Prediagnostic circulating carotenoid levels and the risk of non-Hodgkin lymphoma: the Multiethnic Cohort

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This analysis examined the association of non-Hodgkin lymphoma (NHL) with prediagnostic carotenoid levels, a marker for a diet rich in fruits and vegetables. We conducted a nested case-control study within the Multiethnic Cohort with 271 NHL cases and 538 controls matched on sex, ethnicity, location (Hawaii or Los Angeles), birth year, date and time of blood draw, and hours fasting before blood draw. Serum carotenoid levels were obtained by high-pressure liquid chromatography with photodiode array detection. Conditional logistic regression was used to calculate odds ratios (ORs) according to tertiles of serum carotenoids and trend tests using continuous variables. Higher total serum carotenoids ($OR_{T3\ vs\ T1}$ = 0.66 [0.46-0.96]; P_{trend} = .02), lycopene (OR = 0.54 [0.38-0.78]; P_{trend} = .003), and α-cryptoxanthin (OR = 0.53 [0.36-0.78]; P_{trend} = .003) were associated with a lower risk of NHL. For retinol (OR = 0.90 [0.61-1.33]; P_{trend} = .04), a statistically significant inverse linear trend was detected. Risk estimates remained unchanged with adjustment for NHL risk factors and were similar in analyses stratified by sex and ethnicity; heterogeneity with NHL subtype was detected only for β-carotene. Other carotenoids, including α-carotene, β-carotene, lutein, β-cryptoxanthin, and zeaxanthin, showed no association with risk. These data provide support for a protective role of carotenoid-rich fruits and vegetables in the etiology of NHL. (*Blood*. 2012;119(24): 5817-5823)

Introduction

Non-Hodgkin lymphoma (NHL) is the fifth most common cancer in the United States,[1] consisting of a heterogeneous group of neoplasms that arise from the malignant transformation of B, T, and natural killer cells of the immune system.[2,3] Immune dysfunction is thought to be the underlying basis of lymphoma development, but well-characterized immunosuppressive states (HIV infection and organ transplants) only partially explain the rising NHL risk over the past decades.[4] Based on the hypothesis that lymphoid tissues are vulnerable to nutrient imbalances affecting metabolic pathways and functions necessary to maintain immune functions,[5,6] the association between dietary factors and the risk of NHL has been examined.[7,8] Reports from retrospective case-control studies[9-18] and prospective cohorts[19-21] have found inverse associations between fruit intake, vegetable intake, or both and NHL, although null findings also have been reported.[22-26]

Dietary carotenoids are a class of fat-soluble isoprenoid compounds obtained primarily through the consumption of yellow-orange fruits and vegetables, green leafy vegetables, and tomatoes; they can function as antioxidants and some function as vitamin A precursors.[27] In a Western diet, carrots are the major source of carotenes, spinach and other green leafy vegetables of lutein and zeaxanthin, tomatoes of lycopene, and citrus of cryptoxanthins.[27,28] Carotenoids reduce the production of reactive oxygen species (ROS) in cell membranes and therefore may limit DNA damage and the potential for the malignant transformation of immune cells through the inhibition of radical species.[29-31] Serum levels of carotenoids are considered valid biologic markers for fruit and vegetable intake, and, to date, they have not been investigated in relation to NHL risk.[27] For the present nested case-control study, we examined whether prediagnostic serum levels of total and specific carotenoids were associated with incident diagnoses of NHL among individuals participating in the biospecimen subcohort of the Multiethnic Cohort (MEC) Study.

