alpha-carotene	0.24[a]	0.08–0.75
Lycopene	0.42	0.14–1.30
Lutein + zeaxanthin	0.74	0.28–1.96
Alpha-tocopherol	1.69	0.57–5.01
Retinol	5.12[a]	1.54–17.05
Total carotenoids	0.48	0.16–1.46

[1] Adjusted for age, gender, treatment arm, time-dependent smoking, baseline plasma cholesterol, study site.
[a] $p < 0.05$.

1.02–5.98) were at significantly higher risk of mortality. Transient smokers had nutrient associations with mortality that were intermediate between those for non-smokers and continuous smokers, with the exception of plasma retinol (HR = 3.96, 95% CI 0.89–17.66). In summary, only lycopene was inversely associated with mortality in the full population; other micronutrients (alpha-carotene, total carotenoids) were inversely associated with mortality only in non-smokers. Plasma retinol and alpha-tocopherol were positively associated with mortality in smokers.

DISCUSSION

There has been considerable interest in the relationship between various carotenoids and risk of chronic disease and mortality. Carotenoids are obtained from fruits and vegetables, and plasma carotenoids serve as objective measures of dietary intake. While there are studies that examined the association between plasma beta-carotene and mortality [5], and between individual carotenoids in plasma and risk of cancer or cardiovascular disease, there is a paucity of information on associations between individual carotenoids in plasma and total mortality.

In our full population, persons who had higher plasma concentrations of lycopene but not other carotenoids, vitamin E or retinol were at significantly lower risk of dying during the follow-up period. This association was evident in analyses considering baseline lycopene concentrations, in Cox models using baseline and/or time-dependent lycopene concentrations, and in analyses restricted to the placebo group. The finding was extremely robust, and persisted in essentially all models we ran, with and without adjustment for numerous covariates.

The significant association we observed with regard to lycopene but none of the other carotenoids or nutrients examined in plasma in our study population could well be due to chance. It is notable, however, that preliminary results from one other cohort study substantiate our results. In the Nun study, a cohort study of aging in elderly nuns, only plasma lycopene but not other carotenoids, antioxidants, or nutritional markers in the blood, was associated with a significantly lower risk of all-cause mortality [14]. Our study population consisted primarily of males who had substantial prior exposure to smoking and/or alcohol, so replication of our findings in a population of non-smoking, non-drinking nuns suggests that the finding is potentially generalizable to diverse study populations.

Higher lycopene concentrations in plasma were associated with a lower risk of both cancer mortality and cardiovascular disease mortality, although the confidence intervals around these point estimates did not exclude one. Our results are consistent with results from ecologic studies that indicate that populations with lower lycopene concentrations in plasma have higher cardiovascular death rates [15,16]. The Euramic case-control study used adipose tissue rather than plasma to evaluate the association between micronutrient status and risk of acute

Table 5. Adjusted Hazard Ratios for All-Cause Mortality, Comparing above *versus* below Median Baseline Plasma Nutrient Concentrations, by Smoking Status (Carotene Prevention Trial)

	Hazard Ratio (95% CI)[1]		
	Non-Smokers n = 117	Transient Smokers[2] n = 84	Continuous Smokers[3] n = 58
Beta-carotene	0.49 (0.17–1.43)	0.86 (0.25–2.93)	2.10 (0.87–5.05)
Alpha-carotene	0.25 (0.09–0.73)[a]	0.53 (0.16–1.81)	1.36 (0.63–2.96)
Lycopene	0.08 (0.02–0.36)[a]	0.27 (0.05–1.37)	0.90 (0.39–2.11)
Lutein + zeaxanthin	0.38 (0.14–1.05)	0.62 (0.17–2.23)	1.53 (0.72–3.24)
Alpha-tocopherol	0.71 (0.24–2.13)	1.14 (0.32–4.06)	2.47 (1.02–5.98)[a]
Retinol	0.43 (0.15–1.22)	3.96 (0.89–17.66)	3.56 (1.40–9.09)[a]
Total carotenoids	0.22 (0.07–0.70)[a]	0.56 (0.14–2.28)	0.98 (0.43–2.23)

