

Selected antioxidants and risk of hormone receptor–defined invasive breast cancers among postmenopausal women in the Women’s Health Initiative Observational Study^{1–3}

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ABSTRACT

Background: Few studies have evaluated carotenoids and vitamins C and E in association with the risk of breast cancers defined by estrogen receptor (ER) and progesterone receptor (PR) status.

Objective: We examined the associations between dietary and supplemental intakes of these nutrients and risk of breast cancers jointly defined by both ER and PR status among postmenopausal women.

Design: Our investigation was conducted in the Women’s Health Initiative Observational Study. After following 84 805 women for an average of 7.6 y, 2879 incident invasive breast cancer cases had been ascertained, of whom 2509 had receptor data. We used Cox proportional hazards models to assess the associations of interest.

Results: Dietary α -carotene (highest versus lowest quintile: RR = 0.83; 95% CL = 0.70, 0.99; *P* for trend = 0.019), β -carotene (highest versus lowest quintile: RR = 0.78; 95% CL = 0.66, 0.94; *P* for trend = 0.021), and lycopene (highest versus lowest quintile: RR = 0.85; 95% CL = 0.73, 1.00; *P* for trend = 0.064) were inversely associated with risk of ER+PR+breast cancer, but not with other breast cancer groups jointly defined by ER and PR status. Total or supplemental β -carotene and dietary intakes of lutein+zeaxanthin and β -cryptoxanthin were not associated with breast cancers defined by ER and PR status. Vitamin E (regardless of source) and dietary vitamin C were not associated with breast cancer. However, total and supplemental vitamin C intake had weak positive associations with breast cancer overall.

Conclusion: Dietary intake of certain carotenoids might be differentially associated with risk of invasive breast cancers jointly defined by ER and PR status among postmenopausal women. *Am J Clin Nutr* 2008;87:1009–18.

INTRODUCTION

Epidemiologic studies assessing the associations between antioxidants in fruit and vegetables and breast cancer risk have yielded inconsistent results (1). The discrepancies may have resulted, to some extent, from the fact that breast cancers are heterogeneous, and from the fact that different breast cancers defined by certain characteristics (eg, menopausal status and hormone receptor status) may have different risk profiles. Therefore, studies in which breast cancer is analyzed as a single outcome might dilute or mask the associations for breast cancers defined by these characteristics, and studies conducted in different populations might yield different overall associations, depending on the composition of the breast cancer cases with respect to these characteristics (2).

Estrogen receptor (ER) and progesterone receptor (PR) are the most widely studied markers in breast tissue (2). Compared with ER-positive breast cancers, ER-negative breast cancers often have higher histologic grade, are less responsive to hormonal therapy, and are associated with worse survival (3). Conditional on ER status, PR-negative breast cancers have more aggressive features, are less responsive to hormonal therapy, and have worse survival than PR-positive breast cancers (4–6). These data suggest that hormone receptor–defined breast cancers are clinically distinct diseases and that knowing the ER and PR status has important clinical relevance. However, whether hormone receptor–defined breast cancers are etiologically different is not well understood.

Carotenoids (eg, α -carotene, β -carotene, lutein, zeaxanthin, β -cryptoxanthin, and lycopene) and vitamins C and E are antioxidants that protect DNA from oxidative damage and thereby may protect against carcinogenesis (7–9). In addition, they may protect against carcinogenesis via other mechanisms. For example, lycopene has been found to modulate immune function, and lutein has been found to selectively induce apoptosis of transformed cells (10, 11). Furthermore, α -carotene, β -carotene, and β -cryptoxanthin can be converted to vitamin A in the human body (7). Experimental studies have shown that vitamin A and its derivatives (retinoids) play important roles in regulating the growth, differentiation, and apoptosis of normal and malignant cells (12). However, previous epidemiologic studies of these antioxidants in association with breast cancer risk have yielded inconsistent findings, and few studies have assessed the associations stratified by hormone receptor status (1).

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We conducted a cohort study among postmenopausal women enrolled in the Women's Health Initiative (WHI) Observational Study to investigate the associations between the abovementioned antioxidants and breast cancers jointly defined by both ER and PR status. (See Appendix A for the list of investigators.) ER and PR status were examined jointly given the strong correlation that exists between them.

SUBJECTS AND METHODS

Study population

The study design, study implementation, and characteristics of the study population were described in detail elsewhere (13–15). In brief, the WHI Observational Study comprised a cohort of 93 676 postmenopausal women aged 50–79 y at enrollment. Recruitment of subjects into the study took place between October 1993 and December 1998 at 40 clinical centers in the United States. Women were eligible for inclusion in the study if they planned to live in their clinical center area for ≥ 3 y, had no medical conditions predictive of a survival time of < 3 y, had no conditions or characteristics inconsistent with study participation and adherence, and were not participating in any randomized controlled clinical trials. For the present investigation, women who reported a history of breast cancer ($n = 5298$) or had an estimated daily energy intake of < 600 or > 5000 kcal at baseline ($n = 3759$) were excluded. After these exclusions, 84 805 women were left for the present analysis. The study was approved by appropriate institutional review boards for study of human subjects.

Case ascertainment

Breast cancer patients were identified primarily through annually mailed medical-history update questionnaires. The questionnaires asked the participants to report all cancer-related hospitalization, surgeries or procedures, diagnoses, and outpatient treatments. Each first self-report of breast cancer was verified by medical record, and pathology reports were reviewed by physician adjudicators at each local clinical center. All breast cancers were then adjudicated by blinded review at the WHI Clinical Coordinating Center using the National Cancer Institute's Surveillance, Epidemiology, and End Results coding system (16). After an average follow-up of 7.6 y, 462 incident cases with breast cancer in situ and 2879 incident cases with invasive breast cancer had been identified among the 84 805 participants. Data on ER and PR status in the tumors were only available for cases with invasive breast cancer. Of the invasive breast cancers, 1760 were ER-positive and PR-positive (ER+PR+), 362 were ER-positive and PR-negative (ER+PR-), 37 were ER-negative and PR-positive (ER-PR+), 350 were ER-negative and PR-negative (ER-PR-), and 370 were undefined.

