Longitudinal study of serum carotenoid, retinol, and tocopherol concentrations in relation to breast cancer risk among postmenopausal women[1,2]

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ABSTRACT

Background: Prospective studies have examined the association of serum and plasma carotenoids and micronutrients and breast cancer; however, to date, studies have only assessed exposure at one point in time.

Objective: This study analyzed baseline and repeated serum measurements of carotenoids, retinol, and tocopherols to assess their associations with postmenopausal breast cancer risk.

Design: Serum concentrations of α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein + zeaxanthin, retinol, α-tocopherol, and γ-tocopherol were measured in a 6% sample of women in the Women's Health Initiative clinical trials at baseline and at years 1, 3, and 6 and in a 1% sample of women in the observational study at baseline and at year 3. The association of baseline compounds and breast cancer risk was estimated by Cox proportional hazards models. In addition, repeated measurements were analyzed as time-dependent covariates. Of 5450 women with baseline measurements, 190 incident cases of breast cancer were ascertained over a median of 8.0 y of follow-up.

Results: After multivariable adjustment, risk of invasive breast cancer was inversely associated with baseline serum α-carotene concentrations (hazard ratio for highest compared with the lowest tertile: 0.55; 95% CI: 0.34, 0.90; $P = 0.02$) and positively associated with baseline lycopene (hazard ratio: 1.47; 95% CI: 0.98, 2.22; $P = 0.06$). Analysis of repeated measurements indicated that α-carotene and β-carotene were inversely associated with breast cancer and that γ-tocopherol was associated with increased risk.

Conclusions: The present study, which was the first to assess repeated measurements of serum carotenoids and micronutrients in relation to breast cancer, adds to the evidence of an inverse association of specific carotenoids with breast cancer. The positive associations observed for lycopene and γ-tocopherol require confirmation. This trial was registered at clinicaltrials.gov as NCT00000611. *Am J Clin Nutr* 2009;90:162–9.

INTRODUCTION

Since the early 1980s, there has been great interest in whether the intake of compounds derived from plant sources might protect against common cancers (1). Hence, epidemiologic studies have assessed the association of dietary intake of fruit and vegetables, of specific antioxidant vitamins, and of blood concentrations of specific compounds with risk of breast cancer (1, 2). Such studies are challenging, given the difficulty of accurately characterizing an individual's habitual, predisease intake and circulating concentrations of specific compounds. Current evidence of a protective role of plant-derived compounds in breast carcinogenesis is generally weak (1, 2).

Several previous prospective epidemiologic studies have provided evidence of an inverse association of serum and plasma carotenoids with breast cancer risk (3–6), whereas others found no association (7, 8). In contrast, most prospective studies that have examined the association of serum and plasma vitamin E with breast cancer have been consistently null (3, 5–7), including the 2 studies that examined γ-tocopherol concentrations (5, 6).

Characterizing nutritional status over a long period of time is difficult, and a single measurement may not be adequate for this purpose (9). However, no study to date has included repeated measurements of serum concentrations of carotenoids, retinol, or tocopherols, which might provide a better measure of exposure over time (9). We therefore carried out an analysis of serum carotenoid, retinol, and vitamin E concentrations in relation to breast cancer risk using the 6% sample of subjects in the Women's Health Initiative (WHI) clinical trial who had repeated measurements of these compounds during follow-up and a 1% sample of women in the WHI observational study with measurements at baseline and in year 3.

SUBJECTS AND METHODS

Study population

The WHI is a large, prospective, multicenter study of factors that influence the health of postmenopausal women. It includes

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an observational study (n = 93,676) and 3 clinical trials (n = 68,132) of hormone therapy, dietary modification, and calcium plus vitamin D supplementation (10). Women were recruited at 40 clinical centers throughout the United States, largely via direct mailings, and were eligible to participate if they were postmenopausal, aged 50–79 y, likely to reside in their current residence for $\geq$3 y, and provided written informed consent. Enrollment took place between 1 October 1993 and 31 December 1998. The clinical trials had a number of additional eligibility requirements (11). In general, eligible women were first invited to enroll in the clinical trial component. Women who did not wish to be randomly assigned to an intervention or who were ineligible for the clinical trial component were then invited to participate in the observational study.