[a] $p < 0.05$.
[1] Adjusted for age, gender, treatment arm, time-dependent smoking, baseline plasma cholesterol, study site.
[2] Smoked at diagnosis and quit post-diagnosis, or smoked part but not all of study period.
[3] Smoked at diagnosis and consistently throughout study period.

myocardial infarction. Lycopene but not the other micronutrients was independently associated with risk, with an odds ratio of 0.52 comparing the 90th to 10th percentiles (95% CI 0.33–0.82) [17]. Rissanen *et al*. [18,19] examined the association between plasma lycopene and a marker for early atherosclerosis, the intima-media thickness of the common carotid artery wall. Low plasma lycopene concentrations were associated with increased intima-media thickness in middle-aged men from Finland. Low plasma lycopene was also associated with an increased risk of acute coronary events or stroke [20]. Martin *et al*. [21] examined the effects of the major dietary carotenoids on expression of cell surface adhesion molecules and monocyte binding in human aortic endothelial cell cultures, and concluded that lycopene was the most effective in reducing adhesion to monocytes and expression of adhesion molecules on the cell surface. Low plasma lycopene concentrations have also been associated with increased risk of various cancers [22,23].

Lycopene is derived primarily from consumption of tomatoes and tomato products (watermelon and pink grapefruit also contain lycopene) and in our population the top food sources of lycopene were spaghetti, lasagna and other pasta, followed by tomatoes/tomato juice [10]. Plasma lycopene thus serves as a biomarker for the consumption of tomatoes/tomato products. Tomatoes and tomato products contain a variety of different substances that could have health benefits, including folate and vitamin C, other carotenoids such as phytoene and phytofluene, and trace elements, flavonoids, and phytosterols [24]. Thus, it should be noted that lycopene per se may not be causally associated with lower mortality, but instead may serve as a marker for other factors in tomatoes that are responsible for the observed association. Intake of tomatoes was associated with a significant reduction in the risk of mortality among children in Sudan (RR = 0.52, 95% CI 0.30−0.91) [25], although these findings may be of limited relevance with respect to mortality from chronic disease.

Smokers have been shown to have significantly lower plasma concentrations of some carotenoids, like alpha- and beta-carotene [11,26]. Since smoking is known to be associated with both increased mortality and lower concentrations of plasma carotenoids such as alpha-carotene and beta-carotene, then residual confounding by smoking is a concern for studies that examine associations between plasma carotenoids and mortality. However, we see no evidence of residual confounding by smoking with our beta- and alpha-carotene results, in that associations with these nutrients were positive in smokers, not inverse as would be expected with residual confounding. Also, residual confounding by smoking does not explain the inverse association we observed for plasma lycopene, because smokers have not been shown to have significantly lower concentrations of lycopene than nonsmokers in this study nor in others [10,11,26].

Higher body mass index has also been associated with increased risk of mortality, and has been associated with plasma lycopene in some studies. We examined BMI as a possible determinant of plasma lycopene concentrations in plasma in this population and found no relationship [10]; therefore, confounding by BMI cannot explain these findings.

Measurement error (non-differential) is another possible explanation for why one micronutrient, but not others, might be associated with mortality risk. That is, a nutrient that was measured with inadequate precision might fail to be associated with risk of a disease simply due to measurement error. The coefficient of variation (CV) is an index of measurement error; these nutrients were all measured in one laboratory, and CVs were all less than 10%, with lycopene having the highest CV (greatest amount of measurement error) of the nutrients measured. Based on this, one would have expected that it would be more difficult to detect an association between lycopene and mortality than the other nutrients.

