Plasma carotenoids and risk of breast cancer over 20 y of follow-up[1–3]

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ABSTRACT

Background: Increasing evidence suggests that carotenoids, which are micronutrients in fruit and vegetables, reduce breast cancer risk. Whether carotenoids are important early or late in carcinogenesis is unclear, and limited analyses have been conducted by breast tumor subtypes.

Objectives: We sought to examine issues of the timing of carotenoid exposure as well as associations by breast tumor subtypes.

Design: We conducted a nested case-control study of plasma carotenoids measured by using reverse-phase high-performance liquid chromatography and breast cancer risk in the Nurses' Health Study. In 1989–1990, 32,826 women donated blood samples; in 2000–2002, 18,743 of these women contributed a second blood sample. Between the first blood collection and June 2010, 2188 breast cancer cases were diagnosed (579 cases were diagnosed after the second collection) and matched with control subjects. RRs and 95% CIs were calculated by using conditional logistic regression adjusted for several breast cancer risk factors.

Results: Higher concentrations of α-carotene, β-carotene, lycopene, and total carotenoids were associated with 18–28% statistically significantly lower risks of breast cancer (e.g., β-carotene top compared with bottom quintile RR: 0.72; 95% CI: 0.59, 0.88; P-trend < 0.001). Associations were apparent for total carotenoids measured $\geq$10 y before diagnosis (top compared with bottom quintile RR: 0.69; 95% CI: 0.50, 0.95; P-trend = 0.01) as well as those $<$10 y before diagnosis (RR: 0.79; 95% CI: 0.64, 0.98; P-trend = 0.04, P-interaction = 0.11). Carotenoid concentrations were strongly inversely associated with breast cancer recurrence and death (e.g., β-carotene top compared with bottom quintile RR: 0.32; 95% CI: 0.21, 0.51; P-trend < 0.001) compared with not recurrent and not lethal disease (P-heterogeneity < 0.001).

Conclusion: In this large prospective analysis with 20 y of follow-up, women with high plasma carotenoids were at reduced breast cancer risk particularly for more aggressive and ultimately fatal disease. *Am J Clin Nutr* 2015;101:1197–205.

Keywords: biomarkers, breast cancer, carotenoids, plasma, nested case-control study

INTRODUCTION

Carotenoids, which are essential for plant photosynthesis, provide yellow-red pigments in fruit and vegetables. α-Carotene, β-carotene, β-cryptoxanthin, lutein, zeaxanthin, and lycopene are the most prevalent in the US diet, comprising 90% of circulating carotenoids (1, 2), and hypothesized to be anticarcinogenic through metabolism to retinoids that contribute to cellular differentiation, antioxidation, immuno-enhancement, or the inhibition of tumorigenesis and malignant transformation (3–8). In experimental studies, carotenoids reduce the proliferation in breast cancer cell lines and inhibit tumor progression (9).

Studies of fruit and vegetable intake and, more specifically, carotenoids have been mixed. Most recently, in a pooled analyses of 18 cohort studies, inverse associations were observed between α-carotene, β-carotene, and lutein and zeaxanthin intakes and estrogen receptor (ER)[4]–negative, but not ER-positive, tumors (10). The measurement of circulating carotenoids avoids recalled diet (2) and inaccuracies of nutrient databases (11) and integrates cooking influences (2, 12), geographic and seasonal variation of foods (11), and individual variation in absorption. In our recent pooled analysis (13) of circulating carotenoids and subsequent breast cancer risk (n = 3055 cases), we observed significant 13–22% reduced risks of total breast cancer for the top (compared with bottom) quintiles of α-carotene, β-carotene, lutein and zeaxanthin, lycopene, and total carotenoids and 48% reduced risk of ER-negative tumors for β-carotene.

Although the pooled analysis allowed a comprehensive examination of carotenoids, our ability to examine the importance of exposure timing as well as tumor subtypes and outcomes was limited. Thus, we examined these issues in a nested case-control study within the Nurses' Health Study (NHS) by using blood samples collected 10 y apart with 20 y of follow-up. The pooled analysis included 962 NHS cases; the current analysis was expanded to include 2188 cases.

METHODS

Study population

In 1976, 121,701 female registered nurses aged 30–55 y were enrolled in the NHS. Biennially, participants completed mailed

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[4] Abbreviations used: ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; ICC, intraclass correlation coefficient; NHS, Nurses' Health Study; PMH, postmenopausal hormone; PR, progesterone receptor; WHEL, Women's Health Eating and Living.

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questionnaires on lifestyle, diet, reproductive history, and disease diagnoses. In 1989–1990, 32,826 women aged 43–69 y donated blood samples (14). Briefly, each woman arranged to have her blood drawn and shipped overnight with an ice pack to our laboratory where it was processed and archived in liquid-nitrogen freezers; 97% of samples arrived ≤26 h of collection. In 2000–2002, a second sample was collected by using a similar protocol from 18,743 of these women aged 53–80 y (15). The follow-up rate in the 32,826 women was 97% in 2010. The study was approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women's Hospital; the completion of the self-administered questionnaire and blood collection was considered to imply informed consent.

Case and control selection

Cases had no reported cancer (other than nonmelanoma skin) before blood collection and were diagnosed with breast cancer between the first collection and June 2010. Overall, 2188 breast cancer cases were reported (n = 1750 invasive), which were confirmed by medical record reviews (n = 2,152) or verbally by the nurse (n = 36). The time from blood collection to diagnosis ranged from <1 mo to 20 y (median: 9.3 y) after blood collection. One control was matched per case by using the following factors (at both collections for subjects with 2 samples): age (±2 y), menopausal status and postmenopausal hormone (PMH) use at blood collection and diagnosis (premenopausal, postmenopausal and not taking PMHs, postmenopausal and taking PMHs, and unknown), and month (±1 mo), time of day (±2 h), and fasting status at blood collection (<10 h after a meal or unknown; ≥10h).

Carotenoid assays

Plasma carotenoids were assayed by reverse-phase HPLC (16) at the Harvard T.H. Chan School of Public Health. Assays were conducted in 8 batches; CVs from blinded quality-control replicates (10% of samples) were generally ≤15% except for the following batches for which CVs were ≤20%: α-carotene (n = 2), β-carotene (n = 2), β-cryptoxanthin (n = 1), and lycopene (n = 1). Variation in blinded quality controls across batches was noted, which suggested laboratory variation; all batches were recalibrated to an average standard batch (17).