The present analysis is based on a 6% random sample of women in the clinical trials (n = 4396) who provided fasting blood samples at baseline and at years 1, 3, and 6 of follow-up and a 1% sample of women in the observational study (n = 1,054) who provided fasting blood samples at baseline and in year 3. The 6% random sample was stratified by age, clinical center, and hysterectomy status, with oversampling of minority groups to increase the numbers of black, Hispanic, and Asian-Pacific women. Approval for the WHI was obtained from institutional review boards at all clinical centers. All participants signed informed consent forms. All protocols and procedures were approved by institutional review boards at participating institutions.

Case ascertainment

In the clinical trial, cancer outcomes were ascertained through semiannual self-administered questionnaires and then confirmed by centralized review of pathology reports, discharge summaries, operative and radiology reports, and tumor registry abstracts. In the observational study, cancer outcomes were ascertained annually.

Laboratory methods

Blood samples were collected after an overnight fast (12 h) with minimal stasis and maintained at 4°C until plasma or serum was separated. Plasma or serum aliquots were then frozen at −70°C and sent on dry ice to the WHI central repository (Fisher BioServices, Rockville, MD) for storage at −70°C. Retinol, α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein + zeaxanthin, α-tocopherol, and γ-tocopherol were measured in serum by reverse-phase HPLC (12, 13). After the addition of an internal standard, serum was extracted into hexane and injected onto a C_{18} reverse-phase column. The analytes were measured at wavelengths of 292 and 452 nm. CVs were determined in pooled blood samples from 4 age-eligible female volunteers. The CVs for the 8 analytes ranged from 6.0 (α-tocopherol) to 20.4 (α-carotene).

Statistical analysis

Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% CIs for the associations between serum concentrations of carotenoids and micronutrients and risk of breast cancer, with duration of follow-up (days) as the time scale. For these analyses, study participants were considered to be at risk from their date of enrollment until the date of diagnosis of their breast cancer, termination of follow-up (12 September 2005), loss to follow-up, withdrawal from the study, or death, whichever occurred first. Event times of participants who had not developed breast cancer by the end of follow-up, who had died, or who had withdrawn from the study before the end of follow-up were censored.

Analyses using baseline data

In the first stage of the analysis we estimated the associations of breast cancer with baseline serum carotenoid and micronutrient concentrations. Tertiles of the 8 study variables were created based on their distribution in the total study population. Established risk factors and potential confounding variables included in multivariable analyses were as follows: age (continuous), education (less than high school graduate, high school graduate/some college, college graduate, or postcollege), ethnicity (white, black, or other), body mass index (in kg/m^2; $<$24.7, 24.7 to $<$28.1, 28.1 to $<$32.5, or $\geq$32.5), oral contraceptive use (ever or never), hormone therapy (ever or never), age at menarche (continuous), age at first birth ($<$20 y, 20–29 y, $\geq$30 y, or missing), age at menopause ($<$50 y, $\geq$50 y, or missing), alcohol (servings per week–continuous), family history of breast cancer (yes or no), history of breast biopsy (ever or never), physical activity (metabolic equivalent tasks–continuous), energy intake (continuous), and randomization status (for women in the clinical trial) in hormone therapy, calcium plus vitamin D, and dietary modification trials. Additional adjustment for total serum cholesterol and smoking did not materially affect the results. We analyzed each compound in separate models and in addition included all carotenoids and micronutrients in a single model and used the stepwise procedure to obtain adjusted estimates. Tests for trend were performed by assigning each tertile level its median value and modeling this variable as a continuous variable. All P values were 2-sided.

Intervention status may have affected the postbaseline measurement of the carotenoids and micronutrients or alternatively may have influenced the risk of breast cancer. For this reason, the main analyses were carried out in the total study population and in women who were not randomly assigned to any intervention (comparison group in the dietary modification and placebo groups in the hormone and calcium plus vitamin D trials and women in the observational study). The results were unchanged in the no-intervention group, and we present only the results for the total study population.

Analyses using longitudinal data

In the second stage of the analysis, the repeated measurements of the different biomarkers were analyzed by modeling them as time-dependent covariates in Cox proportional hazards model (14). With this approach, we evaluated the predictive value of measurements obtained 1–3, 2–4, and 3–5 y before the date of diagnosis of breast cancer and the mean of all available measurements. Measurements that were obtained within 1 y of diagnosis were excluded from all analyses because these values may have been influenced by the presence of subclinical disease. All analyses were carried out by using SAS software (version 9.1; SAS Institute, Cary, NC).

