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Authors	O'Reilly, Éilis J.;Bjornevik, Kjetil;Furtado, Jeremy D.;Patel, Alpa V.;Wang, Ying;Kolonel, Laurence N.;Le Marchand, Loïc;Ascherio, Alberto
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Pre-Diagnostic Plasma Carotenoids, Tocopherols and Retinol Levels, and Risk of Amyotrophic Lateral Sclerosis

Éilis J. O'Reilly, ScD,¹ Kjetil Bjornevik, MD, PhD^{1b},² Jeremy D. Furtado, ScD,²

Alpa V. Patel, PhD,³ Ying Wang, PhD,³ Laurence N. Kolonel, MD, PhD,⁴

Loïc Le Marchand, MD, PhD,⁴ and Alberto Ascherio, MD, DrPH^{5,6}

Objective: Our goal was to examine whether pre-diagnostic plasma carotenoids and tocopherols are associated with amyotrophic lateral sclerosis (ALS).

Methods: A nested case-control study within 4 United States cohorts, where 154 participants with pre-diagnostic blood-draw, were diagnosed during follow-up with amyotrophic lateral sclerosis (ALS). Controls were randomly selected from participants alive on the date a case was diagnosed, matched 2:1 by cohort, sex, age, race/ethnicity, fasting-status, time of blood-draw. Carotenoid and tocopherol levels were quantified by high-performance liquid-chromatography with diode array-detector. ALS incidence or death rate ratios (RR) were estimated using conditional logistic regression adjusting for body mass index, smoking status, physical activity, cholesterol, and urate levels.

Results: The association between beta-carotene and ALS varied by sex (p -for-interaction = 0.007). After matched- and multivariable-adjustment, women with higher cis-, trans- and total beta-carotene had lower incidence of ALS (RR for 1-standard deviation [SD] increase in total beta-carotene: 0.68; 95% CI: 0.48–0.98; p = 0.038), whereas a positive association was seen in men (1-SD increase: RR = 1.44; 95% CI: 1.03–2.01; p = 0.033). However, after further adjustment for other correlated carotenoids, the association in men was attenuated, whereas it remained significant in women. Women with higher beta-cryptoxanthin, but not men, had a lower risk of ALS (1-SD increase: RR = 0.67; 95% CI: 0.48–0.94; p = 0.02; p -for-interaction = 0.12). Alpha-carotene, lutein-zeaxanthin, lycopene, retinol, and tocopherols were not associated with ALS, except for a borderline inverse association of gamma-tocopherol in men (RR = 0.74; 95% CI: 0.54–1.01; p = 0.059).

Interpretation: Higher pre-diagnostic plasma levels of beta-carotene and beta-cryptoxanthin in women and gamma-tocopherol in men were suggestively associated with lower ALS risk. Other carotenoids or tocopherols were not clearly associated with ALS.

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Few modifiable factors have been conclusively linked with amyotrophic lateral sclerosis (ALS) risk,¹ but there are some data to suggest that antioxidant nutrients may play a protective role because they inhibit processes implicated in ALS etiology, such as oxidative stress.²

In earlier articles based on longitudinal studies, we reported a lower risk of ALS in individuals with higher

dietary carotenoid intake³ and long-term vitamin E supplement use.^{4,5} Some case-control studies corroborated the relative protective effects of carotenoids or fruit and vegetable intake; but findings were not always consistent across dietary sources or supplement-use, potentially because of small sample size, recall bias, or recording of current diet (after diagnosis), narrow dietary range specific

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Address correspondence to Dr O'Reilly, School of Public Health, University College Cork, Cork, Ireland. E-mail: eilis.oreilly@ucc.ie

From the ¹School of Public Health, University College Cork, Cork, Ireland; ²Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA, USA; ³American Cancer Society, Atlanta, GA, USA; ⁴Epidemiology Program, University of Hawaii Cancer Center, Honolulu, HI, USA; ⁵Departments of Nutrition and Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, USA; and ⁶Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA

to the studied population, or use of controls not representative of the underlying population.^{6–8} Furthermore, inconsistencies in findings may have reflected considerable heterogeneity in absorption and bioavailability of carotenoids, leading to significant interindividual variation in the relationship between intake and levels in peripheral circulation and/or tissues.⁹

To address these limitations, we conducted a nested case–control study in 4 large prospective cohorts from our earlier analyses, all of which had biospecimens available, to determine whether pre-diagnostic plasma carotenoid or tocopherol levels predicted future risk of ALS.