Exposure assessment

On enrollment, all participants completed extensive questionnaire information on demographic characteristics, personal habits, reproductive history, exogenous hormone use, medical history, family history, and dietary and supplement intake. Dietary intake of carotenoids and vitamins C and E was derived from a semiquantitative food-frequency questionnaire (FFQ) completed at baseline, which sought frequency and serving size of 122 food items consumed over the past 3 mo. The daily nutrient

intake from a given food was calculated by multiplying its portion size by the number of servings per day and its nutrient content. The daily nutrient intake for each study subject was then calculated by summing across all food items. The nutrient content values were derived from the University of Minnesota Nutrition Coding Center nutrient database (Nutrition Coordinating Center, Minneapolis, MN) (17). In addition, intakes of multiple vitamins and single supplements were recorded at baseline, from which supplemental intakes of β -carotene and vitamins C and E were estimated.

Statistical analysis

We used Cox proportional hazards models (using time-on-study as the time scale) to estimate hazard ratios (HRs) and 95% confidence limits (CLs) for the associations between carotenoids and vitamins C and E and risk of invasive breast cancer overall and after classification (jointly) by ER and PR status. Breast cancers that were ER-PR+ were not analyzed separately because of the small number of cases ($n = 37$). Cases contributed person-time to the study from their date of enrollment until the date of diagnosis of the invasive breast cancer, and noncases (participants who were censored) contributed person-time from their date of enrollment until the end of follow-up, date of death, date of withdrawal from the study, or date of having bilateral prophylactic mastectomy, whichever came first.

The abovementioned antioxidants were analyzed as total intakes (from diet and supplements combined), and from diet and supplements separately, with adjustment for total energy intake using the residual method (18). When assessing the association of invasive breast cancer risk with dietary intake of a specific antioxidant, its supplemental intake was controlled for in multivariate analysis. Likewise, when the association of invasive breast cancer risk with supplemental intake of a specific antioxidant was assessed, its dietary intake was controlled for. Similar estimates were yielded from models with or without mutual adjustment for dietary and supplemental intakes. Intake was categorized by quintiles to assess the associations of interest. Trend tests were performed by assigning an ordinal number to each category and modeling the resulting variable as a continuous variable. In multivariate analyses, we controlled for energy intake (continuous), age at baseline (continuous), ethnicity (non-Hispanic white, black, Hispanic, other), educational level (below college, some college, postcollege), age at menarche (< 12 , 12, 13, ≥ 14), age at menopause (< 40 , 40 to < 45 , 45 to < 50 , 50 to < 55 , ≥ 55 , missing), parity (nulliparous, 1, 2–4, ≥ 5), age at first full-term pregnancy (nulliparous, < 20 , 20–24, 25–29, ≥ 30 , missing), years of oral contraceptive use (0, $> 0-1$, $> 1-4$, $> 4-8$, > 8), years of postmenopausal hormone use (0, > 0 to < 5 , 5 to < 10 , 10 to < 15 , ≥ 15), body mass index (< 25 , 25 to < 30 , ≥ 30), physical activity (total metabolic equivalents per week: 0, $> 0-10$, $> 10-20$, > 20), alcohol drinking (never, former, current with > 0 to < 5 , 5 to < 10 , 10 to < 15 , or ≥ 15 g/d), dietary folate intake (quartiles), tobacco smoking (ever or never), hysterectomy (yes or no), bilateral oophorectomy (yes or no), history of benign breast disease (yes or no), and family history of breast cancer (yes or no). Because of their magnitude, missing values for age at menopause and age at first full-term pregnancy were analyzed as a separate category for each covariate.

To assess the differential associations of the abovementioned antioxidants with ER+PR+, ER+PR-, and ER-PR- invasive



breast cancers, polytomous logistic regression models were fitted, which allowed us to model the associations with different types of breast cancer simultaneously. Polytomous logistic regression models also allowed us to test the differences between regression coefficients directly, therefore testing the heterogeneity of the associations between the 3 breast cancer subtypes with respect to their associations with the nutrients of interest. Because polytomous logistic regression ignores person-years of follow-up, we also analyzed the data for each hormone receptor-defined invasive breast cancer separately by using Cox proportional hazards models. The results yielded from the Cox proportional hazards models agreed closely with those obtained from the polytomous logistic regression models, and estimates for HRs and 95% CIs yielded from the Cox proportional hazards models are presented. All statistical analyses were performed in SAS 9.1 (SAS Institute, Cary, NC). *P* values are 2-sided.

RESULTS

After an average follow-up of 7.6 y, we had identified 2879 new cases with invasive breast cancer among 84 805 participants eligible for the present analysis. Of these invasive breast cancers, 1760 were ER+PR+, 362 were ER+PR-, 37 were ER-PR+, 350 were ER-PR-, and 370 were undefined. The characteristics of invasive breast cancer cases overall, invasive breast cancer cases with ER+PR+, ER+PR-, and ER-PR-, and noncases are summarized in **Table 1**. Overall, invasive breast cancer cases were more likely than noncases to be non-Hispanic white, to have postcollege education, to have early age at menarche, to be nulliparous, to have late age at first full-term pregnancy, to have used more postmenopausal hormones, to have no history of hysterectomy or bilateral oophorectomy, to have family history of breast cancer, to have had benign breast disease, and to have consumed more tobacco and alcohol. ER+PR+ invasive breast cancer cases generally shared similar characteristics to ER+PR- cancers, whereas ER-PR- invasive breast cancer cases showed some differences from these other 2 types. Specifically, compared with cases with ER+PR+ and ER+PR-, cases with ER-PR- had less education and were more likely to have ≥ 5 children, to have used more oral contraceptives, to have had hysterectomy or bilateral oophorectomy, to be obese (BMI ≥ 30 kg/m²), and to have no history of tobacco smoking or alcohol drinking.