RESULTS

During a median follow-up of 8.0 y, a total of 190 breast cancer cases (153 invasive and 37 in situ) were ascertained among the 5450 women in the cohort. Approximately two-thirds of the women were not in any of the clinical trial intervention groups (136 cases and 3637 noncases).

Cases and noncases were not significantly different with respect to age and anthropometric variables (**Table 1**). Cases were significantly more likely to be non-Hispanic white and had significantly lower levels of physical activity than did noncases.

Pearson correlations between the 8 compounds ranged from 0.57 for α-carotene and β-carotene to −0.38 for α-tocopherol and γ-tocopherol (**Table 2**). For each compound, the correlation between baseline values and values at years 1, 3, and 6 decreased with increasing interval. For example, the correlations were 0.66, 0.53, and 0.49, respectively, for α-carotene and were 0.77, 0.67, 0.58, respectively, for γ-tocopherol.

With few exceptions, mean serum concentrations of carotenoids, retinol, and tocopherols did not differ between cases and noncases (**Table 3**). Mean α-carotene was significantly lower at baseline in cases than in noncases, and mean β-cryptoxanthin was significantly lower in cases than in noncases at year 6.

Women in the highest tertile of α-carotene had a significantly reduced risk of invasive breast cancer in the total sample (HR for highest compared with lowest tertile: 0.55; 95% CI: 0.34, 0.90; $P = 0.02$) but not of all breast cancer (invasive and in situ combined) (**Table 4**). However, after exclusion of cases diagnosed during the first 2 y of follow-up, the reduction in risk of invasive cancer was no longer significant, although it was still suggestive of an inverse association (HR of highest compared with lowest tertile: 0.65; 95% CI: 0.38, 1.13; $P = 0.18$). Baseline lycopene concentrations showed a positive association with invasive breast cancer (HR for highest compared with lowest tertile: 1.47; 95% CI: 0.98, 2.22; $P = 0.06$). However, after exclusion of cases diagnosed during the first 2 y of follow-up, the positive association was reduced and was no longer close to being statistically significant (HR: 1.30; 95% CI: 0.82, 2.07; $P = 0.22$). No other associations of baseline carotenoids or micronutrients with breast cancer risk were statistically significant. Inclusion of additional dietary variables in the model (intakes of fat, fiber, vegetables, and fruit) did not affect the results. Mutual adjustment for other compounds confirmed the inverse association of baseline α-carotene (HR: 0.47; 95% CI: 0.29, 0.79; $P = 0.001$) and the positive association of lycopene (HR: 1.70; 95% CI: 1.12, 2.60; $P = 0.009$) with invasive breast cancer.

Time-dependent covariate analysis showed that more recent measurements of α-carotene and β-carotene were inversely associated with risk of all breast cancers and of invasive breast cancer (**Table 5**), whereas the average of all measurements was not. The inverse association was strongest for 1–3 y before diagnosis and became weaker as the time lag increased. The HR for invasive breast cancer for women in the highest compared with the lowest tertile of α-carotene measured 1–3 y before diagnosis was 0.42 (95% CI: 0.23, 0.75; $P = 0.002$) and for β-carotene the HR was 0.34 (95% CI: 0.19, 0.61; $P = 0.0002$). Borderline inverse associations of lutein + zeaxanthin with invasive breast cancer and of α-tocopherol with all breast cancer and invasive breast were noted for measurements taken 1–3 y before diagnosis only. The average of all γ-tocopherol measurements was significantly and positively associated with risk of all breast cancer and invasive breast cancer: HRs for the highest compared with the lowest tertile were 1.58 (95% CI: 1.03, 2.41; $P = 0.03$) and 1.71 (95% CI: 1.08, 2.73; $P = 0.03$), respectively.