Methods

Study Populations

Participants in this nested case–control study were drawn from 4 prospective cohorts: Health Professionals Follow-up Study (HPFS), Cancer Prevention Study II Nutrition Cohort (CPSII-N), Multiethnic Cohort Study (MEC), and Nurses' Health Study (NHS). Each of the cohorts has been described previously. In short, the NHS registered approximately 121,700 female nurses 30 to 50 years old in 1976.¹⁰ In 1989 to 1990, blood samples were received from 32,826 of the nurses, 39 of whom subsequently developed ALS by end of follow-up (December 2010).

The HPFS began in 1986 enrolling 51,529 male health professionals 40 to 75 years old.¹¹ Between 1993 and 1995 blood samples were received from 18,018 participants. Subsequently, 26 developed ALS by end of follow-up (December 2010). Participants in both cohorts are surveyed biennially with similar self-report questionnaires on medical history and health-related behavior.

In 1992, a sub-cohort of the CPS-II cohort was established to focus on nutrition and comprises 86,406 men and 97,788 women 50 to 74 years old. Questionnaires on lifestyle and diet were administered at baseline (1992) and biennially since 1997. Between 1998 and 2000, blood was collected from 39,380 members, 58 of whom subsequently died of ALS (follow-up until September 2012).¹² The MEC cohort study comprises 96,810 men and 118,441 women who were 45 to 75 years old during enrollment beginning in 1993.¹³ In total, 67,594 participants provided blood samples, mostly collected between 2001 and 2006, and 31 of them were found to have ALS between provision of blood sample and end of the observation period (December 2012). Participants were surveyed on behavior, lifestyle, and medical history at baseline and at 5-year intervals thereafter.

Standard Protocol Approvals, Registrations, and Patient Consents

All cohorts included were reviewed and approved by their respective institutional review boards. This study was approved by the Human Research Committee at Mass General Brigham Health Care and Harvard T. H. Chan School of Public Health. All participants provided informed consent.

Endpoint

Permission was sought from individuals in NHS and HPFS who self-reported ALS on biennial questionnaires (or from a family member if the participant was deceased at the time of writing) to request medical records from their treating neurologist to verify the ALS diagnosis, to confirm date of diagnosis and onset, and to report on a short questionnaire the certainty of diagnosis (definite, probable, or possible). We included definite and probable ALS, according to the final adjudication of all diagnoses made by a neurologist with experience in ALS who reviewed the medical records and questionnaire. When we did not secure a copy of the medical record, or the neurologist's questionnaire to confirm the diagnosis of ALS, participants were included if ALS was on the death certificate.

In CPSII-N and MEC, the National Death Index was used to locate participants who developed ALS during follow-up. The code 335.2 (motor neuron disease), according to the International Classification of Diseases, Ninth Revision (ICD-9), listed as the underlying or contributing cause of death, indicated ALS in the participant. The diagnosis date was set to 3 years before the date of death for participants in CPSII-N and MEC, based on median survival time in ALS.¹⁴ A validation study of death certificates listing code 335.2 as the cause or contributory cause of death showed that 90% had ALS as the primary diagnosis.¹⁵

Of the 154 ALS cases with pre-diagnostic blood samples, 145 were included in the study. For each person with ALS, we chose at random 2 controls from participants within the same cohort alive on the date of the case diagnosis, with matching on birth year (± 1 year), sex and race/ethnicity, and on fasting status and time of blood draw.

Measurement of Levels of Plasma Carotenoids, Retinol, and Tocopherols

Samples from matched triplets were handled identically and assayed in the same batch with random arrangement of the 3 samples within each triplet to conceal case–control status.

Concentrations of retinol, alpha- and beta-carotene, lutein-zeaxanthin (grouped and provided as lutein

+zeaxanthin because they co-elute on chromatogram), beta-cryptoxanthin, lycopene, and tocopherols in plasma samples were quantified by reverse-phase high-performance liquid chromatography using methods previously described^{16,17} in the Biomarker Research Laboratory of the Department of Nutrition at the Harvard School of Public Health. The minimum detection limits in plasma are (ug/L) 3.86 for lutein (which co-elutes with zeaxanthin), 3.88 for beta-cryptoxanthin, 5.44 for lycopene, 4.24 for alpha-carotene, 4.80 for beta-carotene, 8.05 for retinol, 184.0 for α -tocopherol, 38.5 for delta-tocopherol, and 102.5 for gamma-tocopherol. Each run included 2 replicates of a low and 2 replicates of a high plasma pool sample set and when compared within-run and between-run variation was estimated. Overall, coefficients of variation for between-runs ranged from 2.6 to 9.6% for alpha-carotene, 2.5 to 8.9% for beta-carotene, 2.0 to 14.4% for beta-cryptoxanthin, 2.2 to 10.6% for lycopene, 1.0 to 9.0% for lutein-zeaxanthin, 0.4 to 9.6% for retinol, 1.5 to 6.9% for alpha-tocopherol, and 4.1 to 25% for gamma-tocopherol.