Of the carotenoids investigated here, median dietary intake was highest for lycopene (median = 6550, interquartile range = 5595 μ g/d) and β -carotene (median = 3425; interquartile range = 3003 μ g/d), whereas the intakes of lutein+zeaxanthin (median = 1423; interquartile range = 1078 μ g/d), α -carotene (median = 829, interquartile range = 854 μ g/d), and β -cryptoxanthin (median = 112; interquartile range = 101 μ g/d) were considerably lower. Approximately 47% of participants took supplemental β -carotene, at an average daily dose of 4500 μ g, whereas no supplemental α -carotene, lutein+zeaxanthin, β -cryptoxanthin, and lycopene were consumed. In quintile analyses, we observed inverse associations with risk of breast cancer overall for dietary β -carotene (highest versus lowest quintile: RR = 0.86; 95% CI = 0.75, 0.99; *P* for trend = 0.073) and α -carotene (highest versus lowest quintile: RR = 0.89; 95% CI = 0.78, 1.02; *P* for trend = 0.037) (**Table 2**). We also observed inverse associations with risk of ER+PR+ invasive breast cancer for dietary β -carotene (highest versus lowest quintile: RR = 0.78; 95% CI = 0.66, 0.94; *P* for

trend = 0.021), α -carotene (highest versus lowest quintile: RR = 0.83; 95% CI = 0.70, 0.99; *P* for trend = 0.019), and lycopene (highest versus lowest quintile: RR = 0.85; 95% CI = 0.73, 1.00; *P* for trend = 0.064). In contrast, no association between carotenoids and risk of ER+PR- and ER-PR- invasive breast cancers was observed. Decile analyses yielded similar results to those described above, with the exception that they also revealed a suggestive inverse association between dietary lutein+zeaxanthin intake and risk of ER+PR+ invasive breast cancer (highest versus lowest decile: RR = 0.80; 95% CI = 0.63, 1.03; *P* for trend = 0.069) (data not shown). Dietary α -carotene, β -carotene, lutein+zeaxanthin, and lycopene were highly correlated with each other (**Table 3**). When we included α -carotene, β -carotene, lutein+zeaxanthin, and lycopene as covariates, along with risk factors controlled in previous models, their inverse associations with ER+PR+ breast cancer disappeared.

The study participants consumed a daily average of 106 mg vitamin C from diet, and $\approx 60\%$ of them took supplemental vitamin C regularly at an average dose of 350 mg/d. Invasive breast cancer overall and its subtypes were not associated with dietary vitamin C intake (**Table 4**). Nevertheless, there was a positive association of total vitamin C intake with invasive breast cancer overall (highest versus lowest quintile: RR = 1.18; 95% CI = 1.04, 1.34; *P* for trend = 0.0092) and a suggestive positive association of total vitamin C intake with ER+PR+ (highest versus lowest quintile: RR = 1.19; 95% CI = 1.01, 1.41; *P* for trend = 0.087) and ER+PR- (highest versus lowest quintile: RR = 1.38; 95% CI = 0.97, 1.97; *P* for trend = 0.016) invasive breast cancers. Furthermore, supplemental vitamin C was positively associated with invasive breast cancer overall (highest versus lowest quintile: RR = 1.16; 95% CI = 1.04, 1.30; *P* for trend = 0.029), and there was some suggestion also of an increase in risk of ER+PR- invasive breast cancers (highest versus lowest quintile: RR = 1.25; 95% CI = 0.92, 1.69; *P* for trend = 0.062).

On average, the study participants consumed 7 mg/d of dietary vitamin E, and $\approx 60\%$ of them took supplemental vitamin E at an average dose of 230 mg/d. Overall, vitamin E intake, regardless of source, was not associated with altered risk of invasive breast cancer overall or defined by ER and PR status (**Table 4**).

DISCUSSION

In this large cohort study, we observed inverse associations of dietary α -carotene, β -carotene, and lycopene with ER+PR+ breast cancers, but not with other breast cancers defined by both ER and PR status. We also observed weak positive associations of total and supplemental vitamin C with breast cancer overall. However, we observed no associations of dietary intakes of lutein+zeaxanthin, β -cryptoxanthin, and vitamins C and E and supplemental intakes of β -carotene and vitamins C and E with breast cancers defined by ER and PR status.

The results of previous studies that assessed the associations between carotenoids and breast cancer risk have been conflicting. A meta-analysis (19) based on 7 case-control studies and 4 cohort studies published before 1997 concluded that dietary β -carotene was inversely associated with breast cancer risk (RR = 0.82; 95% CI = 0.76, 0.91). However, cohort studies (20–23) have generally observed little association. Although limited by their inconsistent results, studies that assessed the association by menopausal status have provided some evidence of an inverse



TABLE 1Characteristics of the study population in the Women's Health Initiative Observational Study¹