TABLE 1
Baseline characteristics of breast cancer cases and noncases in the Women's Health Initiative

	Cases (n = 190)	Noncases (n = 5260)	P value
Age (y)	62.7 ± 7.2[1]	62.6 ± 6.6	0.55
BMI (kg/m^2)	29.1 ± 5.7	29.1 ± 6.1	0.86
Height (cm)	161.4 ± 6.3	161.0 ± 6.7	0.77
Waist circumference (cm)	88.5 ± 12.9	88.6 ± 14.0	0.88
Waist-to-hip ratio	0.82 ± 0.07	0.82 ± 0.08	0.48
Parity	2.6 ± 1.7	2.7 ± 1.7	0.26
Age at menopause (y)	47.6 ± 6.5	46.7 ± 6.8	0.14
Alcohol (servings/wk)	1.6 ± 3.2	1.7 ± 4.0	0.81
Physical activity (METs)[2]	7.3 ± 10.4	10.0 ± 12.9	0.0002
Oral contraceptive use, ever (%)	37.1	41.7	0.19
Hormone therapy use, ever (%)	50.3	46.6	0.31
History of diabetes, yes (%)	13.6	10.4	0.19
Age at menarche ≤12 y (%)	51.0	46.9	0.38
Age at first birth ≥30 y (%)	10.1	9.0	0.27
Breast cancer in a first-degree family member, yes (%)	16.2	15.4	0.74
Education, some postcollege (%)	27.0	23.9	0.72
Ethnicity, non-Hispanic white (%)	61.9	51.8	0.02
Smoking, current smokers (%)	6.7	8.4	0.65

[1] Mean ± SD (all such values).

[2] METs, metabolic equivalent tasks (defined as caloric need per kilogram of body weight per hour of activity divided by the caloric need per kilogram of body weight per hour at rest) per hour per week.

DISCUSSION

In this prospective cohort study, baseline serum α-carotene was associated with a reduced risk of invasive breast cancer, but not with breast cancer overall (invasive and in situ combined), and lycopene was associated with an increased risk of invasive cancer. Baseline concentrations of the other compounds were not associated with risk. In the time-dependent analyses, both α-carotene and β-carotene concentrations measured 1–3 y before diagnosis showed strong inverse associations with all breast cancer and with invasive breast cancer alone. In contrast with these inverse associations, the average of all γ-tocopherol measurements was positively associated with risk of all breast cancer and invasive breast cancer.

All of the previous cohort studies of the association of serum or plasma carotenoids, retinol, and tocopherols with breast cancer risk used a single baseline measure only and have shown mixed results. Of 6 nested case-control studies with ≥100 breast cancer cases (3–8), 4 showed evidence of significant or borderline inverse associations of specific carotenoids with breast cancer (3–6), whereas 2 studies (7, 8) showed no evidence of an association. Of the studies that suggested an inverse association, the findings differed for specific carotenoids. Dorgan et al (3) reported a significant inverse association of lycopene with breast cancer after adjustment for other carotenoids, nonsignificant inverse associations for lutein + zeaxanthin and β-cryptoxanthin, and no association of retinol or α- or β-carotene.

TABLE 2
Correlations between baseline serum carotenoids, retinol, and tocopherols in the Women's Health Initiative (n = 5450)

	α-Carotene	β-Carotene	β-Cryptoxanthin	Lycopene	Lutein + zeaxanthin	α -Tocopherol	γ-Tocopherol
α-Carotene							
β-Carotene	0.57[1]						
β-Cryptoxanthin	0.28[1]	0.33[1]					
Lycopene	0.20[1]	0.16[1]	0.13[1]				
Lutein + zeaxanthin	0.34[1]	0.29[1]	0.32[1]	0.21[1]			
α-Tocopherol	0.13[1]	0.24[1]	0.15[1]	0.11[1]	0.18[1]		
γ-Tocopherol	−0.25[1]	−0.30[1]	−0.16[1]	0.004[2]	−0.09[1]	−0.38[1]	
Retinol	0.04[3]	0.05[4]	0.04[5]	0.07[1]	0.07[1]	0.35[1]	−0.06[1]

[1] $P < 0.0001$.
[2] $P = 0.004$.
[3] $P = 0.006$.
[4] $P = 0.0002$.
[5] $P = 0.005$.