Some of the cohorts have previously assessed the correlation between dietary assessment and plasma carotenoids. Among non-smokers in NHS and HPFS plasma carotenoids adjusted for age, body mass index (BMI), plasma cholesterol and triglycerides, and total energy were compared to dietary measures assessed by the average of 2 food frequency questionnaires administered at least 1 year apart. In NHS, the adjusted correlations ranged from 0.21 for lycopene, 0.27 for beta-carotene and lutein, 0.32 for beta-cryptoxanthin, 0.41 for alpha-tocopherol, to 0.48 for alpha-carotene. In HPFS, the adjusted correlations were similar, ranging from 0.35 for beta-carotene, 0.40 for lutein, 0.43 for beta-cryptoxanthin, 0.47 for alpha-carotene and lycopene, to 0.51 for alpha-tocopherol.^{18,19} In both cohorts, gamma-tocopherol was inversely correlated with total vitamin E intake: $r = -0.51$ in HPFS and -0.42 in NHS.

Assessment of Covariates

In each cohort, self-reported height and weight, smoking history, and levels of physical activity were taken from the questionnaire most recent before or at the time of blood draw. A small number of participants did not have complete covariate data. Specifically, physical activity was unknown for 4 cases (2.8%) and 10 controls (3.4%), whereas smoking status was unknown for 3 cases (2.1%) and 8 controls (2.8%). Plasma urate concentrations were measured as previously described with colorimetric enzyme assay on the Roche P Modular system (Roche Diagnostics-Indianapolis, IN).²⁰

Statistical Analyses

Log-transformation and standardization (using mean and standard deviations [SDs] from cohort- and sex-specific controls) were applied to carotenoid levels to improve normality and to account for potential impact on levels because of the collection, processing, and storage of samples in each cohort.

Controls selected were cohort-participants who were still alive and “at risk” of becoming a case at the time their matched case developed ALS (ie, risk-set sampling), therefore, incidence rate ratios were estimated with conditional logistic regression.²¹ Biomarkers were modeled as continuous standardized variables (where estimands represent an increase in incidence associated with an increase in carotenoid equivalent to 1 SD increase in controls), which will maximize power if associations are linear. Quartiles were used (based on distribution of the cohort- and sex-specific controls) to explore possible nonlinear associations. To test for a linear trend across biomarker quartiles, the median for each quartile was assigned and modeled as a continuous variable. Potential confounders included BMI (continuous), smoking status (categorical: never smoker, past smoker, current smoker), physical activity (categorical: tertiles of metabolic equivalent hours of total physical activity [NHS, HPFS]; tertiles of the amount of moderate and vigorous physical activity [MEC, CPS-IIN]), and levels of plasma urate, cholesterol, and alpha-linolenic levels (continuous). In sensitivity analyses, we further adjusted for alcohol intake (g/day) and for plasma HDL and LDL levels (continuous) in place of total cholesterol. Effect modification of the association between each biomarker and ALS by sex, BMI (<25 kg/m² vs ≥ 25), smoking (current vs past/never), and time from sample collection to disease diagnosis was explored using pre-defined strata (<5 years and ≥ 5 years to ALS diagnosis). All analyses were conducted using SAS, version 9.4 software (SAS Institute).

Results

Among the 157,808 participants across the 4 cohorts who provided a blood sample, 154 were documented as later developing ALS and 145 had available pre-diagnostic samples for this study. The participants with ALS and their matched controls had similar distribution of baseline characteristics, except cases were more likely to smoke, have lower educational attainment and have a slightly lower BMI (Tables 1 and 2).

As expected, plasma carotenoids were moderately-to-weakly correlated with each other, except retinol, which was correlated with none (Table 3). In particular, beta-

TABLE 1. Age-Standardized Characteristics at Baseline of ALS Cases and Controls Nested in 4 US Cohorts

	Cases, men (n = 69)	Controls, men (n = 138)	Cases, women (n = 76)	Controls, women (n = 152)
Age (yr, mean [SD])	65.0 (8.7)	64.8 (8.4)	61.1 (6.5)	61.0 (6.4)
Fasting $\geq$ 8 h (%)	58	55	42	39
BMI (kg/m ² , mean [SD])	26.1 $\pm$ 3.5	26.4 $\pm$ 3.2	24.6 $\pm$ 3.2	25.5 $\pm$ 5.1
Smoking status				
Never smoker (%)	26	40	51	54
Past smoker (%)	61	51	46	36
Current smoker (%)	9	5	3	9
PMH use				
Pre-meno (%)			7	5
Never use (%)			35	51
Ever use (%)			58	43
Race/ethnicity				
White (%)	72	75	88	86
African American (%)	9	7	1	1
Hispanic (%)	4	4	0	0
Asian (%)	9	9	4	4
Other (%)	1	4	5	8
Physical activity				
Low (%)	42	28	29	29
Medium (%)	15	33	43	33
High (%)	42	39	28	37
Education				
More than high school (%)	4	2	0	0
High school (%)	18	14	18	12
Less than high school (%)	78	84	82	88