	Invasive breast cancer cases				Noncases (n = 81 926)
	All (n = 2879)	ER+PR+ (n = 1760)	ER+PR- (n = 362)	ER-PR- (n = 350)	
Mean age at baseline (y)	64.1	64.4	64.0	63.1	63.5
Ethnicity [n (%)]					
White	2563 (89.3)	1607 (91.7)	330 (91.2)	290 (83.1)	68 847 (84.3)
Black	154 (5.4)	55 (3.1)	16 (4.4)	39 (11.1)	6165 (7.6)
Hispanic	62 (2.1)	33 (1.9)	1 (0.3)	10 (2.9)	3062 (3.7)
Other	92 (3.2)	58 (3.3)	15 (4.1)	10 (2.9)	3630 (4.4)
Missing	8	7	0	1	222
Education [n (%)]					
Below college	766 (26.8)	454 (26.0)	96 (26.6)	105 (30.7)	25 071 (30.8)
Some college	1109 (38.9)	693 (39.7)	123 (34.2)	144 (42.1)	31 186 (38.4)
Postcollege	980 (34.3)	600 (34.3)	141 (39.2)	93 (27.2)	25 008 (30.8)
Missing	24	13	2	8	661
Age at menarche [n (%)]					
<12 y	681 (23.8)	409 (23.4)	84 (23.3)	91 (26.1)	17 934 (22.0)
12 y	747 (26.1)	460 (26.3)	92 (25.5)	93 (26.6)	21 256 (26.0)
13 y	790 (27.6)	490 (28.0)	96 (26.6)	91 (26.1)	23 819 (29.2)
≥14 y	645 (22.5)	390 (22.3)	89 (24.6)	74 (21.2)	18 570 (22.8)
Missing	16	11	1	1	347
Age at menopause [n (%)]					
<40 y	250 (9.5)	138 (8.6)	27 (8.1)	40 (12.5)	9376 (12.5)
40 to <45 y	282 (10.7)	167 (10.4)	38 (11.3)	38 (11.9)	10 113 (13.5)
45 to <50 y	536 (20.4)	329 (20.5)	62 (18.5)	78 (24.4)	16 123 (21.5)
50 to <55 y	981 (37.2)	602 (37.5)	134 (40.0)	103 (32.2)	26 392 (35.2)
≥55 y	584 (22.2)	368 (23.0)	74 (22.1)	61 (19.0)	12 903 (17.3)
Missing	246	156	27	30	7019
Parity [n (%)]					
Nulliparous	424 (14.9)	274 (15.7)	61 (16.9)	33 (9.5)	10 243 (12.6)
1	253 (8.9)	148 (8.5)	40 (11.1)	28 (8.1)	7357 (9.0)
2-4	1878 (65.8)	1165 (66.8)	221 (61.4)	234 (67.2)	52 780 (64.9)
≥5	298 (10.4)	156 (9.0)	38 (10.6)	53 (15.2)	10 950 (13.5)
Missing	26	17	2	2	596
Age at first full-term pregnancy among parous women [n (%)]					
<20 y	261 (11.8)	148 (11.0)	31 (11.8)	32 (11.5)	9217 (14.5)
20-24 y	999 (45.3)	598 (44.4)	125 (47.5)	135 (48.6)	30 144 (47.5)
25-29 y	671 (30.5)	420 (31.2)	76 (28.9)	89 (32.0)	17 981 (28.3)
≥30 y	272 (12.4)	180 (13.4)	31 (11.8)	22 (7.9)	6172 (9.7)
Missing	226	123	36	37	7573
Years of oral contraceptive use [n (%)]					
0 y	1732 (60.1)	1063 (60.4)	209 (57.7)	198 (56.6)	48 837 (59.6)
>0-1 y	326 (11.3)	194 (11.0)	46 (12.7)	37 (10.6)	10 339 (12.6)
>1-4 y	278 (9.7)	170 (9.7)	36 (9.9)	34 (9.7)	7982 (9.8)
>4-8 y	265 (9.2)	167 (9.5)	32 (8.9)	35 (10.0)	6596 (8.0)
>8 y	278 (9.7)	166 (9.4)	39 (10.8)	46 (13.1)	8147 (10.0)
Missing	0	0	0	0	25
Years of postmenopausal hormone use [n (%)]					
0 y	966 (33.6)	548 (31.1)	133 (36.7)	127 (36.3)	32 145 (39.2)
>0 to <5 y	577 (20.0)	350 (19.9)	71 (19.6)	72 (20.6)	17 876 (21.8)
5 to 10 y	466 (16.2)	301 (17.1)	56 (15.5)	54 (15.4)	11 328 (13.8)
10 to <15 y	349 (12.1)	220 (12.5)	53 (14.6)	33 (9.4)	8652 (10.6)
≥15 y	521 (18.1)	341 (19.4)	49 (13.6)	64 (18.3)	11 923 (14.6)
Missing	0	0	0	0	2
Had hysterectomy [n (%)]					
No	1811 (63.0)	1136 (64.6)	233 (64.4)	189 (54.3)	47 737 (58.3)
Yes	1064 (37.0)	622 (35.4)	129 (35.6)	159 (45.7)	34 112 (41.7)
Missing	4	2	0	2	77

(Continued)

TABLE 1 (Continued)

	Invasive breast cancer cases				Noncases (n = 81 926)
	All (n = 2879)	ER+PR+ (n = 1760)	ER+PR- (n = 362)	ER-PR- (n = 350)	
Had bilateral oophorectomy [n (%)]					
No	2300 (81.4)	1416 (82.2)	295 (81.7)	265 (77.3)	63 752 (79.5)
Yes	525 (18.6)	307 (17.8)	66 (18.3)	78 (22.7)	16 428 (20.5)
Missing	54	37	1	7	1746
BMI [n (%)]					
<25 kg/m ²	1155 (40.5)	677 (39.0)	171 (47.4)	145 (41.7)	33 266 (41.1)
25 to <30 kg/m ²	953 (33.5)	614 (35.4)	120 (33.2)	95 (27.3)	27 552 (34.0)
≥30 kg/m ²	741 (26.0)	444 (25.6)	70 (19.4)	108 (31.0)	20 146 (24.9)
Missing	30	25	1	2	962
Physical activity [n (%)]					
0 METs/wk	361 (12.7)	208 (12.0)	47 (13.1)	45 (13.0)	10 890 (13.5)
>0 to 10 METs/wk	1026 (36.1)	630 (36.2)	119 (33.1)	129 (37.3)	30 068 (37.1)
>10 to 20 METs/wk	763 (26.9)	486 (27.9)	109 (30.4)	77 (22.2)	19 863 (24.5)
>20 METs/wk	689 (24.3)	416 (23.9)	84 (23.4)	95 (27.5)	20 152 (24.9)
Missing	40	20	3	4	953
Family history of breast cancer [n (%)]					
No	2198 (76.5)	1360 (77.4)	275 (76.0)	263 (75.4)	67 369 (82.3)
Yes	675 (23.5)	397 (22.6)	87 (24.0)	86 (24.6)	14,461 (17.7)
Missing	6	3	0	1	96
History of benign breast disease [n (%)]					
No	1938 (69.0)	1188 (69.2)	231 (65.1)	254 (70.8)	62 555 (77.8)
Yes	872 (31.0)	530 (30.8)	124 (34.9)	105 (29.2)	17 831 (22.2)
Missing	69	42	7	8	1540
Ever smoked [n (%)]					
No	1344 (47.1)	808 (46.2)	160 (44.8)	180 (51.4)	41 092 (50.6)
Yes	1509 (52.9)	940 (53.8)	196 (55.2)	170 (48.6)	40 105 (49.4)
Missing	26	12	5	0	729
Alcohol drinking [n (%)]					
Never	259 (9.1)	152 (8.7)	31 (8.6)	40 (11.5)	8916 (11.0)
Former	469 (16.5)	280 (16.0)	55 (15.2)	73 (21.0)	15 064 (18.6)
Current					
>0 to <5 g/d	1162 (40.7)	703 (40.3)	128 (35.4)	152 (43.8)	33 645 (41.5)
5 to <10 g/d	332 (11.6)	202 (11.6)	54 (14.9)	35 (10.1)	8947 (11.0)
10 to <15 g/d	202 (7.1)	130 (7.4)	29 (8.0)	20 (5.8)	4835 (6.0)
≥15 g/d	428 (15.0)	279 (16.0)	65 (17.9)	27 (7.8)	9618 (11.9)
Missing	27	14	0	3	901
Mean dietary folate (μg/d)	258	259	268	252	254
Mean total energy (kcal)	1562	1584	1591	1573	1580