Toniolo et al (4) observed significant inverse associations of total carotenoids, α-carotene, β-carotene, lutein, and β-cryptoxanthin with breast cancer, whereas zeaxanthin, lycopene, and retinol were not associated with risk; however, individual associations were not adjusted for the effects of the other carotenoids and micronutrients. Sato et al (5) found a significant inverse association for β-carotene and nonsignificant inverse associations for lycopene and total carotenoids. However, as in the study by Toniolo et al (4), the individual associations were not mutually adjusted. Finally, Tamimi et al (6) reported a significant inverse association principally for α-carotene after controlling for other compounds. Our baseline results are generally consistent with those of Tamimi et al (6). Also in agreement with previous studies, we found no association of baseline serum retinol (3–7) or α- or γ-tocopherol (3, 5–7) with risk of breast cancer.

TABLE 3
Mean serum retinol, carotenoid, and tocopherol concentrations at baseline and years 1, 3, and 6 according to case and noncase status in the Women's Health Initiative[1]

	Year			
	0[2]	1	3	6
Retinol (μg/mL)				
Cases	0.61 ± 0.15 (190)	0.62 ± 0.16 (135)	0.61 ± 0.16 (95)	0.67 ± 0.18 (43)
Noncases	0.60 ± 0.16 (5260)	0.60 ± 0.15 (3883)	0.60 ± 0.16 (3414)	0.63 ± 0.17 (3157)
α-Carotene (μg/mL)				
Cases	0.075 ± 0.052[3] (190)	0.076 ± 0.059 (135)	0.053 ± 0.071 (95)	0.060 ± 0.055 (43)
Noncases	0.086 ± 0.083 (5260)	0.080 ± 0.075 (3882)	0.066 ± 0.70 (3414)	0.069 ± 0.069 (3156)
β-Carotene (μg/mL)				
Cases	0.30 ± 0.29 (190)	0.29 ± 0.24 (135)	0.29 ± 0.31 (95)	0.28 ± 0.26 (43)
Noncases	0.33 ± 0.33 (5260)	0.32 ± 0.30 (3882)	0.31 ± 0.34 (3414)	0.33 ± 0.37 (3156)
β-Cryptoxanthin (μg/mL)				
Cases	0.10 ± 0.10 (190)	0.09 ± 0.07 (135)	0.10 ± 0.08 (95)	0.09 ± 0.07[4] (43)
Noncases	0.10 ± 0.11 (5260)	0.10 ± 0.10 (3882)	0.11 ± 0.10 (3414)	0.12 ± 0.14 (3156)
Lycopene (μg/mL)				
Cases	0.42 ± 0.19 (190)	0.38 ± 0.19 (135)	0.35 ± 0.20 (95)	0.36 ± 0.21 (43)
Noncases	0.41 ± 0.20 (5260)	0.39 ± 0.19 (3885)	0.37 ± 0.20 (3414)	0.37 ± 0.20 (3156)
Lutein + zeaxanthin (μg/mL)				
Cases	0.22 ± 0.10 (190)	0.23 ± 0.10 (135)	0.20 ± 0.08 (95)	0.19 ± 0.09 (43)
Noncases	0.22 ± 0.11 (5260)	0.22 ± 0.11 (3883)	0.21 ± 0.10 (3414)	0.20 ± 0.11 (3256)
α-Tocopherol (μg/mL)				
Cases	16.79 ± 7.38 (190)	17.14 ± 7.53 (135)	17.40 ± 8.00 (95)	18.99 ± 8.66 (43)
Noncases	16.60 ± 7.72 (5260)	16.84 ± 7.74 (3883)	17.95 ± 8.25 (3414)	18.38 ± 8.32 (3157)
γ-Tocopherol (μg/mL)				
Cases	2.16 ± 1.28 (190)	1.78 ± 1.01 (135)	1.69 ± 0.96 (95)	1.51 + 0.98 (43)
Noncases	2.11 ± 1.43 (5260)	1.93 ± 1.40 (3882)	1.75 ± 1.32 (3414)	1.71 + 1.27 (3157)

[1] All values are means ± SDs; n in parentheses. Cases include both invasive and in situ breast cancer. Only incident cases diagnosed after blood drawing are included.
[2] Baseline.
[3] $P = 0.005$.
[4] $P = 0.02$.