ALS = amyotrophic lateral sclerosis; BMI = body mass index; SD = standard deviation; US = United States; yr = year.

carotene was moderately correlated with alpha-carotene ($\rho = 0.61$; $p < 0.0001$) and lycopene ($\rho = 0.61$; $p < 0.0001$) in controls, and slightly more weakly ($\rho = 0.39$; $p < 0.0001$ and $\rho = 0.54$; $p < 0.0001$, respectively) in cases. Alpha-tocopherol was positively correlated with delta-tocopherol ($\rho = 0.23$; $p < 0.001$) and inversely associated with gamma-tocopherol ($\rho = -0.42$; $p < 0.0001$). In addition, alpha-tocopherol was weakly correlated with all carotenoids, except beta-cryptoxanthin (Table 3).

Carotenoids

The association between beta-carotene and ALS varied by sex (p for interaction = 0.007). In women, total beta-carotene in pre-diagnostic plasma was associated with lower incidence of ALS in both continuous (1 SD increase in total beta-carotene: relative risk [RR] = 0.68; [95% CI: 0.48–0.98]) and categorical analyses (Q4 vs Q1: RR = 0.35; [95% CI: 0.13–0.93]) adjusted for matching factors, total cholesterol, BMI, smoking, physical activity, cholesterol, and urate levels. Further adjustment for alpha-

TABLE 2. Age-Standardized Levels of Carotenoids and Tocopherols in ALS Cases and Controls Nested in 4 US Cohorts

	Cases, men (n = 69)	Controls, men (n = 138)	Cases, women (n = 76)	Controls, women (n = 152)
Urate (mg/dL) ^a	5.67 ± 1.3	5.98 ± 1.3	4.64 ± 1.23	4.60 ± 1.11
Alpha-linolenic (ug/L)	0.56 ± 0.20	0.65 ± 0.26	0.56 ± 0.24	0.57 ± 0.27
Alpha-carotene (ug/L)	77.3 ± 54.8	71.1 ± 46.9	83.9 ± 98.6	78.6 ± 63.5
Beta-carotene (ug/L)	386 ± 358	286 ± 250	294 ± 221	359 ± 359
Beta-cryptoxanthin (ug/L)	137 ± 108	131 ± 110	116 ± 69	132 ± 96
Lutein/zeaxanthin (ug/L)	175 ± 97	170 ± 78	171 ± 79	173 ± 77
Lycopene (ug/L)	427 ± 283	359 ± 165	402 ± 155	400 ± 272
Retinol (ug/L)	575 ± 123	590 ± 116	562 ± 118	537 ± 113
Alpha-tocopherol (ug/L)	13.549 ± 6.102	14.129 ± 6.102	14.319 ± 5.644	13.894 ± 5.777
Delta-tocopherol (ug/L)	306 ± 227	310 ± 210	294 ± 174	288 ± 180
Gamma-tocopherol (ug/L)	1.297 ± 950	1.481 ± 862	1.653 ± 1.253	1.538 ± 1.005

ALS = amyotrophic lateral sclerosis; SD = standard deviation; US = United States.

^aValues are means ± SD.

linolenic acid did not change the association. In men, there appeared to be a positive association with total beta carotene (1 SD increase: RR = 1.44; [95% CI: 1.03–2.01]) (Table 4). However, the positive association was evident among men in MEC (1 SD increase: RR = 2.20; [95% CI: 1.03–4.68], $p = 0.04$), but not among HPFS and CPS men (RR = 1.25; [95% CI: 0.85–1.84]).

Because beta-carotene was moderately correlated with lycopene and alpha-carotene, we repeated the multivariable analyses including these as continuous variables separately and together. In women, the associations were deattenuated (adjusting for both: 1 SD increase: RR = 0.58; [95% CI: 0.38–0.89]; and Q4 vs Q1: RR = 0.23; [95% CI: 0.08–0.70]), while in men the positive association was no longer significant when adjusted for alpha-carotene, total lycopene, or both (1 SD increase: RR = 1.39; [95% CI: 0.86–2.24]). Similar associations were observed when cis- and trans-beta-carotene were considered separately.

In analyses stratified on time from blood draw to ALS diagnosis, the beta-carotene—ALS associations in women were observed when restricted to an interval between blood draw and diagnosis that was 5 years or under (1 SD increase: RR = 0.55; [95% CI: 0.31–0.96]; $p = 0.0345$ for <5 years compared to 0.79 [95% CI: 0.51–1.22] for ≥5 years; p for interaction = 0.29).