¹ ER, estrogen receptor; PR, progesterone receptor; MET, metabolic equivalents.

association for premenopausal women, but not for postmenopausal women. Three (22, 24, 25) of 4 (20, 22, 24, 25) studies among premenopausal women reported an inverse association between dietary β -carotene and breast cancer risk, whereas only 2 (26, 27) of 9 (22–24, 26–30) studies among postmenopausal women reported an inverse association. Although 2 case-control studies (31, 32) observed inverse associations of breast cancer risk with dietary intake of α -carotene, lutein+zeaxanthin, β -cryptoxanthin, and lycopene, other case-control studies (33–35) and cohort studies (20–22, 36) observed little association, especially among postmenopausal women. Similarly, studies of carotenoids measured in blood (29, 36–47) or in breast adipose tissue (48, 49) have also yielded inconsistent results for breast cancer risk, with null or inverse associations observed. Therefore, the cumulative data to date do not provide unequivocal support for inverse associations between carotenoids and breast cancer risk (50).

To date, only one study (1) has been published that evaluated the association between carotenoids and breast cancer risk by menopausal status and by both ER and PR status. This population-based case-control study observed no associations between dietary carotenoids and premenopausal breast cancer risk, but observed inverse associations for dietary α -carotene, β -carotene, lutein, and lycopene among postmenopausal women. Further analyses among postmenopausal women showed inverse associations between β -carotene and ER+PR+ and ER-PR- breast cancers, between lutein and ER+PR+ breast cancer, and between lycopene and ER-PR- breast cancer, although these analyses were limited by small number of cases. In addition, another study (36) found no association of dietary and plasma lycopene with breast cancer overall and with breast cancers positive for ERs and PRs. Our study results suggest inverse associations with dietary α -carotene, β -carotene, and lycopene only for ER+PR+ breast cancers among postmenopausal

TABLE 2

Associations between carotenoids and risk of invasive breast cancer overall, and by receptor status, among postmenopausal women in the Women's Health Initiative Observational Study¹