Questionnaire, tumor, and outcome data

Information on breast cancer risk factors, including anthropometric measures, reproductive history, and diet, was collected from biennial and blood collection questionnaires. Detailed information on case characteristics, including invasiveness, histologic grade, ER, progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) status, was extracted from pathology reports. In addition, cases with available tumor tissue included in tumor microarrays were immunostained for ER, PR, and HER2 and read manually by a study pathologist (18).

Breast cancer recurrences were documented from reported second cancer diagnoses. If the second cancer was reported in the lung, liver, bone, or brain, it was assumed that breast cancer recurred because these are the most-common sites of breast cancer recurrence; medical records were reviewed to exclude primary lung cancer (19). Deaths were reported by the family or post office. Nonresponders were searched in the National Death Index. More than 98% of deaths in the NHS have been identified by these methods. Physician reviewers blinded to exposure information ascertained the cause of death from death certificates, which were supplemented with medical records if necessary.

Statistical analysis

Included in the analysis were 2188 distinct cases and 2188 controls. Of these, 2147 cases and 2150 controls had at least one carotenoid measure by using the first collection; 579 cases and 580 controls had 2 carotenoids measures from the first and second collections. With the use of all available data, 1828 cases had carotenoids measured <10 y before diagnosis, and 895 cases had carotenoids measured ≥10 y before diagnosis.

We calculated intraclass correlation coefficients (ICCs) over 10 y in women with 2 blood collections. Quintile cutoffs for carotenoids were established in controls. RRs and 95% CIs were calculated from conditional logistic regression models adjusted for breast cancer risk factors. Tests for trend were conducted by using the Wald test on quintile medians modeled continuously. We examined the possibly nonlinear relation between carotenoids and risk of breast cancer nonparametrically with restricted cubic splines (20). Tests for nonlinearity used the likelihood ratio test for comparison of the model with only the linear term to the model with the linear and cubic spline terms.

We conducted separate analyses for carotenoids measured <10 and ≥10 y before diagnosis by using all available measures (i.e., first and second blood samples). In women with 2 blood samples, we simultaneously modeled carotenoid concentrations in the first (1989–1990) and second (2000–2002) collections. We also conducted analyses of all available cases and controls by using one carotenoids measure or, when available, the average of 2 measures. Unconditional logistic regression models, which were additionally adjusted for matching factors, were used for stratified analyses.

We investigated interactions between carotenoids and follow-up time and lifestyle factors, including BMI (in kg/m^2), smoking, alcohol intake, PMH use, and menopausal status, by using likelihood ratio tests. We assessed associations with carotenoids by ER status and luminal A (ER-positive or PR-positive, HER2-negative, and grade 1 or 2), luminal B (ER positive or PR positive and either HER2-positive or HER2-negative and grade 3), and triple-negative tumors (ER-negative, PR-negative, and HER2-negative) (18). We also examined associations by tumor invasiveness, histologic grade, tumor size, and lymph node status. To test whether associations differed by tumor subtype, we used polychotomous logistic regression (21) with a likelihood ratio test for the comparison of a model with separate slopes for carotenoids in each case group to one with a common slope. We conducted a survival analyses in breast cancer cases by using Cox proportional hazards models, calculating the person-time from diagnosis to the first of recurrence or breast cancer death, other death, or end of follow-up (June 2010). Primary survival analyses were adjusted for breast cancer risk factors; secondary analyses were further adjusted for tumor stage, ER and PR status, hormone therapy, chemotherapy, and radiation therapy. All P values were based on 2-sided tests and considered statistically significant if ≤0.05; interactions with P > 0.05 but ≤0.10 were considered suggestive. Analyses were conducted

with SAS version 9 software (SAS Institute) or STATA version 12.1 software (StataCorp).

RESULTS

At the first collection, cases were more likely than controls to be nulliparous, have a history of benign breast disease, and have a family history of breast cancer (**Table 1**). By the second collection, women were, on average, 10 y older, slightly heavier, and less likely to smoke. Approximately two-thirds of women were postmenopausal at the first blood collection; nearly all women were postmenopausal at the second collection. Ten-year ICCs ranged from 0.30 (β-carotene) to 0.54 (lutein and zeaxanthin).

Significant inverse associations were observed between α-carotene, β-carotene, lycopene, and total carotenoids and breast cancer risk with overall 18–28% lower risk in the top compared with bottom quintiles (**Table 2**). Results were similar between simple and multivariate conditional logistic regression models (data not shown). Additional adjustment for physical activity, plasma cholesterol concentrations, or vitamin D intake did not alter the results (data not shown). The association with lycopene was suggestively stronger with measures 10–20 compared with <10 y before diagnosis [top compared with bottom quintile RRs (95% CIs): ≥10 y, 0.69 (0.50, 0.94; *P*-trend = 0.01); <10 y: 0.87 (0.70, 1.07; *P*-trend = 0.14, *P*-heterogeneity = 0.09]. Associations with β-carotene and total carotenoids were apparent for measures both 10–20 and <10 y before diagnosis (*P*-interaction = 0.55 and 0.11, respectively). Analyses restricted to women with 2 blood samples yielded similar results (data not shown). Tests for nonlinearity were NS for any individual carotenoids or total carotenoids (data not shown).

The correlation between carotenoids was highest in the pro–vitamin A carotenoids (α-carotene, β-carotene, and β-cryptoxanthin; Spearman r = 0.48–0.76). Associations for α-carotene (except when adjusted for β-carotene) and β-carotene remained when adjusted for the other carotenoids (data not shown). The lycopene association was attenuated with adjustment for α-carotene or β-carotene.

Associations between individual carotenoids and breast cancer risk were similar by alcohol intake, PMH use, and menopausal status at blood collection and in women who received screening mammograms within 2 y of blood collection (data not shown). The associations of β-carotene and total carotenoids with breast cancer risk differed significantly by BMI (**Table 3**). Significant inverse associations were observed in lean women [BMI <25 top compared with bottom quintile RRs (95% CIs): β-carotene, 0.62 (0.47, 0.83; *P*-trend < 0.001); total carotenoids, 0.64 (0.48, 0.84; *P*-trend < 0.001)], whereas no association was observed for overweight or obese women [e.g., BMI ≥30 RRs: β-carotene, 0.96 (*P*-trend = 0.86, *P*-interaction = 0.04); total carotenoids, 0.98 (*P*-trend = 0.75, *P*-interaction = 0.02)]. Although β-cryptoxanthin was not associated with breast cancer overall, it was significantly inversely associated with risk in lean women (top compared with bottom quintile RR: 0.70; 95% CI: 0.53, 0.92; *P*-trend = 0.05, *P*-heterogeneity = 0.08). The association of α-carotene with breast cancer was significantly stronger in nonsmokers (comparable RR: 0.74; 95% CI: 0.60, 0.92; *P*-trend = 0.01) than in current smokers (RR: 1.23; 95% CI: 0.54, 2.80; *P*-trend = 0.22, *P*-interaction = 0.03).