Methods

Study design and population

The MEC is a longitudinal study designed to investigate the associations of dietary, life style, and genetic factors with the incidence of cancer and was previously described in detail.[32] In brief, the cohort was established from 1993 to 1996 by mailing a self-administered, 26-page questionnaire to men and women ages 45 to 75 years residing in Hawaii and California. To obtain a multiethnic sample of blacks, Japanese Americans, Latinos, Native Hawaiians, and whites, the primary sampling frame included drivers' license records in both states, supplemented with voter registration lists in Hawaii and Medicare files in California. More than 215 000 men and women voluntarily completed the baseline questionnaire, indicating their consent to participate in the study. The questionnaire included queries on demographic characteristics, anthropometric measures, medical history, family history of cancer, smoking history, reproductive and menstrual history for women, cancer screening, occupational history, and physical activity, as well as a food frequency questionnaire (FFQ).[33]

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The prospective MEC biospecimen subcohort was established from 2001 to 2006 by asking the ~ 136 000 surviving and geographically accessible cohort members to provide specimens of blood and urine.[34] Blood samples were drawn and processed within 4 hours of collection by centrifugation. Then, the components were aliquoted by automation into 0.5-mL cryotubes and stored in the vapor phase of liquid nitrogen (−186°C). For ~ 95% of the participants contributing to the biorepository, fasting blood samples (≥ 8 hours) were obtained. In total, 67 594 cohort members (49.7% of eligible) contributed to the biorepository from which the cases and controls were selected for the present study. comparing individuals who provided specimens with those who did not, no substantial difference by several demographic characteristics and cancer risk factors, including family history of cancer, body mass index (BMI), fat and vegetable intake, and physical activity, was detected; this suggests that the biospecimen repository participants are broadly representative of all cohort members. The study protocol was approved by the Institutional Review Boards of the University of Hawaii and the University of Southern California.

Case ascertainment and control selection

Cases for this analysis were participants from the 5 main ethnic groups, black, white, Japanese American, Native Hawaiian, and Latino, who contributed a prediagnostic fasting blood sample to the MEC biorepository and received a diagnosis of NHL before February 28, 2010. Regular linkages of the cohort to the Surveillance, Epidemiology, and End Results cancer registries for Hawaii and California were conducted to identify incident NHL cases diagnosed over the follow-up period. In an investigation within the entire cohort, the average out-migration rate for members was only 3.7% after 7 years and 7.6% after 15 years of follow-up, with California as the primary destination for Hawaii participants. Therefore, case ascertainment is thought to be close to complete. Diagnoses of NHL were classified according to the International Classification of Diseases for Oncology, Third Edition and aggregated into common NHL subtypes in accordance with the proposed hierarchical classification of lymphoid neoplasms for epidemiologic research.[3]

Two controls per case were randomly selected from the eligible pool of participants who were alive and free of a diagnosis of NHL at the age of the case's diagnosis and who matched the case on year of birth (± 1 year), location (Hawaii or California), ethnicity, date of blood draw (± 1 year), time of blood draw (± 2 hours), and hours fasting before blood draw (< 6, 6 to < 8, 8 to < 10, ≥ 10). In total, 275 cases were identified over the follow-up period and matched to 549 controls. Insufficient sample for analysis led to missing values for 4 cases and 3 controls. After their exclusion, as well as that of any sets with no cases or controls, 271 cases and 538 controls were available for the present analysis.

Laboratory assays

All assays were performed at the University of Hawaii Cancer Center in the laboratory of Dr Adrian Franke. Frozen serum samples were retrieved from the MEC biorepository in matched case-control sets, thawed, and analyzed together within batches. The laboratory personnel were blinded to the case-control status of the samples. High-pressure liquid chromatography with photodiode array detection, according to our previous protocol,[35-37] was used to obtain carotenoid concentrations. This assay is continuously validated by quality assurance programs organized by the US National Institute of Standards and Technology. In addition, replicate samples of pooled serum were included in each analysis batch for quality control. Based on 27 duplicate and 9 triplet samples, the average intrabatch coefficient of variation (CV) was less than 4.1% and the average interbatch CV was less than 9.1% for all analytes. Serum triglycerides and cholesterol were measured using an automated chemical analyzer (Cobas, MiraPlus, Roche Diagnostics).