Considering carotenoids other than lycopene, current smoking was found to significantly modify their association with mortality, as evidenced by significant interaction terms in the models. In stratified analyses, it became apparent that both total carotenoids and specific carotenoids were inversely associated with mortality only among non-smokers. Higher plasma concentrations of carotenoids other than lycopene were of no apparent benefit to smokers in our study population, nor did they convey any risk. Of note, much higher plasma concentrations of beta-carotene (resulting from high-dose supplementation) have been associated with a reduced risk of lung cancer in never/former smokers, while being associated with increased risk of both lung cancer and cardiovascular disease in smokers [27,28]. Since diet intervention trials aimed at increasing fruit and vegetable intake are known to increase plasma concentrations of several different carotenoids/total carotenoids [29,30], our results suggest that non-smokers might be more likely to benefit from such interventions, at least with regard to mortality. Replication of these smoking and carotenoid interactions in other, more generalizable cohorts, however, is needed.

Continuous smokers who had higher plasma retinol concentrations, in particular, at baseline were at more than threefold higher risk of death during the study period. Unlike the other micronutrients studied, plasma retinol is not well correlated with retinol intake. Plasma retinol concentrations are regulated by the body, and are altered by infection, protein and zinc intake, hepatic function, renal function and other factors [31]. In the Carotene and Retinol Efficacy Trial (CARET), retinol supplementation (along with beta-carotene) to current and former smokers produced increases in cancer mortality, cardiovascular mortality, and total mortality. Retinol supplementation is also known to elevate both cholesterol and triacylglycerol concentrations in plasma [32]. It is not obvious why an apparent adverse effect of higher plasma retinol concentrations was seen in smokers but not non-smokers. Smokers with higher plasma alpha-tocopherol concentrations were also at higher risk

for mortality, although this association was of borderline statistical significance and may be a chance finding. Alpha-tocopherol in the diet comes from vegetable fats (margarines, mayonnaise, salad dressings etc.). Similar associations might not be expected when the source of vitamin E is a supplement; clinical intervention trials of supplemental vitamin E in smokers do not report increased mortality [28].

Participants in this study also completed food frequency questionnaires at baseline in order to estimate intake of carotenoids and other nutrients, as described elsewhere [10]. Dietary intake of lycopene was significantly correlated with plasma lycopene concentrations in our subjects ($r = 0.29$, $p = 0.002$) [10]. However, plasma lycopene concentrations better reflect in vivo exposure as compared to dietary intake, since lycopene bioavailability is strongly influenced by factors such as food processing/preparation, and poorly characterized host factors. Previous analyses on study participants in this cohort [10] showed that lycopene from spaghetti/lasagna/other pasta had 1.7 times the apparent bioavailability as compared to lycopene from tomatoes/tomato juice (not cooked, not generally consumed with added fats), consistent with the results of feeding studie [33]. Thus, it may not be surprising that dietary lycopene intake was not significantly associated with mortality in these subjects (data not shown), since overall lycopene intake is a poorer measure of exposure as compared to plasma lycopene concentrations.

Our results add to a growing body of literature suggesting health benefits associated with increased exposure to lycopene. No subjects were consuming lycopene supplements during this study period, so it should be noted that this association pertains only to lycopene as consumed from food sources (primarily tomatoes/tomato products). Most of the variance in plasma lycopene concentrations, however, is not explained by lycopene intake or known factors [10], so plasma lycopene concentrations may reflect other as yet unrecognized factors associated with mortality. One such factor could be chronic inflammation; there is some evidence to suggest that relationships between serum nutrient concentrations and disease risk might be confounded by inflammation [34].

CONCLUSIONS

This prospective serological study demonstrated that higher concentrations of plasma lycopene are associated with a reduced risk of dying during the follow-up period. Smoking status was found to modify the association between other plasma nutrient concentrations and mortality; alpha-carotene and total carotenoids were inversely associated with mortality in non-smokers only. Lycopene concentrations in plasma reflect consumption of tomatoes/tomato products, as well as host factors, so lycopene per se may not be causally related to the observed protection, but instead serve as a biomarker of subsequent mortality risk. Replication of this finding in other cohorts is necessary to better understand the relationship between lycopene/tomatoes/tomato products and mortality.

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