TABLE 1
Characteristics of breast cancer cases and matched controls at each blood collection in the NHS[1]

	1989–1990 blood draw		2000–2002 blood draw	
	Cases	Controls	Cases	Controls
n[2]	2147	2150	579	580
Age at blood draw, y	56.4 ± 7.0[3]	56.5 ± 7.0	66.3 ± 6.9	66.5 ± 6.8
BMI at age 18 y, kg/m^2	21.1 ± 2.7	21.3 ± 2.9	—[4]	—
BMI at blood draw, kg/m^2	25.5 ± 4.5	25.3 ± 4.7	26.8 ± 5.0	26.5 ± 5.3
Age at menarche, y	12.5 ± 1.4	12.6 ± 1.4	—	—
Nulliparous, %	10.4	8.6	—	—
Age at first birth, y	24.8 ± 3.3	24.7 ± 3.1	—	—
Postmenopausal, %	65.7	65.8	98.1	98.1
Age at menopause, y	49.3 ± 4.5	48.7 ± 5.0	50.1 ± 4.5	49.2 ± 4.9
Current smokers, %	12.6	10.5	5.2	4.0
Alcohol consumption, g/d	6.3 ± 9.7	5.6 ± 8.4	6.9 ± 10.8	5.7 ± 9.1
History of benign breast disease, %	45.3	36.7	62.9	54.5
Family history of breast cancer, %	15.6	10.2	22.1	14.3
Plasma carotenoids[5], μg/dL				
α-Carotene	61.3 (26.7, 140)	63.7 (27.6, 149)	57.5 (23.4, 149)	61.7 (25.6, 158)
β-Carotene	216 (92.9, 519)	233 (93.8, 558)	224 (88.5, 652)	245 (94.9, 584)
β-Cryptoxanthin	75.7 (38.3, 149)	78.4 (37.5, 154)	87.8 (37.3, 174)	86.0 (42.8, 184)
Lutein and zeaxanthin	164 (95.9, 272)	168 (95.0, 278)	166 (92.7, 284)	167 (95.1, 291)
Lycopene	398 (217, 642)	413 (217, 675)	373 (186, 627)	385 (205, 652)
Total carotenoids	966 (577, 1567)	1007 (581, 1649)	985 (545, 1673)	1020 (593, 1698)

[1]NHS, Nurses' Health Study.
[2]Mismatched case and control numbers were due to missing carotenoid measures for first or second blood collection.
[3]Mean ± SD (all such values).
[4]Unchanged between first and second blood collection (all such values).
[5]All values are medians; 10th, 90th percentiles in parentheses.

TABLE 2
RRs (95% CIs) of breast cancer according to quintile of plasma carotenoids in the NHS by follow-up period (≤10 compared with >10 y)[1]

Carotenoid	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	*P*-trend
α-Carotene, μg/dL	<37.7	37.7 to <54.4	54.4 to <75.8	75.8 to <111	≥111	—
<10 y						
Cases/controls, *n*	430/388	354/367	370/360	371/352	303/361	—
RR (95% CI)	1.00	0.84 (0.68, 1.03)	0.92 (0.75, 1.13)	0.95 (0.77, 1.17)	0.73 (0.59, 0.91)	0.02
≥10 y						
Cases/controls, *n*	194/157	173/179	184/183	186/193	157/185	—
RR (95% CI)	1.00	0.78 (0.58, 1.06)	0.85 (0.63, 1.15)	0.81 (0.60, 1.10)	0.77 (0.56, 1.05)	0.21
Overall[2]						
Cases/controls, *n*	483/431	413/430	468/430	424/431	363/430	—
RR (95% CI)	1.00	0.82 (0.67, 0.99)	0.95 (0.78, 1.15)	0.86 (0.71, 1.05)	0.74 (0.60, 0.91)	0.01
β-Carotene, μg/dL	<136	136 to <198	198 to <282	282 to <419	≥419	—
<10 y						
Cases/controls, *n*	414/379	358/366	397/340	332/362	327/382	—
RR (95% CI)	1.00	0.91 (0.74, 1.12)	1.09 (0.89, 1.35)	0.85 (0.69, 1.06)	0.77 (0.62, 0.96)	0.01
≥10 y						
Cases/controls, *n*	215/167	186/180	196/206	165/183	133/164	—
RR (95% CI)	1.00	0.83 (0.62, 1.12)	0.80 (0.60, 1.07)	0.78 (0.57, 1.06)	0.70 (0.51, 0.97)	0.05
Overall						
Cases/controls, *n*	492/430	449/432	456/431	396/429	358/431	—
RR (95% CI)	1.00	0.91 (0.76, 1.11)	0.96 (0.79, 1.16)	0.83 (0.68, 1.01)	0.72 (0.59, 0.88)	<0.001
β-Cryptoxanthin, μg/dL	<50.9	50.9 to <70.3	70.3 to <92.1	92.1 to <126	≥126	
<10 y						
Cases/controls, *n*	386/384	372/350	357/365	366/357	343/372	—
RR (95% CI)	1.00	1.06 (0.87, 1.31)	0.97 (0.78, 1.19)	1.00 (0.81, 1.23)	0.90 (0.72, 1.11)	0.21
≥10 y						
Cases/controls, *n*	199/161	208/196	154/181	185/188	149/174	—
RR (95% CI)	1.00	0.92 (0.68, 1.23)	0.72 (0.53, 0.98)	0.86 (0.63, 1.16)	0.76 (0.55, 1.05)	0.12
Overall						
Cases/controls, *n*	455/431	462/430	413/432	428/429	391/431	—
RR (95% CI)	1.00	1.02 (0.84, 1.23)	0.90 (0.74, 1.10)	0.94 (0.77, 1.14)	0.86 (0.70, 1.06)	0.12
Lutein and zeaxanthin, μg/dL	<120	120 to <152	152 to <187	187 to <233	≥233	—
<10 y						
Cases/controls, *n*	387/369	354/365	383/372	344/360	359/364	—
RR (95% CI)	1.00	0.90 (0.73, 1.10)	0.96 (0.78, 1.18)	0.89 (0.72, 1.10)	0.91 (0.74, 1.13)	0.47
≥10 y						
Cases/controls, *n*	214/177	163/182	184/174	180/184	154/183	—
RR (95% CI)	1.00	0.75 (0.55, 1.01)	0.91 (0.68, 1.23)	0.84 (0.62, 1.14)	0.70 (0.52, 0.96)	0.07
Overall						
Cases/controls, *n*	458/431	439/431	427/431	414/430	413/431	—
RR (95% CI)	1.00	0.91 (0.75, 1.10)	0.90 (0.74, 1.10)	0.89 (0.73, 1.09)	0.86 (0.70, 1.05)	0.19
Lycopene, μg/dL	<288	288 to <371	371 to <449	449 to <563	≥563	—
<10 y						
Cases/controls, *n*	418/385	374/363	352/362	338/352	341/360	—
RR (95% CI)	1.00	0.94 (0.77, 1.15)	0.88 (0.71, 1.08)	0.86 (0.70, 1.06)	0.87 (0.70, 1.07)	0.14
≥10 y						
Cases/controls, *n*	184/159	196/181	192/182	179/192	142/184	—
RR (95% CI)	1.00	0.93 (0.69, 1.26)	0.94 (0.70, 1.28)	0.82 (0.60, 1.11)	0.69 (0.50, 0.94)	0.01
Overall						
Cases/controls, *n*	457/429	487/428	394/430	412/430	397/429	—
RR (95% CI)	1.00	1.03 (0.85, 1.25)	0.80 (0.66, 0.98)	0.87 (0.71, 1.06)	0.82 (0.67, 1.01)	0.02
Total carotenoids, μg/dL	<729	729 to <917	917 to <1124	1124 to <1379	≥1379	—
<10 y						
Cases/controls, *n*	420/380	375/363	340/355	351/360	328/360	—
RR (95% CI)	1.00	0.91 (0.74, 1.12)	0.84 (0.68, 1.04)	0.87 (0.70, 1.07)	0.79 (0.64, 0.98)	0.04
≥10 y						
Cases/controls, *n*	198/163	193/180	205/187	156/183	137/183	—
RR (95% CI)	1.00	0.91 (0.67, 1.22)	0.97 (0.72, 1.30)	0.72 (0.53, 0.99)	0.69 (0.50, 0.95)	0.01
Overall						
Cases/controls, *n*	485/428	450/429	443/429	379/428	384/429	—
RR (95% CI)	1.00	0.89 (0.74, 1.09)	0.89 (0.72, 1.09)	0.77 (0.63, 0.94)	0.77 (0.63, 0.94)	0.005