	All cases		ER+PR+		ER+PR-		ER-PR-		P for heterogeneity ³
	n	Adjusted RR (95% CL) ²	n	Adjusted RR (95% CL) ²	n	Adjusted RR (95% CL) ²	n	Adjusted RR (95% CL) ²	
Total β -carotene intake (μg) ⁴									
0 to <2775	548	1.0 (ref)	332	1.0 (ref)	65	1.0 (ref)	71	1.0 (ref)	
2775 to <4694	586	0.98 (0.87, 1.11)	341	0.92 (0.78, 1.08)	80	1.03 (0.73, 1.46)	74	1.09 (0.77, 1.54)	
4694 to <7061	567	0.95 (0.83, 1.07)	371	0.99 (0.85, 1.16)	59	0.77 (0.53, 1.11)	59	0.88 (0.60, 1.27)	0.62
7061 to <9903	588	0.97 (0.86, 1.10)	365	0.99 (0.84, 1.16)	77	0.92 (0.64, 1.31)	75	1.07 (0.75, 1.54)	
≥ 9903	590	0.95 (0.84, 1.09)	351	0.92 (0.78, 1.09)	81	0.96 (0.67, 1.38)	71	1.00 (0.68, 1.45)	
P for trend		0.51		0.71		0.65		0.96	
Dietary β -carotene intake (μg) ⁵									
0 to <2155	569	1.0 (ref)	358	1.0 (ref)	71	1.0 (ref)	63	1.0 (ref)	
2155 to <3043	568	0.95 (0.84, 1.07)	354	0.94 (0.81, 1.10)	58	0.68 (0.47, 0.97)	68	1.02 (0.71, 1.47)	
3043 to <4099	605	0.98 (0.86, 1.11)	363	0.92 (0.78, 1.08)	74	0.85 (0.60, 1.21)	78	1.25 (0.87, 1.79)	0.36
4099 to <5835	598	0.95 (0.83, 1.08)	372	0.94 (0.80, 1.11)	83	0.92 (0.65, 1.31)	67	1.02 (0.69, 1.50)	
≥ 5835	539	0.86 (0.75, 0.99)	313	0.78 (0.66, 0.94)	76	0.84 (0.57, 1.22)	74	1.12 (0.75, 1.68)	
P for trend		0.073		0.021		0.96		0.62	
Supplemental β -carotene intake (μg) ⁶									
0	1445	1.0 (ref)	866	1.0 (ref)	184	1.0 (ref)	183	1.0 (ref)	
>0 to <4430	350	1.03 (0.91, 1.16)	209	1.01 (0.86, 1.18)	39	0.90 (0.64, 1.27)	47	1.13 (0.80, 1.58)	
4430 to <4504	350	1.04 (0.92, 1.17)	237	1.15 (0.99, 1.34)	36	0.81 (0.56, 1.17)	31	0.79 (0.53, 1.19)	0.55
4504 to <5443	362	1.05 (0.93, 1.18)	235	1.11 (0.96, 1.30)	52	1.08 (0.78, 1.50)	42	1.08 (0.76, 1.53)	
≥ 5443	372	1.07 (0.95, 1.21)	213	1.03 (0.88, 1.20)	51	1.09 (0.79, 1.50)	47	1.14 (0.81, 1.60)	
P for trend		0.20		0.21		0.67		0.62	
Dietary α -carotene intake (μg) ⁴									
0 to <461	560	1.0 (ref)	346	1.0 (ref)	61	1.0 (ref)	61	1.0 (ref)	
461 to <718	574	0.96 (0.85, 1.09)	352	0.93 (0.80, 1.08)	76	1.13 (0.80, 1.61)	69	1.17 (0.81, 1.68)	
718 to <1020	625	1.00 (0.89, 1.13)	381	0.95 (0.81, 1.11)	72	0.96 (0.67, 1.38)	84	1.40 (0.97, 2.00)	0.64
1020 to <1531	553	0.88 (0.77, 1.00)	338	0.84 (0.71, 0.99)	75	0.98 (0.67, 1.41)	66	1.12 (0.76, 1.65)	
≥ 1531	567	0.89 (0.78, 1.02)	343	0.83 (0.70, 0.99)	78	1.00 (0.68, 1.46)	70	1.19 (0.80, 1.77)	
P for trend		0.037		0.019		0.70		0.55	
Dietary β -cryptoxanthin intake (μg) ⁴									
0 to <60	554	1.0 (ref)	336	1.0 (ref)	73	1.0 (ref)	60	1.0 (ref)	
60 to <96	545	0.97 (0.86, 1.10)	325	0.95 (0.81, 1.11)	60	0.77 (0.54, 1.09)	76	1.30 (0.91, 1.85)	
96 to <134	579	1.00 (0.88, 1.13)	364	1.03 (0.88, 1.20)	69	0.84 (0.60, 1.19)	60	0.99 (0.67, 1.45)	0.21
134 to <184	605	1.04 (0.91, 1.17)	359	1.02 (0.87, 1.20)	89	1.02 (0.73, 1.43)	82	1.39 (0.97, 2.01)	
≥ 184	596	1.08 (0.95, 1.23)	376	1.14 (0.97, 1.34)	71	0.83 (0.58, 1.19)	72	1.30 (0.89, 1.92)	
P for trend		0.14		0.08		0.88		0.18	
Dietary lutein and zeaxanthin intake (μg) ⁴									
0 to <1000	563	1.0 (ref)	359	1.0 (ref)	71	1.0 (ref)	62	1.0 (ref)	
1000 to <1290	598	1.04 (0.92, 1.17)	354	0.97 (0.83, 1.13)	73	0.93 (0.66, 1.31)	72	1.16 (0.81, 1.67)	
1290 to <1646	594	0.99 (0.87, 1.13)	373	0.98 (0.84, 1.16)	64	0.72 (0.49, 1.04)	79	1.28 (0.89, 1.86)	0.32
1646 to <2281	574	0.95 (0.83, 1.08)	353	0.93 (0.78, 1.10)	69	0.79 (0.54, 1.16)	68	1.06 (0.72, 1.58)	
≥ 2281	550	0.93 (0.80, 1.07)	321	0.86 (0.72, 1.04)	85	1.01 (0.69, 1.49)	69	1.06 (0.70, 1.62)	
P for trend		0.14		0.13		0.88		0.99	
Dietary lycopene intake (μg) ⁴									
0 to <4223	560	1.0 (ref)	355	1.0 (ref)	56	1.0 (ref)	69	1.0 (ref)	
4223 to <5973	569	0.98 (0.87, 1.10)	346	0.91 (0.78, 1.06)	75	1.30 (0.91, 1.85)	64	0.88 (0.61, 1.26)	
5973 to <7894	634	1.09 (0.97, 1.22)	382	1.00 (0.86, 1.16)	73	1.21 (0.84, 1.75)	80	1.21 (0.86, 1.69)	0.26
7894 to <10 824	561	0.95 (0.84, 1.08)	345	0.88 (0.75, 1.03)	72	1.16 (0.80, 1.68)	71	1.09 (0.76, 1.54)	
$\geq 10 824$	555	0.93 (0.82, 1.06)	332	0.85 (0.73, 1.00)	86	1.41 (0.99, 2.03)	66	0.91 (0.63, 1.32)	
P for trend		0.25		0.064		0.17		0.95	

¹ ER, estrogen receptor; PR, progesterone receptor; ref, referent; RR, relative risk; CL, confidence limit.

² RRs and 95% CLs were estimated in the Cox proportional hazards models.

³ Based on comparison of the 3 types of invasive breast cancers (ER+PR+, ER+PR-, and ER-PR-) and were calculated in the polytomous logistic regression models.

⁴ Adjusted for energy intake, age at baseline, ethnicity, educational level, age at menarche, age at menopause, parity, age at first full-term pregnancy, oral contraceptive use, postmenopausal hormone use, BMI, physical activity, alcohol drinking, dietary folate intake, tobacco smoking, hysterectomy, bilateral oophorectomy, history of benign breast disease, and family history of breast cancer.

⁵ Further adjustment for supplemental β -carotene intake.

⁶ Further adjustment for dietary β -carotene intake.



TABLE 3

Correlations between dietary α -carotene, β -carotene, lutein+zeaxanthin, and lycopene and their associations with estrogen-receptor-positive, progesterone-receptor-positive invasive breast cancer with mutual adjustment in the Women's Health Initiative Observational Study¹

	Correlation coefficient ²			RR (95% CL): highest vs lowest quintile ³	P for trend
	β -Carotene	Lutein+zeaxanthin	Lycopene		
α -Carotene	0.83	0.49	0.24	0.91 (0.71, 1.17)	0.31
β -Carotene		0.67	0.31	0.88 (0.66, 1.18)	0.66
Lutein+zeaxanthin			0.29	0.99 (0.79, 1.25)	0.99
Lycopene				0.89 (0.75, 1.05)	0.20

¹ RR, relative risk; CL, confidence limit.

² $P < 0.0001$ for all.