Statistical analysis

Data analyses were performed using SAS Version 9.2 statistical software. All tests were 2-sided; P less than .05 was considered statistically significant. Characteristics of cases and controls were compared using χ^2 tests for categorical variables, t tests for normally distributed continuous variables, and the nonparametric Wilcoxon rank-sum test for non-normally distributed variables. Spearman correlation coefficients were computed between serum carotenoids and intake estimates derived from the baseline FFQ.[33]

Conditional logistic regression with matched sets as strata was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for NHL. Tertiles of serum carotenoids were examined in all models using cut-points based on the exposure distribution of the total study population to optimize the distribution of cases and controls within strata. The lowest exposure group served as the referent in all models. Linear trends were assessed by Wald tests of the parameter estimates for the natural log–transformed continuous variables. Years of education; alcohol consumption; pack-years of cigarette smoking; BMI, physical activity; history of blood transfusion; history of asthma; use of antihistamine, aspirin, and other nonsteroidal anti-inflammatory drugs; and serum triglycerides, and high-density, low-density, and total cholesterol were examined as potential confounders but were not included in the final models because they were not found alone, or in combination, to change the risk estimates by more than 10%.[38] Sensitivity analyses were performed by excluding participants diagnosed with NHL within 1 year after the date of blood draw.

Associations between serum carotenoids and the risk of NHL also were examined in analyses stratified by sex, ethnicity, and common NHL subtypes.[3] Common subtypes included diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), chronic lymphocytic leukemia or small lymphocytic lymphoma (CLL/SLL), T-cell lymphoma (all types), and all other NHL subtypes. No separate ORs were calculated for the latter 2 categories because of the limited number of T-cell cases (n = 15) and the subtype heterogeneity for the cases classified as "other." Heterogeneity in the risk estimates by sex and ethnicity was assessed using a Wald test of the cross-product terms and across the 3 NHL subtypes using a Wald test of the parameter estimates obtained from unconditional polytomous logistic regression adjusting for the matching factors and comparing case subgroups to all controls.

Results

The mean age at blood draw of both cases and controls was 70 years (Table 1), and the median time from the date of blood draw to the date of diagnosis was 2.7 years. Whites comprised the largest ethnic group (28%), followed by Japanese Americans (26%), Latinos (23%), blacks (17%), and Native Hawaiians (6%). No differences in the levels of educational attainment, BMI, pack-years of cigarette smoking, physical activity, alcohol consumption, or positive history of blood transfusion were detected between cases and controls ($P > .05$). The serum concentrations of total carotenoids, lycopene, dihydrolycopene, and α-cryptoxanthin were significantly lower for cases than for controls ($P < .05$); the differences in levels by case-control status did not reach statistical significance for β-carotene and *trans*-lutein ($P = .08$ and .09, respectively) or for α-carotene, β-cryptoxanthin, *trans*-zeaxanthin, and retinol. The majority of NHL cases were of B-cell origin (> 94%) and classified as diffuse DLBCL (29%), CLL/SLL (19%), FL (18%), or other NHL B-cell subtypes (29%), with a small number of T-cell lymphomas (6%) diagnosed over the follow-up period.

Higher serum levels of total carotenoids ($OR_{T3\ vs\ T1} = 0.66$ [0.46-0.96]; $P_{trend} = .02$), lycopene (OR = 0.54 [0.38-0.78]; $P_{trend} = .003$), dihydrolycopene (OR = 0.51 [0.35-0.73]; $P_{trend} = .007$), and α-cryptoxanthin (OR = 0.53 [0.36-0.78]; $P_{trend} = .003$) were associated with a lower risk of NHL (Table 2). A statistically significant inverse trend was detected for retinol ($P_{trend} = .04$); however, risk estimates did not depart from unity in