[1]Multivariate conditional logistic regression models were adjusted for BMI at age 18 y; weight gain since age 18 y; ages at menarche, first birth, and menopause; parity; alcohol intake; history of benign breast disease; and family history of breast cancer. Overall values use the 1990 blood collection or the average of 1990 and 2000 blood collections if available. *n* cases: <10 y, 1828; ≥10 y, 895; overall, 2147. *P*-heterogeneity <10 compared with ≥10 y: α-carotene, 0.85; β-carotene, 0.55; β-cryptoxanthin, 0.47; lutein and zeaxanthin, 0.20; lycopene, 0.09; total carotenoids, 0.11. NHS, Nurses' Health Study.

TABLE 3
RRs (95% CIs) of breast cancer and according to quintile of plasma carotenoids in the NHS by BMI and smoking status[1]

	n		RR (95% CI)						
	Cases	Controls	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	*P*-trend	*P*-interaction
α-Carotene (μg/dL)									
BMI (in kg/m^2) <25	1121	1209	1.00	0.74 (0.55, 1.00)	0.90 (0.68, 1.19)	0.77 (0.58, 1.01)	0.65 (0.49, 0.86)	0.005	—
BMI from 25 to <30	695	644	1.00	0.85 (0.61, 1.19)	0.93 (0.67, 1.30)	1.05 (0.74, 1.49)	0.94 (0.65, 1.37)	0.87	—
BMI ≥30	334	298	1.00	1.00 (0.65, 1.54)	1.06 (0.66, 1.73)	0.96 (0.56, 1.63)	0.76 (0.37, 1.55)	0.52	0.08
Nonsmokers	1880	1926	1.00	0.86 (0.69, 1.06)	0.96 (0.78, 1.18)	0.85 (0.69, 1.06)	0.74 (0.60, 0.92)	0.01	—
Current smokers	271	226	1.00	0.67 (0.41, 1.11)	1.08 (0.62, 1.88)	1.45 (0.78, 2.70)	1.23 (0.54, 2.80)	0.22	0.03
β-Carotene (μg/dL)									
BMI <25	1121	1209	1.00	0.85 (0.63, 1.15)	0.78 (0.58, 1.03)	0.73 (0.55, 0.97)	0.62 (0.47, 0.83)	<0.001	—
BMI from 25 to <30	696	644	1.00	0.90 (0.66, 1.25)	1.06 (0.75, 1.49)	0.91 (0.64, 1.30)	0.86 (0.59, 1.26)	0.48	—
BMI ≥30	333	299	1.00	0.95 (0.63, 1.45)	1.40 (0.86, 2.28)	0.79 (0.45, 1.38)	0.96 (0.45, 2.04)	0.86	0.04
Nonsmokers	1880	1927	1.00	0.94 (0.76, 1.16)	0.96 (0.78, 1.18)	0.84 (0.68, 1.04)	0.73 (0.59, 0.91)	0.002	—
Current smokers	271	226	1.00	0.83 (0.51, 1.38)	0.94 (0.55, 1.62)	0.90 (0.51, 1.59)	0.99 (0.45, 2.16)	0.95	0.23
β-Cryptoxanthin (μg/dL)									
BMI <25	1121	1209	1.00	0.79 (0.59, 1.05)	0.77 (0.58, 1.03)	0.86 (0.65, 1.13)	0.70 (0.53, 0.92)	0.05	—
BMI from 25 to <30	695	644	1.00	1.39 (1.00, 1.93)	1.14 (0.81, 1.59)	1.00 (0.71, 1.42)	1.12 (0.76, 1.67)	0.84	—
BMI ≥30	332	299	1.00	1.10 (0.72, 1.69)	0.90 (0.54, 1.48)	0.87 (0.51, 1.48)	1.41 (0.74, 2.68)	0.62	0.08
Nonsmokers	1880	1927	1.00	1.01 (0.82, 1.25)	0.93 (0.75, 1.15)	0.99 (0.80, 1.22)	0.89 (0.71, 1.10)	0.25	—
Current smokers	269	226	1.00	1.28 (0.78, 2.11)	1.07 (0.60, 1.90)	0.70 (0.38, 1.30)	0.97 (0.45, 2.11)	0.53	0.98
Lutein and zeaxanthin (μg/dL)									
BMI <25	1121	1210	1.00	0.70 (0.52, 0.94)	0.79 (0.59, 1.05)	0.78 (0.59, 1.04)	0.74 (0.56, 0.98)	0.22	—
BMI from 25 to <30	695	644	1.00	1.03 (0.74, 1.44)	0.89 (0.64, 1.25)	0.98 (0.69, 1.38)	0.98 (0.68, 1.42)	0.84	—
BMI ≥30	334	299	1.00	1.34 (0.86, 2.08)	1.24 (0.78, 1.99)	0.90 (0.54, 1.49)	1.17 (0.59, 2.33)	0.95	0.34
Nonsmokers	1881	1928	1.00	0.97 (0.79, 1.20)	0.99 (0.80, 1.21)	0.96 (0.78, 1.19)	0.97 (0.78, 1.20)	0.78	—
Current smokers	270	226	1.00	0.83 (0.50, 1.38)	0.75 (0.43, 1.32)	0.78 (0.44, 1.38)	0.50 (0.24, 1.04)	0.07	0.11
Lycopene (μg/dL)									
BMI <25	1118	1206	1.00	1.01 (0.77, 1.32)	0.77 (0.58, 1.01)	0.85 (0.65, 1.12)	0.72 (0.55, 0.94)	0.006	—
BMI from 25 to <30	696	640	1.00	1.17 (0.83, 1.66)	0.94 (0.66, 1.34)	1.11 (0.78, 1.59)	1.15 (0.80, 1.65)	0.56	—
BMI ≥30	332	299	1.00	0.85 (0.54, 1.34)	0.75 (0.45, 1.26)	0.54 (0.32, 0.91)	1.14 (0.66, 1.97)	0.64	0.09
Nonsmokers	1876	1923	1.00	1.06 (0.86, 1.29)	0.80 (0.65, 0.99)	0.89 (0.72, 1.09)	0.91 (0.74, 1.11)	0.15	—
Current smokers	271	223	1.00	0.92 (0.52, 1.60)	1.17 (0.65, 2.10)	0.88 (0.49, 1.59)	0.60 (0.33, 1.11)	0.12	0.30
Total carotenoids (μg/dL)									
BMI <25	1118	1204	1.00	0.80 (0.59, 1.07)	0.77 (0.58, 1.03)	0.61 (0.46, 0.82)	0.64 (0.48, 0.84)	<0.001	—
BMI from 25 to <30	693	640	1.00	1.02 (0.73, 1.42)	1.09 (0.78, 1.51)	1.01 (0.71, 1.44)	1.08 (0.74, 1.58)	0.73	—
BMI ≥30	329	298	1.00	0.82 (0.54, 1.25)	0.79 (0.50, 1.25)	0.98 (0.53, 1.81)	0.98 (0.49, 1.97)	0.75	0.02
Nonsmokers	1873	1920	1.00	0.91 (0.74, 1.12)	0.94 (0.76, 1.16)	0.81 (0.65, 1.00)	0.82 (0.66, 1.01)	0.04	—
Current smokers	268	223	1.00	0.91 (0.54, 1.53)	0.73 (0.43, 1.23)	0.71 (0.38, 1.31)	0.60 (0.28, 1.28)	0.09	0.45