³ Adjusted for energy intake, age at baseline, ethnicity, educational level, age at menarche, age at menopause, parity, age at first full-term pregnancy, oral contraceptive use, postmenopausal hormone use, BMI, physical activity, alcohol drinking, dietary folate intake, tobacco smoking, hysterectomy, bilateral oophorectomy, history of benign breast disease, family history of breast cancer, and the nutrients listed in the table in the Cox proportional hazards models.

women. However, because of correlations between these nutrients, we were not able to separate their independent associations. The biological mechanisms for the observed differential associations with breast cancers jointly defined by ER and PR status are not well understood, although some *in vitro* studies (51, 52) have shown that vitamin A–derived retinoids inhibit the proliferation of ER-positive breast cancer cells, but not ER-negative breast cancer cells. Given the fact that an inverse association with ER+PR+ breast cancer was observed for dietary β -carotene but not for supplemental β -carotene, our study suggests that foods rich in β -carotene, rather than β -carotene itself, might account for these findings.

A meta-analysis (19) of 9 studies published before 1998 concluded that vitamin C was inversely associated with breast cancer risk (RR = 0.80; 95% CL = 0.68, 0.95). The inverse association was mainly driven by case-control studies. Indeed, cohort studies included in this meta-analysis (23, 28, 30, 53, 54) and other published cohort studies (20, 22, 23, 28) have yielded uniformly null results. Among postmenopausal women, Gaudet et al (1) found an inverse association with dietary vitamin C intake for ER+PR+ breast cancer, but not for other breast cancer groups defined by both ER and PR status. Our study observed little association between dietary vitamin C intake and postmenopausal breast cancer overall or by combined ER/PR status. However, we observed weak positive associations of vitamin C intake from all sources combined and supplemental vitamin C with breast cancer overall. There was some evidence of a slightly increased risk in association with vitamin C intake from all sources combined for ER+PR+ and ER+PR– breast cancers. Although vitamin C can increase oxidative damage under certain conditions, the overall evidence thus far supports no significant oxidative DNA damage with high consumption of supplemental vitamin C (8, 55). Therefore, our results were possibly a chance finding. Nevertheless, the possible increase in breast cancer risk among women with a high intake of supplemental vitamin C warrants further investigation in large cohort studies.

An inverse association between vitamin E intake and breast cancer risk has been observed in some case-control studies (24, 25, 27, 29, 31, 32, 56), but not all (33, 57, 58). In contrast, cohort studies (20, 22, 23, 28, 30, 53, 54) have consistently observed no association. Moreover, studies that have investigated breast cancer risk in association with vitamin E detected in blood (29, 37, 39, 40, 42, 44, 45) or in breast adipose tissue (49) also observed

no association. A large randomized controlled trial (59) showed that daily supplementation with natural-source vitamin E provided no benefit with regard to breast cancer incidence (RR = 1.00; 95% CI = 0.90, 1.12). The study by Gaudet et al (1) observed no association between dietary vitamin E and breast cancer overall, or stratified by menopausal status and hormone receptor status. In accordance with the study by Gaudet and colleagues, our study demonstrated no association between vitamin E intake and breast cancer overall or when defined by ER and PR status among postmenopausal women.

To our knowledge, this is the first cohort study to assess the association between carotenoids, vitamins C and E, and risk of postmenopausal breast cancer jointly defined by both ER and PR status. The strengths of our study include the prospective study design, the large sample size, essentially complete follow-up of the cohort, and comprehensive questionnaire data. We were able to collect detailed information on potential risk or protective factors for breast cancer. Estimates from adjusted analyses presented here were rather different from crude estimates (data not shown), which demonstrated the importance of controlling for potential confounders. The limitations of our study include the potential for error in measuring nutrient intake using FFQs (60), missing data on ER or PR status for 13% of the invasive breast cancer cases, and the possibility of residual confounding. Although invasive breast cancer cases with or without data on ER or PR status shared similar distributions for most breast cancer risk factors, they differed with respect to ethnic composition, use of oral contraceptives and postmenopausal hormones, body size, and physical activity. Compared with invasive breast cancer cases with ER or PR data, those without receptor data had a lower proportion of whites, were less likely to have used oral contraceptives and postmenopausal hormones, had a higher BMI, and were less physically active. Potential selection bias incurred by missing data for some of the breast cancer cases cannot be excluded. Dietary carotenoids and vitamins C and E are highly correlated with each other because they share common rich sources, which have limited our ability to separate their independent effects. Indeed, when we mutually adjusted for α -carotene, β -carotene, lutein+zeaxanthin, and lycopene in multivariate models, their inverse associations with ER+PR+ breast cancer disappeared. Furthermore, vegetables and fruit contain other biologically active compounds (eg, flavonoids), which might be correlated with the nutrients of interest, but were not controlled

TABLE 4Associations between vitamins C and E and risk of invasive breast cancer overall, and by receptor status, among postmenopausal women in the Women's Health Initiative Observational Study¹