Table 1. Characteristics of participants in the NHL nested case-control study

Characteristic	Cases (n = 271)	Controls (n = 538)	P*
Mean age at blood draw, y (SD)†	70.0 (7.4)	70.0 (7.5)	
Hours fasting before blood draw, mean (SD)†	12.0 (4.0)	12.3 (4.1)	
Male, n (%)†	157 (57.9)	312 (58.0)	
Ethnicity, n (%)†			
Black	46 (17.0)	92 (17.1)	
Native Hawaiian	17 (6.3)	33 (6.1)	
Japanese American	72 (26.6)	142 (26.4)	
Latino	62 (22.9)	123 (22.9)	
White	74 (27.3)	148 (27.5)	
Years of education, mean (SD)	13.7 (3.0)	13.7 (3.2)	.88
Mean BMI, kg/m^2 (SD)	26.9 (5.3)	26.4 (4.3)	.16
Pack-years of cigarette smoking, mean (SD)	10.5 (15.1)	9.8 (15.2)	.54
Mean physical activity, h/d (SD)	1.2 (1.2)	1.3 (1.4)	.29
Mean alcohol intake, g/d (SD)	9.7 (24.4)	11.2 (28.4)	.43
Positive history of blood transfusion, n (%)	23 (8.5)	53 (9.9)	.53
NHL subtype, n (%)			
DLBCL	78 (28.8)		
Follicular lymphoma	49 (18.1)		
T-cell lymphomas	15 (5.5)		
SLL/CLL	51 (18.8)		
Other types	78 (28.8)		
Total carotenoids, ng/mL‡	1184 (862-1659)	1326 (965-1769)	.02
α-carotene, ng/mL	48 (29-81)	54 (32-83)	.20
β-carotene, ng/mL	224 (118-440)	264 (147-430)	.08
Lycopene, ng/mL	370 (275-521)	439 (320-583)	< .01
Dihydrolycopene, ng/mL	93 (67-127)	107 (79-138)	< .01
trans-lutein, ng/mL	96 (73-129)	104 (78-138)	.09
α-cryptoxanthin, ng/mL	30 (22-41)	33 (26-43)	< .01
β-cryptoxanthin, ng/mL	156 (103-270)	168 (106-295)	.45
trans-zeaxanthin, ng/mL	20 (16-26)	20 (16-26)	.87
Retinol, ng/mL	648 (527-798)	661 (562-780)	.19

DLBCL indicates diffuse large B-cell lymphoma; and SLL/CLL, small lymphocytic lymphoma/chronic lymphocytic lymphoma.

*P values obtained by χ^2 test for categorical variables; t tests were performed for all continuous variables except for the non-normally distributed carotenoids, for which a nonparametric Wilcoxon rank-sum test was used.

†Matching variable; cases and controls were matched on sex, birth year (± 1 year), location (Hawaii or California), ethnicity, date of blood draw (± 1 year), time of blood draw (± 2 hours), and hours fasting before blood draw (< 6, 6 to < 8, 8 to < 10, ≥ 10).

‡Medians and 25th-75th percentiles are presented for carotenoids and retinol.

models examining tertiles of exposure. The risk estimates for β-carotene (OR = 0.77 [0.53-1.12]; P_{trend} = .06) and *trans*-lutein (OR = 0.71 [0.48-1.04]; P_{trend} = .08) included 1. No statistically significant associations were detected for α-carotene, β-cryptoxanthin, and *trans*-zeaxanthin. The associations were similar in a sensitivity analyses excluding the cases (n = 53) diagnosed with NHL within 1 year after the date of blood draw and their matched controls; however, the risk estimate for total carotenoids (OR = 0.72 [0.48-1.10]; P_{trend} = .11) no longer departed from unity.

No heterogeneity in the risk estimates was detected in analyses stratified by sex (P = .77) or by ethnicity (P = .99; data not shown). After stratification by common NHL subtype (Table 3), the inverse association for higher serum levels of total carotenoids, β-carotene, lycopene, and dihydrolycopene was confined to DLBCL. At the same time, α-cryptoxanthin remained inversely associated with DLBCL and CLL/SLL and serum retinol with the risk of DLBCL and FL. However, statistically significant heterogeneity in risk estimates across histologic subtypes was detected only for β-carotene (P = .0001).