[1]Multivariate unconditional logistic regression models were adjusted for matching factors; BMI at age 18 y; weight gain since age 18 y; ages at menarche, first birth, and menopause; parity; alcohol intake; history of benign breast disease; and family history of breast cancer. NHS, Nurses' Health Study.

Associations did not differ by tumor size, invasiveness, or nodal involvement (data not shown). RRs were similar for ER-positive and ER-negative cases (e.g., β-carotene top compared with bottom quintile RRs: 0.70 and 0.72, respectively) although the number of ER-negative cases was limited (n = 291–292) (**Table 4**). Associations with β-carotene were suggestively stronger in poorly differentiated tumors (P-heterogeneity = 0.08). Associations with α-carotene and β-carotene were significantly inverse for luminal B, but not luminal A, tumors, although tests for heterogeneity were NS [e.g., β-carotene top compared with bottom quintile RRs (95% CIs): luminal B, 0.47 (0.28, 0.77); P-trend = 0.003; luminal A: 0.80 (0.59, 1.09); P-trend = 0.08; P-heterogeneity = 0.32]. No significant associations were observed in triple-negative tumors (n = 107–108).

α-Carotene, β-carotene, β-cryptoxanthin, and total carotenoids were strongly inversely associated with risk of breast tumors that recurred or were ultimately lethal (Table 4). Risks in the top quintile (compared with bottom quintile) were 46–68% lower for α-carotene (RR: 0.54; 95% CI: 0.35, 0.83; P-trend = 0.01), β-carotene (RR: 0.32; 95% CI: 0.21, 0.51; P-trend < 0.001), and total carotenoids (RR: 0.48; 95% CI: 0.31, 0.73; P-trend = 0.001) (P-heterogeneity compared with nonrecurrent and nonlethal cases = 0.08, <0.001, and 0.02, respectively). β-Cryptoxanthin, although not associated with overall breast cancer risk, was associated with significantly lower risk of recurrent or lethal disease (RR: 0.68; 95% CI: 0.45, 1.04; P-trend = 0.008); however, the test for heterogeneity was NS (P = 0.30). β-Carotene and total carotenoid concentrations were significantly inversely associated with recurrence and breast cancer death in survival analyses in cases [RRs (95% CIs): 0.47 (0.31, 0.71; P-trend = 0.002) and 0.65 (0.43, 0.96; P-trend = 0.04), respectively] (data not shown). Associations were slightly attenuated with additional adjustment for tumor and treatment characteristics although the β-carotene association remained significant (RR: 0.52; 95% CI: 0.34, 0.79; P-trend = 0.003) (data not shown). When restricted to breast cancer deaths only (n = 176), point estimates were similar, but CIs were wider because of to the smaller sample size (data not shown).