In multivariable analyses of beta-cryptoxanthin, women with higher plasma levels had lower risk of ALS with an RR = 0.67; (95% CI: 0.48–0.94) for a 1 SD increase and an RR = 0.25 comparing Q4 versus Q1 (95% CI: 0.09–0.69). However, there was no association in men (with p for sex interaction = 0.12). In analyses that mutually adjusted for beta-carotene and beta-cryptoxanthin, both were slightly attenuated.

Plasma levels of lutein-zeaxanthin, cis-, trans- and total lycopene, alpha-carotene, and retinol were not associated with risk of ALS in analyses including all participants (Table 5), in men and women separately (data not shown), or stratified by time from blood draw, smoking, or BMI.

Tocopherols

In men, there was an association between gamma-tocopherol and ALS in matched continuous analyses (1 SD increase: RR = 0.72; [95% CI: 0.54–0.96]; $p = 0.025$). Findings were materially the same in multivariable models (RR = 0.74; [95% CI: 0.54–1.01] $p = 0.059$). Because gamma-tocopherol was moderately correlated with beta-carotene and alpha-tocopherol, we repeated the multivariable analyses including these as continuous variables: the association was attenuated when adjusted for beta-carotene (RR = 0.81; [95% CI: 0.59–

TABLE 3. Correlation between Plasma Carotenoids, Retinol, and Tocopherols in ALS Cases and Controls Nested in 4 US Cohorts

	Alpha-carotene	Beta-carotene	Beta-cryptoxanthin	Lutein-zeaxanthin	Lycopene	Retinol	Alpha-tocopherol	Delta-tocopherol	Gamma-tocopherol
Alpha-carotene	—	0.391 (<i>p</i> < 0.001)	0.475 (<i>p</i> < 0.001)	0.145 (<i>p</i> = 0.08)	0.237 (<i>p</i> = 0.004)	−0.087 (<i>p</i> = 0.30)	−0.037 (<i>p</i> = 0.66)	0.115 (<i>p</i> = 0.17)	−0.042 (<i>p</i> = 0.61)
Beta-carotene	0.611 (<i>p</i> < 0.001)	—	0.397 (<i>p</i> < 0.001)	0.226 (<i>p</i> = 0.006)	0.545 (<i>p</i> < 0.001)	−0.0654 (<i>p</i> = 0.44)	0.083 (<i>p</i> = 0.32)	0.203 (<i>p</i> = 0.015)	−0.271 (<i>p</i> = 0.001)
Beta-cryptoxanthin	0.365 (<i>p</i> < 0.001)	0.387 (<i>p</i> < 0.001)	—	0.332 (<i>p</i> < 0.001)	0.331 (<i>p</i> < 0.001)	−0.071 (<i>p</i> = 0.40)	0.067 (<i>p</i> = 0.42)	0.321 (<i>p</i> < 0.001)	−0.253 (<i>p</i> = 0.002)
Lutein-zeaxanthin	0.316 (<i>p</i> < 0.001)	0.215 (<i>p</i> = 0.002)	0.240 (<i>p</i> < 0.001)	—	0.172 (<i>p</i> = 0.04)	0.006 (<i>p</i> = 0.95)	0.019 (<i>p</i> = 0.82)	0.196 (<i>p</i> = 0.018)	−0.060 (<i>p</i> = 0.47)
Lycopene	0.403 (<i>p</i> < 0.001)	0.613 (<i>p</i> < 0.001)	0.318 (<i>p</i> < 0.001)	0.136 (<i>p</i> = 0.021)	—	−0.031 (<i>p</i> = 0.71)	0.016 (<i>p</i> = 0.84)	0.177 (<i>p</i> = 0.033)	−0.028 (<i>p</i> = 0.74)
Retinol	0.087 (<i>p</i> = 0.14)	0.028 (<i>p</i> = 0.63)	−0.004 (<i>p</i> = 0.95)	0.079 (<i>p</i> = 0.18)	0.023 (<i>p</i> = 0.69)	—	0.157 (<i>p</i> = 0.06)	−0.067 (<i>p</i> = 0.42)	0.018 (<i>p</i> = 0.83)
Alpha-tocopherol	0.119 (<i>p</i> = 0.043)	0.289 (<i>p</i> < 0.001)	0.058 (<i>p</i> = 0.32)	0.112 (<i>p</i> = 0.06)	0.147 (<i>p</i> = 0.01)	0.197 (<i>p</i> = 0.008)	—	0.133 (<i>p</i> = 0.11)	−0.311 (<i>p</i> < 0.001)
Delta-tocopherol	0.074 (<i>p</i> = 0.21)	0.071 (<i>p</i> = 0.23)	0.264 (<i>p</i> < 0.001)	0.114 (<i>p</i> = 0.05)	0.021 (<i>p</i> = 0.73)	−0.044 (<i>p</i> = 0.46)	0.230 (<i>p</i> < 0.001)	—	−0.04 (<i>p</i> = 0.60)
Gamma-tocopherol	−0.146 (<i>p</i> = 0.013)	−0.183 (<i>p</i> = 0.002)	−0.187 (<i>p</i> = 0.001)	−0.131 (<i>p</i> = 0.026)	0.009 (<i>p</i> = 0.880)	−0.064 (<i>p</i> = 0.28)	−0.416 (<i>p</i> < 0.0001)	0.027 (<i>p</i> = 0.64)	—