A comparison of serum levels with energy-adjusted nutrient intake levels based on the baseline FFQ showed Spearman correlation coefficients of 0.21 for total carotenoids, 0.29 for α-carotene, 0.27 for β-carotene, and 0.16 for lycopene and lutein (P < .0001 for all).

Discussion

In this nested case-control study, prediagnostic total carotenoids measured in serum were associated with a reduced risk of NHL; in particular, lycopene and α-cryptoxanthin were associated with a 30% to 50% lower risk of disease. Risk estimates for β-carotene, lutein, and retinol were also suggestive of a protective effect. When stratified by common NHL subtype, significant associations were generally confined to DLBCL, probably reflecting the small number of cases for the other subtypes examined. Given the lack of an a priori hypothesis, the subtype heterogeneity for β-carotene may represent a chance finding and requires replication.

To the best of our knowledge, no previous study has directly investigated the association between prediagnostic serum carotenoid levels and the risk of NHL. Previous studies, in agreement with our findings in blood, have reported inverse associations for individual carotenoids estimated from FFQs,[9,10,12-14,18,21] as well as for carotenoid-rich foods, including green leafy vegetables[9-15] and yellow-orange vegetables.[9,10,17,20,21] Associations for the intake of fruits in relation to the risk of NHL have been less consistent.[9,16,19] In addition to the potential for recall bias in retrospective case-controls studies of diet, the difficulty in obtaining precise estimates of carotenoids levels when measured by a FFQ raises concerns

Table 2. Association of NHL with tertiles of serum carotenoids

Biomarker	T_1	T_2	T_3	P_{trend}*
Total carotenoids, ng/mL				
Tertile cut-points	< 1040.49	1040.49-1550.33	≥ 1550.34	
Cases/controls	109/159	76/195	86/184	
OR (95% CI)	1.00	0.56 (0.38-0.81)	0.66 (0.46-0.96)	.02
α-carotene, ng/mL				
Tertile cut-points	< 38.53	38.53-68.75	≥ 68.76	
Cases/controls	102/169	80/190	89/179	
OR (95% CI)	1.00	0.70 (0.49-1.00)	0.81 (0.56-1.17)	.34
β-carotene, ng/mL				
Tertile cut-points	< 167.52	167.52-353.05	≥ 353.06	
Cases/controls	101/164	79/188	91/186	
OR (95% CI)	1.00	0.67 (0.47-0.97)	0.77 (0.53-1.12)	.06
Lycopene, ng/mL				
Tertile cut-points	< 345.70	345.70-510.43	≥ 510.44	
Cases/controls	109/155	87/184	75/199	
OR (95% CI)	1.00	0.68 (0.48-0.97)	0.54 (0.38-0.78)	.003
Dihydrolycopene, ng/mL				
Tertile cut-points	< 85.14	85.14-122.47	≥ 122.48	
Cases/controls	110/155	88/182	73/201	
OR (95% CI)	1.00	0.69 (0.48-0.98)	0.51 (0.35-0.73)	.007
***trans*-lutein, ng/mL**				
Tertile cut-points	< 84.33	84.33-122.36	≥ 122.37	
Cases/controls	95/173	98/174	78/191	
OR (95% CI)	1.00	1.03 (0.73-1.46)	0.71 (0.48-1.04)	.08
α-cryptoxanthin, ng/mL				
Tertile cut-points	< 27.16	27.16-37.72	≥ 37.73	
Cases/controls	112/158	79/187	80/193	
OR (95% CI)	1.00	0.57 (0.40-0.82)	0.53 (0.36-0.78)	.003
β-cryptoxanthin, ng/mL				
Tertile cut-points	< 126.45	126.45-232.90	≥ 232.91	
Cases/controls	90/181	100/169	81/188	
OR (95% CI)	1.00	1.18 (0.83-1.68)	0.84 (0.57-1.24)	.55
***trans*-zeaxanthin, ng/mL**				
Tertile cut-points	< 16.92	16.92-24.05	≥ 24.06	
Cases/controls	88/180	96/177	87/181	
OR (95% CI)	1.00	1.12 (0.78-1.60)	0.98 (0.68-1.43)	.66
Retinol, ng/mL				
Tertile cut-points	< 587.75	587.75-740.43	≥ 740.44	
Cases/controls	98/176	84/186	89/176	
OR (95% CI)	1.00	0.81 (0.56-1.16)	0.90 (0.61-1.33)	.04