	All cases		ER+PR+		ER+PR-		ER-PR-		P for heterogeneity ³
	n	Adjusted RR (95% CL) ²	n	Adjusted RR (95% CL) ²	n	Adjusted RR (95% CL) ²	n	Adjusted RR (95% CL) ²	
Total vitamin C intake (mg) ⁴									
0 to <97	508	1.0 (ref)	296	1.0 (ref)	58	1.0 (ref)	72	1.0 (ref)	
97 to <151	551	1.02 (0.90, 1.16)	346	1.11 (0.94, 1.31)	59	0.90 (0.61, 1.31)	66	0.89 (0.62, 1.27)	
151 to <240	594	1.11 (0.97, 1.26)	377	1.21 (1.02, 1.43)	71	1.04 (0.71, 1.51)	75	1.07 (0.75, 1.53)	0.62
240 to <686	573	1.07 (0.94, 1.22)	355	1.14 (0.96, 1.34)	77	1.13 (0.78, 1.62)	64	0.93 (0.64, 1.34)	
≥686	653	1.18 (1.04, 1.34)	386	1.19 (1.01, 1.41)	97	1.38 (0.97, 1.97)	73	1.05 (0.73, 1.51)	
P for trend		0.0092		0.087		0.016		0.71	
Dietary vitamin C intake (mg) ⁵									
0 to <67	544	1.0 (ref)	332	1.0 (ref)	61	1.0 (ref)	63	1.0 (ref)	
67 to <95	536	0.97 (0.85, 1.10)	332	0.98 (0.83, 1.15)	68	1.00 (0.69, 1.45)	63	1.02 (0.70, 1.48)	
95 to <123	607	1.04 (0.91, 1.18)	369	1.03 (0.87, 1.22)	81	1.13 (0.78, 1.63)	77	1.20 (0.83, 1.75)	0.92
123 to <158	615	1.06 (0.92, 1.21)	369	1.06 (0.89, 1.26)	81	1.04 (0.71, 1.53)	67	1.09 (0.73, 1.63)	
≥158	577	1.06 (0.92, 1.22)	358	1.09 (0.90, 1.31)	71	0.99 (0.65, 1.49)	80	1.41 (0.93, 2.13)	
P for trend		0.23		0.25		0.97		0.12	
Supplemental vitamin C intake (mg) ⁶									
0	1089	1.0 (ref)	645	1.0 (ref)	132	1.0 (ref)	151	1.0 (ref)	
>0 to <61	429	1.03 (0.91, 1.16)	286	1.15 (0.99, 1.32)	42	0.79 (0.55, 1.14)	42	0.76 (0.53, 1.09)	
61 to <347	420	0.99 (0.88, 1.11)	264	1.03 (0.89, 1.20)	53	0.92 (0.66, 1.29)	49	0.88 (0.63, 1.23)	0.26
347 to <711	443	1.04 (0.92, 1.16)	270	1.04 (0.90, 1.21)	66	1.21 (0.90, 1.64)	55	1.00 (0.72, 1.39)	
≥711	498	1.16 (1.04, 1.30)	295	1.13 (0.98, 1.30)	69	1.25 (0.92, 1.69)	53	1.03 (0.74, 1.42)	
P for trend		0.029		0.23		0.062		0.80	
Total vitamin E intake (mg) ⁴									
0 to <7.5	528	1.0 (ref)	300	1.0 (ref)	62	1.0 (ref)	75	1.0 (ref)	
7.5 to <22.2	574	1.03 (0.91, 1.16)	346	1.07 (0.91, 1.26)	73	1.14 (0.81, 1.62)	77	1.02 (0.73, 1.42)	
22.2 to <40.2	555	0.98 (0.86, 1.11)	356	1.08 (0.92, 1.26)	70	1.02 (0.72, 1.45)	62	0.83 (0.58, 1.19)	0.88
40.2 to <419	607	1.06 (0.94, 1.20)	393	1.16 (0.99, 1.35)	73	1.05 (0.74, 1.49)	64	0.94 (0.66, 1.33)	
≥419	615	1.04 (0.92, 1.17)	365	1.05 (0.90, 1.24)	84	1.15 (0.82, 1.62)	72	0.95 (0.67, 1.35)	
P for trend		0.45		0.36		0.62		0.64	
Dietary vitamin E intake (mg) ⁷									
0 to <6.2	553	1.0 (ref)	332	1.0 (ref)	74	1.0 (ref)	74	1.0 (ref)	
6.2 to <7.1	585	0.98 (0.86, 1.10)	349	0.97 (0.83, 1.13)	67	0.79 (0.56, 1.12)	80	1.01 (0.72, 1.41)	
7.1 to <8.0	589	1.03 (0.91, 1.17)	371	1.07 (0.92, 1.25)	65	0.85 (0.60, 1.19)	68	0.89 (0.63, 1.26)	0.39
8.0 to <9.4	577	1.03 (0.91, 1.17)	356	1.06 (0.91, 1.24)	71	0.96 (0.68, 1.34)	68	0.87 (0.61, 1.23)	
≥9.4	575	1.03 (0.91, 1.17)	352	1.04 (0.88, 1.23)	85	1.18 (0.84, 1.66)	60	0.85 (0.59, 1.23)	
P for trend		0.41		0.34		0.18		0.24	
Supplemental vitamin E intake (mg) ⁸									
0	1070	1.0 (ref)	626	1.0 (ref)	135	1.0 (ref)	147	1.0 (ref)	
>0 to <30	410	0.98 (0.87, 1.10)	253	1.01 (0.87, 1.18)	47	0.87 (0.62, 1.23)	49	0.89 (0.63, 1.26)	
30 to <232	472	1.05 (0.94, 1.18)	313	1.16 (1.00, 1.33)	60	1.00 (0.72, 1.37)	50	0.91 (0.65, 1.27)	0.49
232 to <424	457	1.04 (0.92, 1.16)	296	1.11 (0.96, 1.29)	50	0.84 (0.60, 1.18)	49	0.90 (0.63, 1.27)	
≥424	470	1.01 (0.90, 1.14)	272	0.97 (0.84, 1.13)	70	1.11 (0.82, 1.51)	55	0.96 (0.69, 1.33)	
P for trend		0.55		0.57		0.81		0.64	

¹ ER, estrogen receptor; PR, progesterone receptor; CL, confidence limit; RR, relative risk; ref, referent.² RRs and 95% CLs were estimated in the Cox proportional hazards models.³ Based on comparison of the 3 types of invasive breast cancers (ER+PR+, ER+PR-, and ER-PR-) and were calculated in the polytomous logistic regression models.⁴ Adjusted for energy intake, age at baseline, ethnicity, educational level, age at menarche, age at menopause, parity, age at first full-term pregnancy, oral contraceptive use, postmenopausal hormone use, BMI, physical activity, alcohol drinking, dietary folate intake, tobacco smoking, hysterectomy, bilateral oophorectomy, history of benign breast disease, and family history of breast cancer.⁵ Further adjustment for supplemental vitamin C intake.⁶ Further adjustment for dietary vitamin C intake.⁷ Further adjustment for supplemental vitamin E intake.⁸ Further adjustment for dietary vitamin E intake.

for in the study. Last, although a large cohort of women were followed in this study, analytic power might still be limited for breast cancer subtypes other than ER+PR+.

In conclusion, in this large cohort of postmenopausal women, dietary intakes of α -carotene, β -carotene, and lycopene were inversely associated with invasive breast cancer positive for both



ERs and PRs, but not with other invasive breast cancer groups jointly defined by ER and PR status. Further investigations are warranted to assess the differential associations of hormone receptor-defined breast cancers with dietary carotenoids and carotenoids measured in blood or in breast adipose tissue.

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APPENDIX

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