TABLE 4
Adjusted hazard ratios (HRs) and 95% CIs for the association of baseline fasting serum retinol, carotenoids, and tocopherols with breast cancer in the Women's Health Initiative[1]

	All cases (n = 190)		Invasive cases (n = 153)
Baseline analyte	Age-adjusted HR (95% CI)	MV-adjusted HR (95% CI)[2]	MV-adjusted HR (95% CI)[2]
Retinol			
<0.53 μg/mL	1.00 (ref)	1.00 (ref)	1.00 (ref)
0.53 to <0.65 μg/mL	1.12 (0.79, 1.59)	1.11 (0.76, 1.61)	1.11 (0.73, 1.69)
≥0.65 μg/mL	1.12 (0.78, 1.60)	1.00 (0.68, 1.48)	0.96 (0.62, 1.49)
P for trend	0.54	0.98	0.84
α-Carotene			
<0.04 μg/mL	1.00 (ref)	1.00 (ref)	1.00 (ref)
0.04 to <0.09 μg/mL	1.13 (0.80, 1.58)	0.99 (0.68, 1.44)	1.00 (0.67, 1.49)
≥0.09 μg/mL	0.85 (0.60, 1.22)	0.75 (0.49, 1.15)	0.55 (0.34, 0.90)
P for trend	0.40	0.19	0.02
β-Carotene			
<0.17 μg/mL	1.00 (ref)	1.00 (ref)	1.00 (ref)
0.17 to <0.33 μg/mL	1.04 (0.74, 1.46)	1.06 (0.73, 1.54)	1.01 (0.67, 1.53)
≥0.33 μg/mL	0.82 (0.57, 1.18)	0.95 (0.63, 1.43)	0.78 (0.49, 1.24)
P for trend	0.29	0.82	0.30
β-Cryptoxanthin			
<0.06 μg/mL	1.00 (ref)	1.00 (ref)	1.00 (ref)
0.06 to <0.10 μg/mL	1.14 (0.80, 1.63)	1.24 (0.85, 1.82)	1.17 (0.76, 1.78)
≥0.10 μg/mL	1.16 (0.81, 1.65)	1.28 (0.86, 1.92)	1.14 (0.73, 1.79)
P for trend	0.42	0.23	0.56
Lycopene			
<0.30 μg/mL	1.00 (ref)	1.00 (ref)	1.00 (ref)
0.30 to <0.47 μg/mL	1.11 (0.77, 1.59)	1.02 (0.69, 1.51)	0.96 (0.62, 1.50)
≥0.47 μg/mL	1.30 (0.91, 1.84)	1.34 (0.92, 1.94)	1.47 (0.98, 2.22)
P for trend	0.14	0.12	0.06
Lutein + zeaxanthin			
<0.16 μg/mL	1.00 (ref)	1.00 (ref)	1.00 (ref)
0.16 to <0.24 μg/mL	0.86 (0.60, 1.21)	0.76 (0.52, 1.12)	0.71 (0.46, 1.09)
≥0.24 μg/mL	0.93 (0.66, 1.31)	1.00 (0.68, 1.46)	0.91 (0.59, 1.38)
P for trend	0.69	0.97	0.62
α-Tocopherol			
<12.36 μg/mL	1.00 (ref)	1.00 (ref)	1.00 (ref)
12.36 to <17.23 μg/mL	1.03 (0.72, 1.46)	1.05 (0.72, 1.52)	0.94 (0.62, 1.43)
≥17.23 μg/mL	1.04 (0.73, 1.48)	0.92 (0.62, 1.36)	0.88 (0.57, 1.35)
P for trend	0.84	0.67	0.55
γ-Tocopherol			
<1.30 μg/mL	1.00 (ref)	1.00 (ref)	1.00 (ref)
1.30 to <2.55 μg/mL	1.13 (0.79, 1.61)	1.11 (0.76, 1.63)	1.07 (0.70, 1.64)
≥2.55 μg/mL	1.27 (0.89, 1.81)	1.26 (0.85, 1.88)	1.34 (0.86, 2.07)
P for trend	0.18	0.25	0.20

[1] MV, multivariate; ref, reference.

[2] Adjusted for the following variables: age (continuous), education (less than high school, high school graduate/some college, college graduate, or postcollege), ethnicity (white, black, or other), BMI (in kg/m^2; <24.7, 24.7 to <28.1, 28.1 to <32.5, or ≥32.5), oral contraceptive use (ever or never), hormone therapy (ever or never), age at menarche (continuous), age at first birth (<20 y, 20–29 y, ≥30 y, or missing), age at menopause (<50 y, ≥50 y, or missing), alcohol (servings per week—continuous), family history of breast cancer (yes or no), history of breast biopsy (ever or never), physical activity (metabolic equivalent tasks), energy intake (continuous), and randomization status in hormone therapy, calcium plus vitamin D, and dietary modification trials.