ORs and 95% CI estimated from conditional logistic regression with matched sets as strata. Cases and controls were matched on sex, age (± 1 year), location (Hawaii or California), ethnicity, date of blood draw (± 1 year), time of blood draw (± 2 hours), and hours fasting before blood draw (< 6, 6 to < 8, 8 to < 10, ≥ 10).

*P value for the Wald χ^2 test of H_0: $\beta = 0$ when modeling the natural log–transformed continuous variable.

regarding previous findings. As shown by the modest correlations of 0.16 to 0.29 in our population, serum carotenoid levels do not represent the same exposure as FFQ-based dietary intakes. Thus, prospective analyses of circulating carotenoid levels probably provide for a more valid assessment. Interactions between the intake of vegetables and genetic polymorphisms in pathways involved in oxidative stress, DNA repair, and carcinogen metabolism in relation to NHL risk may influence the results of individual studies, in particular those with null findings,[22-26] and warrant further examination.[39,40]

Despite our lack of a priori hypothesis and the limited evidence of heterogeneity across common NHL subtypes examined in our analysis, the finding of significant risk estimates primarily confined to DLBCL agrees with several previous reports.[12-14,23,24] However, inverse associations with dietary intake of carotenoids have been reported for other NHL subtypes.[11,21]

Multiple mechanisms of action for carotenoids in relation to NHL have been proposed. Carotenoids, functioning as antioxidants, may reduce the potential for malignant transformation of lymphoid cells by limiting DNA damage induced by ROS and reactive nitrogen species. In addition to genotoxic effects, ROS have been implicated in tumor promotion and may adversely affect the rates of cellular proliferation and differentiation.[41] Carotenoids also may influence the risk of NHL through immune-mediated pathways.[31,42] For example, elderly subjects who received supplementation with carotenoid-containing vitamins and trace elements showed a higher production of the cytokine IL-2 and a lower rate of infections,[43] and a carotenoid-rich vegetable intervention suppressed the secretion of IL-2 and IL-4.[44,45]

This study had several strengths, including the prospective design that allowed for the prediagnostic assessment of serum carotenoid levels, the ethnic diversity of the study sample, and the population-based sampling frame allowing for generalizability of results. Although NHL diagnoses could not be confirmed by the rereview of pathology records, the majority of histologic subtypes are expected to be correctly classified within the Surveillance, Epidemiology, and End Results registry; all cases were diagnosed after the International Classification of Diseases for Oncology,