Control correlations are shaded lower left and cases correlations in upper right of the table.

ALS = amyotrophic lateral sclerosis; US = United States.

1.12]) but not alpha-tocopherol (RR = 0.64; [95% CI: 0.44–0.93]). In women, the risk of ALS did not vary with gamma-tocopherol levels (*p* for interaction by sex = 0.38). Analyses including all participants are shown in Table 6.

Plasma levels of alpha- or delta-tocopherol were not associated with risk of ALS in analyses including all participants (Tables 5 and 6), in men and women separately (data not shown), or stratified by time from blood draw, smoking, or BMI.

Findings from sensitivity analyses that replaced circulating total cholesterol with levels of HDL and LDL or included alcohol intake (g/day) were materially unchanged.

Discussion

We have found that pre-diagnostic beta-carotene and beta-cryptoxanthin levels were associated with lower risk of ALS in women, whereas in men gamma-tocopherol levels were suggestively associated with lower risk of ALS. On the other hand, there appeared to be higher pre-diagnostic beta-carotene levels in plasma of men who developed ALS.

We previously reported associations between dietary carotenoid intake and ALS risk in these cohorts among

1,100,910 participants with 1,153 ALS deaths during follow-up.³ Similar to the findings reported here for plasma levels, dietary alpha-carotene, lycopene, beta-cryptoxanthin were not associated with ALS.³ Dietary beta-carotene was modestly associated with lower ALS risk in both men and women: a 2,500μg increase was associated with an 8% decrease in risk. However, beta-carotene supplement users did not have a lower risk of ALS (pooled multivariate RR = 0.88; [95% CI: 0.65–1.20]). In a randomized controlled trial of antioxidant supplements (including 20mg/day beta-carotene) designed to assess associations with lung cancer,²² 50 of the 29,127 smoking men enrolled in the study were recorded as having ALS based on national hospital discharge registry data or death certificates during 16 to 19 years of follow-up. Neither pre-randomization beta-carotene serum levels nor randomization to short-term, high-dose supplementation was associated with later ALS diagnosis.²³ Men who had pre-randomization alpha-tocopherol serum above the median (≥11.6mg/l) had lower ALS risk, but high-dose supplementation did not change ALS risk.²³

In our dietary carotenoid study,³ lutein was associated with lower risk of ALS: the pooled multivariable RR comparing individuals in the top quintile with those in the bottom was reported as 0.79 (95% CI: 0.64–0.96; *p* =

TABLE 4. Multivariable-Adjusted Relative Risks of ALS According to Quartiles of Beta-Carotene

	Q1	Q2	Q3	Q4	1-SD increase
Men					
No. of controls	34	35	35	34	
No. of cases	17	19	9	24	
Age, matching		1.12 (0.47–2.65)	0.47 (0.17–1.26)	1.50 (0.66–3.39)	1.35 (1.02–1.80)
RR (95% CI) ^a				<i>p</i> -trend = 0.47	<i>p</i> = 0.037
Multivariable		0.90 (0.34–2.36)	0.47 (0.16–1.41)	1.53 (0.60–3.89)	1.44 (1.03–2.01)
RR (95% CI) ^b				<i>p</i> -trend = 0.40	<i>p</i> = 0.033
Women					
No. of controls	37	39	39	37	
No. of cases	25	13	22	16	
Age, matching		0.47 (0.20–1.09)	0.83 (0.40–1.70)	0.63 (0.29–1.37)	0.83 (0.62–1.11)
RR (95% CI) ^a				<i>p</i> -trend = 0.43	<i>p</i> = 0.21
Multivariable		0.27 (0.10–0.73)	0.56 (0.24–1.31)	0.35 (0.13–0.93)	0.68 (0.48–0.98)
RR (95% CI) ^b				<i>p</i> -trend = 0.12	<i>p</i> = 0.038
<i>p</i> -interaction by sex ^b = 0.007					

P-trend calculated using median value for each quartile.

ALS = amyotrophic lateral sclerosis; CI = confidence interval; RR = relative risk; SD = standard deviation.