TABLE 4
RRs of breast cancer and 95% CIs according to quintile of plasma carotenoids in the NHS by tumor subtype[1]

		Quintile (μg/dL)						
	Cases, *n*	1	2	3	4	5	*P*-trend	*P*-heterogeneity[2]
α-Carotene								
ER+	1316	1.00	0.84 (0.68, 1.05)	1.01 (0.82, 1.26)	0.88 (0.70, 1.09)	0.74 (0.59, 0.93)	0.02	0.94
ER−	292	1.00	0.71 (0.48, 1.05)	0.90 (0.62, 1.31)	0.83 (0.56, 1.22)	0.68 (0.45, 1.02)	0.15	—
Well differentiated	350	1.00	0.87 (0.61, 1.25)	1.04 (0.73, 1.48)	0.90 (0.62, 1.30)	0.82 (0.56, 1.20)	0.34	0.52
Moderately differentiated	596	1.00	0.97 (0.73, 1.29)	0.84 (0.63, 1.13)	0.83 (0.61, 1.12)	0.89 (0.66, 1.20)	0.39	—
Poorly differentiated	373	1.00	0.69 (0.47, 0.99)	1.32 (0.95, 1.84)	0.98 (0.69, 1.39)	0.58 (0.39, 0.86)	0.02	—
Luminal A	646	1.00	0.92 (0.69, 1.22)	1.10 (0.83, 1.45)	0.94 (0.71, 1.26)	0.80 (0.59, 1.08)	0.13	0.73
Luminal B	216	1.00	0.71 (0.45, 1.10)	1.02 (0.67, 1.54)	0.75 (0.48, 1.18)	0.54 (0.33, 0.88)	0.02	—
Triple negative	108	1.00	1.01 (0.53, 1.92)	1.12 (0.60, 2.11)	1.25 (0.67, 2.33)	0.91 (0.46, 1.80)	0.84	—
Nonrecurrent and nonlethal	1850	1.00	0.85 (0.70, 1.04)	0.95 (0.78, 1.15)	0.90 (0.74, 1.10)	0.78 (0.63, 0.96)	0.04	0.08
Recurrent or lethal	301	1.00	0.67 (0.46, 0.99)	1.04 (0.73, 1.50)	0.74 (0.50, 1.09)	0.54 (0.35, 0.83)	0.01	—
β-Carotene								
ER+	1316	1.00	0.93 (0.75, 1.16)	0.90 (0.72, 1.12)	0.86 (0.69, 1.08)	0.70 (0.56, 0.89)	0.002	0.28
ER−	292	1.00	0.82 (0.55, 1.21)	1.07 (0.73, 1.56)	0.81 (0.54, 1.22)	0.72 (0.47, 1.10)	0.14	—
Well differentiated	350	1.00	0.86 (0.60, 1.23)	1.04 (0.73, 1.49)	0.87 (0.60, 1.26)	0.79 (0.54, 1.16)	0.26	0.08
Moderately differentiated	596	1.00	0.87 (0.66, 1.16)	0.74 (0.55, 1.00)	0.85 (0.64, 1.14)	0.74 (0.55, 1.01)	0.11	—
Poorly differentiated	373	1.00	1.06 (0.76, 1.49)	1.07 (0.76, 1.51)	0.80 (0.56, 1.16)	0.53 (0.35, 0.80)	<0.001	—
Luminal A	646	1.00	1.03 (0.78, 1.36)	1.02 (0.77, 1.36)	0.94 (0.70, 1.26)	0.80 (0.59, 1.09)	0.08	0.32
Luminal B	216	1.00	0.89 (0.59, 1.33)	0.63 (0.40, 0.99)	0.73 (0.47, 1.14)	0.47 (0.28, 0.77)	0.003	—
Triple negative	108	1.00	0.89 (0.46, 1.70)	1.40 (0.77, 2.54)	0.97 (0.50, 1.87)	0.81 (0.40, 1.62)	0.48	—
Nonrecurrent and nonlethal	1850	1.00	1.01 (0.83, 1.23)	0.97 (0.79, 1.18)	0.90 (0.73, 1.10)	0.82 (0.67, 1.02)	0.03	<0.001
Recurrent or lethal	301	1.00	0.53 (0.36, 0.78)	0.86 (0.61, 1.21)	0.53 (0.36, 0.79)	0.32 (0.21, 0.51)	<0.001	—
β-Cryptoxanthin								
ER+	1313	1.00	1.07 (0.86, 1.33)	1.02 (0.82, 1.28)	1.02 (0.81, 1.27)	0.93 (0.74, 1.17)	0.36	0.86
ER−	292	1.00	0.97 (0.66, 1.42)	0.74 (0.49, 1.12)	1.03 (0.70, 1.51)	0.90 (0.60, 1.35)	0.79	—
Well differentiated	349	1.00	1.06 (0.73, 1.54)	1.16 (0.80, 1.68)	1.21 (0.84, 1.76)	1.09 (0.74, 1.59)	0.64	0.35
Moderately differentiated	595	1.00	0.95 (0.71, 1.27)	1.02 (0.77, 1.37)	0.94 (0.70, 1.27)	0.82 (0.60, 1.12)	0.19	—
Poorly differentiated	373	1.00	1.28 (0.92, 1.78)	0.69 (0.47, 1.01)	0.96 (0.67, 1.37)	0.93 (0.65, 1.35)	0.40	—
Luminal A	646	1.00	1.16 (0.87, 1.55)	1.32 (0.99, 1.75)	1.12 (0.83, 1.50)	1.06 (0.78, 1.44)	0.93	0.98
Luminal B	215	1.00	1.19 (0.77, 1.83)	0.73 (0.45, 1.19)	1.07 (0.68, 1.67)	0.98 (0.61, 1.58)	0.85	—
Triple negative	108	1.00	0.95 (0.51, 1.76)	0.84 (0.44, 1.61)	1.21 (0.66, 2.22)	0.91 (0.48, 1.76)	0.99	—
Nonrecurrent and nonlethal	1849	1.00	0.99 (0.81, 1.20)	0.94 (0.77, 1.14)	0.98 (0.80, 1.20)	0.89 (0.72, 1.10)	0.29	0.30
Recurrent or lethal	300	1.00	1.32 (0.92, 1.89)	0.84 (0.57, 1.25)	0.78 (0.52, 1.18)	0.68 (0.45, 1.04)	0.008	—
Lutein and zeaxanthin								
ER+	1315	1.00	0.96 (0.77, 1.20)	0.95 (0.76, 1.19)	0.96 (0.77, 1.20)	0.83 (0.66, 1.05)	0.13	0.06
ER−	292	1.00	1.01 (0.68, 1.50)	0.87 (0.58, 1.32)	0.92 (0.61, 1.39)	1.41 (0.95, 2.09)	0.08	—
Well differentiated	350	1.00	1.25 (0.86, 1.81)	1.14 (0.77, 1.67)	1.42 (0.98, 2.05)	0.99 (0.66, 1.47)	0.91	0.86
Moderately differentiated	596	1.00	0.99 (0.74, 1.33)	1.02 (0.76, 1.37)	0.87 (0.64, 1.18)	0.92 (0.68, 1.25)	0.43	—
Poorly differentiated	372	1.00	0.74 (0.52, 1.05)	0.79 (0.56, 1.13)	0.75 (0.52, 1.07)	0.93 (0.66, 1.33)	0.92	—
Luminal A	646	1.00	1.16 (0.87, 1.54)	1.09 (0.82, 1.46)	1.18 (0.88, 1.57)	0.91 (0.67, 1.23)	0.42	0.25
Luminal B	216	1.00	0.55 (0.35, 0.86)	0.67 (0.43, 1.05)	0.76 (0.49, 1.17)	0.68 (0.43, 1.07)	0.37	—
Triple negative	108	1.00	1.24 (0.66, 2.32)	1.26 (0.66, 2.41)	0.87 (0.43, 1.77)	1.70 (0.90, 3.22)	0.20	—
Nonrecurrent and nonlethal	1851	1.00	0.93 (0.77, 1.14)	0.90 (0.73, 1.10)	0.94 (0.77, 1.15)	0.85 (0.69, 1.05)	0.17	0.65
Recurrent or lethal	300	1.00	0.93 (0.63, 1.37)	1.20 (0.82, 1.76)	0.68 (0.44, 1.05)	1.03 (0.69, 1.54)	0.83	—
Lycopene								
ER+	1313	1.00	1.07 (0.86, 1.32)	0.79 (0.63, 0.99)	0.90 (0.72, 1.12)	0.80 (0.64, 1.01)	0.02	0.53
ER−	291	1.00	1.09 (0.74, 1.62)	0.91 (0.61, 1.37)	0.88 (0.58, 1.32)	1.09 (0.74, 1.62)	0.92	—
Well differentiated	350	1.00	0.89 (0.64, 1.25)	0.75 (0.53, 1.07)	0.74 (0.52, 1.05)	0.56 (0.38, 0.83)	0.002	0.12
Moderately differentiated	595	1.00	1.24 (0.94, 1.65)	0.82 (0.60, 1.11)	1.07 (0.80, 1.44)	0.90 (0.67, 1.22)	0.26	—
Poorly differentiated	371	1.00	0.88 (0.62, 1.26)	0.75 (0.52, 1.08)	0.86 (0.60, 1.23)	1.00 (0.70, 1.41)	0.91	—
Luminal A	645	1.00	1.10 (0.84, 1.45)	0.87 (0.66, 1.16)	0.93 (0.70, 1.23)	0.75 (0.56, 1.01)	0.02	0.24
Luminal B	216	1.00	1.04 (0.68, 1.60)	0.71 (0.44, 1.14)	1.00 (0.64, 1.54)	0.79 (0.50, 1.26)	0.31	—
Triple negative	107	1.00	0.91 (0.48, 1.74)	0.73 (0.38, 1.43)	0.76 (0.39, 1.48)	1.36 (0.75, 2.44)	0.27	—
Nonrecurrent and nonlethal	1848	1.00	1.03 (0.85, 1.26)	0.89 (0.73, 1.09)	0.90 (0.74, 1.11)	0.87 (0.71, 1.07)	0.08	—
Recurrent or lethal	299	1.00	1.01 (0.71, 1.45)	0.58 (0.38, 0.88)	0.79 (0.53, 1.16)	0.78 (0.53, 1.16)	0.11	0.13
Total carotenoids								
ER+	1309	1.00	0.93 (0.75, 1.15)	0.85 (0.68, 1.06)	0.78 (0.62, 0.98)	0.73 (0.58, 0.92)	0.003	0.20
ER−	291	1.00	0.79 (0.53, 1.18)	0.94 (0.64, 1.39)	0.83 (0.55, 1.24)	0.90 (0.60, 1.35)	0.76	—
Well differentiated	349	1.00	0.79 (0.56, 1.13)	0.82 (0.57, 1.16)	0.76 (0.53, 1.09)	0.61 (0.41, 0.89)	0.02	0.45