Table 3. Association of NHL subtypes with tertiles of serum carotenoids

		DLBCL		FL		CLL/SLL		
	Controls	Cases	OR (95% CI)	Cases	OR (95% CI)	Cases	OR (95% CI)	$P_{heterogeneity}$*
Total carotenoids, ng/mL								
< 1040.49	159	37	1.00	15	1.00	20	1.00	
1040.49-1550.33	195	20	0.42 (0.23-0.76)	15	0.86 (0.40-1.86)	15	0.59 (0.29-1.21)	
≥ 1550.34	184	21	0.46 (0.25-0.84)	19	1.07 (0.51-2.27)	16	0.73 (0.36-1.49)	
P_{trend}†			< 0.01		0.27		0.34	.29
α-carotene, ng/mL								
< 38.53	169	35	1.00	16	1.00	20	1.00	
38.53-68.75	190	19	0.47 (0.25-0.85)	14	0.69 (0.32-1.49)	17	0.85 (0.42-1.71)	
≥ 68.76	179	24	0.62 (0.34-1.11)	19	0.96 (0.46-2.03)	14	0.77 (0.36-1.62)	
P_{trend}			0.24		0.48		0.64	.89
β-carotene, ng/mL								
< 167.52	164	38	1.00	15	1.00	18	1.00	
167.52-353.05	188	21	0.44 (0.24-0.79)	16	0.94 (0.44-2.02)	15	0.78 (0.37-1.62)	
≥ 353.06	186	19	0.38 (0.20-0.71)	18	1.04 (0.48-2.26)	18	0.95 (0.46-1.96)	
P_{trend}			< 0.01		0.89		0.84	< .01
Lycopene, ng/mL							3w1112	
< 345.70	155	34	1.00	18	1.00	17	1.00	
345.70-510.43	184	22	0.54 (0.30-0.96)	18	0.85 (0.42-1.72)	22	1.11 (0.56-2.20)	
≥ 510.44	199	22	0.51 (0.28-0.91)	13	0.53 (0.25-1.14)	12	0.52 (0.24-1.15)	
P_{trend}			< 0.01		0.12		0.19	.44
Dihydrolycopene, ng/mL								
< 85.14	155	32	1.00	18	1.00	19	1.00	
85.14-122.47	182	26	0.68 (0.39-1.20)	17	0.84 (0.41-1.73)	21	0.92 (0.47-1.80)	
≥ 122.48	/201	20	0.49 (0.27-0.89)	14	0.54 (0.26-1.16)	11	0.45 (0.20-0.99)	
P_{trend}			0.01		0.11		0.23	.59
***trans*-lutein, ng/mL**								
< 84.33	173	28	1.00	21	1.00	18	1.00	
84.33-122.36	174	3	1.06 (0.60-1.86)	13	0.59 (0.28-1.26)	22	1.21 (0.62-2.37)	
≥ 122.37	191	20	0.62 (0.33-1.17)	15	0.64 (0.31-1.35)	11	0.59 (0.26-1.30)	
P_{trend}			0.16		0.28		0.10	.93
α-cryptoxanthin, ng/mL								
< 27.16	158	36	1.00	19	1.00	25	1.00	
27.16-37.72	187	25	0.57 (0.32-1.00)	12	0.48 (0.22-1.03)	9	0.27 (0.12-0.60)	
≥ 37.73	193	17	0.35 (0.19-0.68)	18	0.76 (0.37-1.57)	17	0.47 (0.23-0.95)	
P_{trend}			0.02		0.10		0.07	.99
β-cryptoxanthin, ng/mL								
< 126.45	181	33	1.00	15	1.00	16	1.00	
126.45-232.90	171	21	0.64 (0.35-1.16)	22	1.50 (0.74-3.05)	21	1.45 (0.72-2.93)	
≥ 232.91	186	24	0.66 (0.36-1.21)	12	0.70 (0.31-1.61)	14	1.04 (0.47-2.28)	
P_{trend}			0.49		0.74		0.71	.98
***trans*-zeaxanthin, ng/mL**								
< 16.92	180	25	1.00	16	1.00	19	1.00	
16.92-24.05	177	30	1.21 (0.68-2.15)	14	0.85 (0.39-1.83)	21	1.19 (0.61-2.32)	
≥ 24.06	181	23	0.90 (0.48-1.66)	19	1.25 (0.60-2.60)	11	0.60 (0.27-1.32)	
P_{trend}			0.87		0.89		0.24	.67
Retinol, ng/mL								
< 587.75	176	27	1.00	26	1.00	18	1.00	
587.75-740.43	186	30	1.03 (0.59-1.81)	13	0.49 (0.24-0.99)	14	0.69 (0.33-1.44)	
≥ 740.44	176	21	0.75 (0.40-1.40)	10	0.47 (0.21-1.04)	19	0.83 (0.41-1.67)	
P_{trend}			0.02		0.03		0.73	.48

ORs and 95% CI estimated from conditional logistic regression with matched sets as strata. Cases and controls were matched on sex, age (± 1 year), study site (Hawaii or California), ethnicity, date of blood draw (± 1 year), time of blood draw (± 2 hours), and hours fasting prior to blood draw (< 6, 6 to < 8, 8 to < 10, ≥ 10).