^aAdjusted for age (years) and matching factors.

^bAdding body mass index, smoking status (never, past, current), physical activity (low, medium, high), plasma cholesterol and urate levels.

for-trend = 0.01), in contrast to the current study where there was no association with plasma lutein and ALS. However, plasma carotenoid levels may not reflect accurately CNS levels. Lutein, for example, is only sourced from diet and appears to concentrate in the brain and retina despite not being the main dietary carotenoid. This active concentration is evidence of specific functionality of carotenoids in the CNS. In a small study, the correlation between serum and brain lutein was modest at 0.28 ($p < 0.0001$).²⁴ Therefore, it is plausible that long-term dietary lutein and zeaxanthin intake, but not plasma levels, could better predict ALS.

In our study of dietary and supplemental vitamin E in the current cohorts, the risk of ALS was similar in users and non-users of vitamin E supplements, but risk was lower with longer duration of use.⁵ This is consistent with the current study where plasma alpha-tocopherol, measured at 1 point in time, was not associated with ALS. We also reported that the multivariable-adjusted RR was 0.79 (95% CI: 0.61–1.03; $p = 0.08$) comparing participants in the highest dietary vitamin E quartile with participants

in the lowest. An inverse dose-response was seen in women but not in men.⁵

Gamma-tocopherol is the isoform of vitamin E most abundant in United States (US) diets, and plasma gamma-levels are inversely correlated with plasma alpha-tocopherol. In the current analysis, among men only, plasma gamma-tocopherol was associated with lower risk of ALS.

A number of case-control studies considered diet and nutrient intake in relation to risk of ALS. Results were inconsistent possibly because of biases inherent in case-control studies of long-term exposures. For example, among 72 people with ALS in a Korean study intake of fruits and beta-carotene, but not vegetables, were lower compared to healthy controls,⁷ while a study in Japan of 153 ALS patients found lower intake of fruit and vegetables, but not beta-carotene.⁶ Similarly, a population case-control study in Italy comprising 212 ALS patients found lower intake of raw vegetables and citrus fruit, but not beta-carotene, compared to controls.

A particular strength of this study is the nested case-control design, which makes artefactual differences

TABLE 5. Multivariable-Adjusted Relative Risks of ALS According to Quartiles of Plasma Carotenoids Men and Women Combined

	Q1	Q2	Q3	Q4	1-SD increase
Alpha-carotene					
No. controls /cases	71/37	74/37	74/32	71/39	
Age, matching		0.97 (0.56–1.68)	0.82 (0.46–1.45)	1.08 (0.61–1.89)	1.04 (0.85–1.27)
RR (95% CI) ^a				<i>p</i> -trend = 0.91	
Multivariable		0.94 (0.53–1.67)	0.82 (0.44–1.53)	1.02 (0.54–1.94)	1.03 (0.82–1.29)
RR (95% CI) ^b				<i>p</i> -trend = 0.96	
Beta-cryptoxanthin					
No. controls/cases	71/42	74/24	74/44	71/35	
Age, matching		0.57 (0.32–1.02)	1.02 (0.61–1.70)	0.87 (0.50–1.50)	0.93 (0.77–1.12)
RR (95% CI) ^a				<i>p</i> -trend = 0.88	
Multivariable		0.49 (0.26–0.91)	0.90 (0.52–1.55)	0.75 (0.41–1.37)	0.88 (0.71–1.09)
RR (95% CI) ^b				<i>p</i> -trend = 0.64	
Lycopene					
No. controls/cases	71/31	74/36	74/38	71/40	
Age, matching		1.10 (0.63–1.93)	1.18 (0.66–2.13)	1.32 (0.73–2.38)	1.13 (0.92–1.38)
RR (95% CI) ^a				<i>p</i> -trend = 0.47	
Multivariable		1.07 (0.60–1.92)	1.12 (0.61–2.05)	1.31 (0.69–2.48)	1.14 (0.92–1.42)
RR (95% CI) ^b				<i>p</i> -trend = 0.56	
Lutein-zeaxanthine					
No. controls/cases	71/34	74/37	74/37	71/37	
Age, matching		1.05 (0.59–1.86)	1.05 (0.59–1.86)	1.09 (0.61–1.97)	1.01 (0.83–1.23)
RR (95% CI) ^a				<i>p</i> -trend = 0.99	
Multivariable		0.93 (0.51–1.72)	0.92 (0.50–1.69)	0.95 (0.50–1.80)	0.97 (0.78–1.20)
RR (95% CI) ^b				<i>p</i> -trend = 0.62	
Retinol					
No. controls/cases	71/28	74/51	74/36	71/30	
Age, matching		1.84 (1.01–3.37)	1.30 (0.70–2.40)	1.08 (0.55–2.10)	1.07 (0.86–1.32)
RR (95% CI) ^a				<i>p</i> -trend = 0.84	
Multivariable		1.82 (0.96–3.44)	1.35 (0.70–2.59)	1.05 (0.51–2.16)	1.08 (0.86–1.37)
RR (95% CI) ^b				<i>p</i> -trend = 0.86	

P-trend calculated using median value for each quartile.