(Continued)

TABLE 4 (*Continued*)

		Quintile (μg/dL)						
	Cases, *n*	1	2	3	4	5	*P*-trend	*P*-heterogeneity[2]
Moderately differentiated	594	1.00	0.94 (0.71, 1.25)	0.84 (0.63, 1.13)	0.68 (0.50, 0.93)	0.86 (0.64, 1.16)	0.15	—
Poorly differentiated	370	1.00	0.92 (0.65, 1.30)	0.91 (0.64, 1.30)	0.88 (0.61, 1.26)	0.69 (0.47, 1.01)	0.06	—
Luminal A	645	1.00	0.99 (0.76, 1.31)	1.00 (0.76, 1.32)	0.80 (0.60, 1.08)	0.77 (0.57, 1.04)	0.03	0.15
Luminal B	215	1.00	0.91 (0.60, 1.39)	0.88 (0.57, 1.36)	0.69 (0.43, 1.09)	0.61 (0.38, 1.00)	0.02	—
Triple negative	107	1.00	1.11 (0.58, 2.14)	1.24 (0.65, 2.37)	0.91 (0.45, 1.83)	1.40 (0.73, 2.70)	0.41	—
Nonrecurrent and nonlethal	1844	1.00	0.92 (0.76, 1.13)	0.92 (0.75, 1.12)	0.79 (0.64, 0.97)	0.83 (0.68, 1.02)	0.04	0.02
Recurrent or lethal	297	1.00	0.75 (0.52, 1.09)	0.79 (0.54, 1.14)	0.68 (0.46, 1.00)	0.48 (0.31, 0.73)	0.001	—

[1]Multivariate unconditional logistic regression models were adjusted for matching factors; BMI at age 18 y; weight gain since age 18 y; ages at menarche, first birth, and menopause; parity; alcohol intake; history of benign breast disease; and family history of breast cancer. ER, estrogen receptor; NHS, Nurses' Health Study.

[2]*P*-heterogeneity across subtypes from polychotomous logistic regression.

DISCUSSION

In this large, nested, case-control study of plasma carotenoid concentrations and breast cancer risk, we observed significant modest inverse associations with α-carotene, β-carotene, and total carotenoids and a suggestive inverse association with lycopene. Associations were apparent for measures both <10 and ≥ 10 y before the diagnosis of breast cancer although associations with some carotenoids were suggestively stronger with distant measures. Associations were stronger in leaner women. Inverse associations with carotenoids were not different by ER status but were suggestively stronger for more-aggressive tumors, including poorly differentiated and luminal B tumors. α-Carotene, β-carotene, β-cryptoxanthin, and total carotenoids were associated with strong, significant reduced risks of recurrent or lethal tumors.

This study built on our recent pooled analysis (13) of plasma carotenoids and breast cancer risk with more than double the number of NHS cases and the investigation of carotenoid exposure timing and breast cancer subtypes. Our current observations of inverse associations with plasma α-carotene, β-carotene, and total carotenoid concentrations overall were in agreement with the pooled results. However, we did not observe a significant association with lutein and zeaxanthin concentrations in the current study and only a suggestive association with lycopene concentrations. Our results were also consistent with one (22) of 2 (22, 23) recent studies that were not included in the pooled analysis in which inverse associations were observed with α-carotene and β-carotene concentrations.