*P value for the test of heterogeneity in the parameter estimates for the log-transformed continuous variables across the NHL subtypes.

†P value for the Wald χ^2 of H_0: $\beta = 0$ when modeling the natural log–transformed continuous variable.

Third Edition classification had been adopted.[3] There were also limitations. Only a single biologic sample was available for this analysis. Because seasonal variations have been observed for these nutrients,[36] misclassification may have attenuated the risk estimates. However, in contrast to other nutrients, intraindividual circulating carotenoid levels seem to be relatively stable over time.[46,47] For example, an intraclass correlation coefficient of 0.82 to 0.84 over 2 to 4 weeks was reported previously.[46] The fat-soluble nature and the relatively long half-life of carotenoids, estimated as 26 to 76 days in 1 report,[48] contribute to this stability. Despite the potential for higher sun exposure in Hawaii and California, we consider it unlikely that vitamin D intake influenced the findings through competitive binding with retinol because a large pooling project of cohort studies did not detect an association between circulating vitamin D and NHL risk and vitamin D levels within the MEC were comparable with other sites.[49]

Furthermore, the median follow-up time from the date of blood draw to the date of diagnosis for this analysis was less than 2.7 years. Despite this relatively short follow-up period, no differences in the risk estimates were detected in sensitivity analyses excluding cases diagnosed within the first year of providing a biologic specimen. Longer follow-up of the MEC and other cohorts is needed to confirm these findings with greater statistical power. Although information on HIV status, family history of lymphoma, presence of autoimmune disease, immunosuppression, or agricultural exposure was not available and could not be examined as potential confounders, these conditions are expected to be rare in this relatively healthy cohort population. In addition, the small number of cases limited our ability to detect statistically significant associations in subgroup analyses. Finally, the characteristics of our study population with a mean age of 70 years and relatively high carotenoid levels limit the generalizability of our findings to younger individuals and to those with lower carotenoid exposure. Compared with a value of 1284 ng/mL in the MEC, the 2003 to 2006 US data show median levels of ~ 770 ng/mL for total carotenoids in adults 60 years of age or older.[50]

In conclusion, our findings provide support for the hypothesis that higher levels of circulating carotenoids, presumably from the consumption of carotenoid-rich foods, are associated with a lower risk of NHL. Additional prospective studies and longer follow-up of the MEC and other cohorts over time are needed to confirm these findings in larger populations and to establish long-term relations between serum carotenoids and NHL risk. To further elucidate the role of prediagnostic circulating carotenoids for specific common NHL subtypes, pooled analyses of prospective data are needed. In addition, future studies focused on investigating whether circulating carotenoids may influence the risk of NHL through oxidative stress pathways or through immune function may provide further insights. Non-invasive approaches to measure carotenoid levels, eg, dermal resonance Raman spectroscopy, may facilitate exposure assessment for such epidemiologic studies in the future.[51]

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Authorship

Contribution: N.J.O. performed the statistical analysis and drafted the manuscript; G.M. planned the study, procured funding, planned the statistical analysis, and finalized the paper; S.M.C. and Y.M. participated in the statistical analysis and contributed to the writing of the paper; A.A.F. and R.V.C. were responsible for the laboratory assays, the quality control, and the analytical methods section. L.R.W. was in charge of the selection of cases and controls, directed the specimen assembly, and supervised the statistical analysis; L.L.M., M.T.G., B.Y.H., B.E.H., and L.N.K. participated in planning the project and contributed to the analysis and to the writing; and all authors approved the final manuscript.

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