ALS = amyotrophic lateral sclerosis; CI = confidence interval; RR = relative risk; SD = standard deviation.

^aAdjusted for age (years) and matching factors.

^bAdding body mass index, smoking status (never, past, current), physical activity (low, medium, high), plasma cholesterol and urate levels.

TABLE 6. Multivariable-adjusted Relative Risks of ALS According to Quartiles of Plasma Tocopherols Men and Women Combined

	Q1	Q2	Q3	Q4	1-SD increase
Alpha-tocopherol					
No. controls/cases	71/34	74/33	74/48	71/30	
Age, matching		0.94 (0.54–1.64)	1.38 (0.79–2.42)	0.92 (0.51–1.67)	1.04 (0.84–1.29)
RR (95% CI) ^a				<i>p</i> -trend = 0.80	
Multivariable		0.90 (0.50–1.62)	1.25 (0.70–2.25)	0.82 (0.43–1.54)	1.00 (0.79–1.26)
RR (95% CI) ^b				<i>p</i> -trend = 0.50	
Delta-tocopherol					
No. controls/cases	100/52	45/23	74/37	71/33	
Age, matching		0.97 (0.51–1.84)	0.94 (0.53–1.67)	0.88 (0.50–1.55)	1.01 (0.83–1.24)
RR (95% CI) ^a				<i>p</i> -trend = 0.82	
Multivariable		1.00 (0.52–1.94)	0.94 (0.52–1.70)	0.86 (0.48, 1.56)	1.03 (0.83–1.26)
RR (95% CI) ^b				<i>p</i> -trend = 0.81	
Gamma-tocopherol^c					
No. controls/cases	71/43	74/46	74/24	71/32	
Age, matching		1.04 (0.61–1.77)	0.49 (0.26–0.93)	0.75 (0.42–1.32)	0.88 (0.73–1.07)
RR (95% CI) ^a				<i>p</i> -trend = 0.09	
Multivariable		1.18 (0.68–2.06)	0.51 (0.27–0.98)	0.82 (0.45–1.48)	0.92 (0.75–1.12)
RR (95% CI) ^b				<i>p</i> -trend = 0.17	

P-trend calculated using median value for each quartile.

ALS = amyotrophic lateral sclerosis; CI = confidence interval; RR = relative risk; SD = standard deviation.

^aAdjusted for age (years) and matching factors.

^bAdding body mass index, smoking status (never, past, current), physical activity (low, medium, high), plasma cholesterol and urate levels.

^cIn men, RR for 1 SD increase in gamma tocopherol was 0.74 (95% CI: 0.54, 1.01) *p* = 0.059; when adjusted for beta-carotene RR = 0.81 (95% CI: 0.59, 1.12) and for alpha-tocopherol RR = 0.64 (95% CI: 0.44, 0.93). In women, the risk of ALS did not vary with gamma-tocopherol levels (*p* interaction by sex = 0.038).

unlikely because cases and controls are from the same source population with the same methods of blood collection and processing. Within each cohort, the blood samples from cases and controls were assayed as a triplet, blind to case-status, and in random order. Although 4 large cohorts were included, the number of participants who developed ALS after blood draw was 154, and of these 145 had usable pre-diagnostic blood samples, which may have limited the statistical power to detect small differences across biomarkers and made estimates vulnerable to random variation. Each of the 4 cohorts are based in the United States, therefore, it is possible that these findings are not generalizable to populations with different dietary/supplement-use patterns or underlying risk profiles. As in any observational study, residual or unmeasured confounding cannot be ruled out.

In summary, using plasma drawn from healthy individuals in the years before ALS diagnosis, we found that women with higher levels of beta-carotene and beta-cryptoxanthin, and men with higher gamma-tocopherol may be at lower risk of ALS. Other plasma carotenoids, retinol, and tocopherols were not associated with the risk of ALS.

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Author Contributions

E.O.R., K.B., and A.A. contributed to the conception and design of the study. E.O.R., K.B., A.A., J.F., A.V.P., Y.Y., L.N.K., and L.L.M. contributed to the acquisition and analysis of data. E.O.R., K.B., J.F., and A.A. contributed to drafting the text and tables/figures.

Potential Conflicts of Interest

Nothing to report.

Data Availability

The datasets analyzed in the current study are not publicly available because of restricted access, but further information about the datasets is available from the corresponding author on request.

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