We investigated whether the carotenoid–breast cancer associations varied across exposures that may have contributed to oxidative stress because women with a greater likelihood of oxidative stress, such as smokers, may be more likely to benefit from high amounts of antioxidants such as carotenoids. Similar to the pooled analysis (13), we did not observe interactions with alcohol intake, menopausal status, or PMH use. Our ability to detect interactions with smoking was limited by the small number of current smokers. Although adiposity contributes to oxidative stress (24), in both pooled (13) and current analyses, we observed stronger inverse associations with plasma carotenoid concentrations in lean women than in overweight and obese women. However, in contrast to the pooled (13) results, we did not observe positive associations in overweight or obese women. Because fat-soluble carotenoids are stored in adipose tissue (25), and BMI is inversely associated with plasma carotenoid concentrations (2, 13), circulating carotenoid concentrations in overweight women likely incorporate more exposure misclassification. Although it is not clear if adipose carotenoids are bioavailable, there is evidence to suggest that an exchange with other tissue sources contributes to plasma concentrations (26).

To our knowledge, the Women's Health Initiative (22) is the only other study of multiple measures of carotenoids and breast cancer risk, although the time between measures ($\leq$6 y) and follow-up time (median: 8 y; maximum: 12 y) were shorter than in our study. Although associations with more-recent measures of α-carotene and β-carotene in the Women's Health Initiative were suggestively stronger, CIs for each time period (1–3, 2–4, and 3–5 y) before diagnosis were wide given the small number of cases (n = 190), and baseline α-carotene concentrations also were associated with significantly lower risk. In the current analysis, with the unique advantage of 2 samples collected 10 y apart, we examined proximate (<10 y before diagnosis) and distant (10–20 y before diagnosis) carotenoid concentrations. Although we reported very good reproducibility of carotenoids over a 2–3-y period (ICCs: 0.73–0.88) (27), the 10-y reproducibility was attenuated (ICCs: 0.39–0.54), which was similar to 15-y correlations published previously (28), which suggests that distant measures are not simply a proxy for recent exposure. Because of this, and because we observed inverse associations with both proximate and distant measures, carotenoids may play important roles both early and late in carcinogenesis. However, associations with lycopene and total carotenoids were suggestively stronger for distant than for proximate measures, which is consistent with the stronger dose-response observed with total carotenoids measured 10–15 y before diagnosis (compared with 1–9 y before diagnosis) in a previous publication of 295 cases (29). Together, these results suggest carotenoids may inhibit tumor initiation, which is compatible with hypothesized mechanisms, including the conversion of pro–vitamin A carotenoids to retinol, which regulates cell growth, differentiation, and apoptosis (8, 30, 31), and the antioxidant capacity to scavenge reactive oxygen species and prevent DNA damage (5–7, 32, 33). Although experimental evidence also suggested that carotenoids may inhibit progression after initiation (4–6), the observed suggested association with distant carotenoids measures warrants additional investigation.

Although we did not observe significant heterogeneity by ER status, we had fewer ER-negative cases than in the pooled analysis (n = 292 compared with 417) (13). RRs were comparably inverse for both ER-positive and ER-negative tumors, and carotenoids have inhibited the growth of both ER-positive and ER-negative cell lines (9). These findings support carotenoids as a modifiable risk factor for ER-negative as well as ER-positive breast cancer.

To our knowledge, this is the first study to investigate the associations with prediagnostic plasma carotenoid concentrations by molecular subtype or recurrent or lethal breast cancer. Although heterogeneity was NS, associations with plasma carotenoids tended to be stronger for luminal B than for luminal A tumors. For the subset of tumors that recurred or caused death, inverse associations with plasma carotenoids were considerably stronger with a >46% reduction in risk in top quintiles of α-carotene, β-carotene, β-cryptoxanthin, and total carotenoids. Although increased fruit and vegetable intake after diagnosis was not associated with breast cancer recurrence in the Women's Health Eating and Living (WHEL) Study, reduced risk of recurrence was observed with higher baseline (34) and cumulative (35) plasma carotenoids, which suggested that early concentrations may be associated with longer-term response. Although the WHEL Study assessed postdiagnostic carotenoid concentrations, it is possible, given that one measure of carotenoids is representative of longer-term exposure, that WHEL results also were reflective of prediagnostic concentrations as were assessed in our analysis. This issue of timing relative to diagnosis and breast cancer survival deserves additional study. The combination of these results and results of our prior pooled analysis (13) suggest that carotenoids may be particularly important for the prevention of aggressive, and deadly, breast tumors.

With many breast cancer cases, our study had several strengths. To our knowledge, it is the first comprehensive study of the importance of carotenoid exposure timing with blood samples collected 10 y apart and with 20 y of follow-up. It is also the first investigation of the associations by molecular subtype and recurrent or lethal breast cancer. However, there were also limitations in our study. Although we could not eliminate the possibility of residual confounding, the comprehensive information on breast cancer risk factors in the NHS allowed for thorough adjustment for potential confounders. Although only one blood sample was available for the majority of women, reproducibility over a 2–3-y period in the NHS is very good (27). In addition, we reduced measurement error by averaging the values of 2 blood samples 10 y apart when available. We had too few cases of HER2-type and basal-like tumors to examine these subtypes separately; future pooled analyses of these rarer subtypes would be beneficial. Although there are biologically plausible mechanisms through which carotenoids may reduce breast cancer risk (4–8, 31–33), it is possible that the observed association may have been due to other phytochemicals in fruit and vegetables correlated with carotenoids or an interaction between various phytochemicals (36).

In conclusion, the results of this large prospective analysis suggest that women with higher circulating carotenoid concentrations are at reduced breast cancer risk, particularly for tumors that are more aggressive and have worse prognosis. In addition, carotenoid measures both close to and more than a decade before diagnosis appear protective but may be particularly important for preventing tumor initiation. Although additional work is necessary to confirm the causal role of carotenoids, and the use of specific carotenoid supplements is not advised (37, 38), circulating carotenoid concentrations are responsive to changes in dietary intake of carotenoid-rich fruit and vegetables, such as carrots, sweet potatoes, leafy greens, and tomatoes (39–41). Because intake of fruit and vegetables is beneficial for many reasons, the association of higher plasma carotenoid concentrations with lower risk of aggressive and lethal breast cancer may encourage women to increase their consumption of carotenoid-rich fruit and vegetables.

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