Many factors may be responsible for the weak and inconsistent results of previous studies, including possible confounding by dietary and nondietary factors and the inadequacy of a single measurement to represent average concentrations over a period of years (9). Two previous studies have examined the reproducibility of serum carotenoids and micronutrients over periods of 3 y (4) and 15 y (15), respectively, and reported generally high levels of reliability. Intraclass correlation coefficients in the present study ranged from 0.51 for lycopene to 0.68 for retinol, which indicated moderate reliability. This level of reliability suggests that a single baseline measurement would be an imperfect indicator of a subject's underlying "true" value.

Our time-dependent covariate analysis yielded a somewhat different picture from the baseline analysis. More recent measurements of α-carotene (1–3 y before diagnosis) showed a stronger inverse association with both all breast cancer and invasive cancer alone than baseline concentrations. Furthermore, serum β-carotene concentrations 1–3 y before diagnosis were also strongly predictive of reduced risk of all breast cancer and invasive breast cancer, whereas baseline β-carotene concentrations

TABLE 5
Adjusted hazard ratios (HRs) and 95% CIs for the association of fasting serum retinol, carotenoids, and tocopherols with breast cancer in the Women's Health Initiative[1]

Analyte	HR (95% CI) All cases (n = 190)	Invasive cases (n = 153)
Retinol		
Average		
<0.53 μg/mL	1.00 (ref)	1.00 (ref)
0.53 to <0.65 μg/mL	0.89 (0.60, 1.31)	0.88 (0.57, 1.37)
≥0.65 μg/mL	0.98 (0.66, 1.45)	1.02 (0.66, 1.58)
P for trend	0.93	0.89
1–3 y		
<0.53 μg/mL	1.00 (ref)	1.00 (ref)
0.53 to <0.65 μg/mL	1.06 (0.66, 1.68)	1.07 (0.65, 1.78)
≥0.65 μg/mL	0.91 (0.56, 1.48)	0.86 (0.50, 1.47)
P for trend	0.69	0.58
2–4 y		
<0.53 μg/mL	1.00 (ref)	1.00 (ref)
0.53 to <0.65 μg/mL	0.97 (0.59, 1.61)	1.13 (0.64, 1.98)
≥0.65 μg/mL	1.03 (0.62, 1.70)	1.10 (0.62, 1.94)
P for trend	0.91	0.76
3–5 y		
<0.53 μg/mL	1.00 (ref)	1.00 (ref)
0.53 to <0.65 μg/mL	0.84 (0.51, 1.38)	0.80 (0.45, 1.43)
≥0.65 μg/mL	0.94 (0.57, 1.54)	1.02 (0.59, 1.78)
P for trend	0.79	0.93
α-Carotene		
Average		
<0.04 μg/mL	1.00 (ref)	1.00 (ref)
0.04 to <0.09 μg/mL	0.98 (0.67, 1.43)	0.99 (0.66, 1.50)
≥0.09 μg/mL	0.86 (0.56, 1.34)	0.67 (0.40, 1.12)
P for trend	0.51	0.14
1–3 y		
<0.04 μg/mL	1.00 (ref)	1.00 (ref)
0.04 to <0.09 μg/mL	0.64 (0.41, 1.01)	0.54 (0.33, 0.89)
≥0.09 μg/mL	0.48 (0.28, 0.82)	0.42 (0.23, 0.75)
P for trend	0.005	0.002
2–4 y		
<0.04 μg/mL	1.00 (ref)	1.00 (ref)
0.04 to <0.09 μg/mL	0.94 (0.58, 1.52)	0.80 (0.47, 1.37)
≥0.09 μg/mL	0.70 (0.40, 1.23)	0.59 (0.31, 1.11)
P for trend	0.23	0.10
3–5 y		
<0.04 μg/mL	1.00 (ref)	1.00 (ref)
0.04 to <0.09 μg/mL	0.96 (0.59, 1.57)	0.90 (0.53, 1.54)
≥0.09 μg/mL	0.84 (0.48, 1.44)	0.59 (0.31, 1.11)
P for trend	0.53	0.11
β-Carotene		
Average		
<0.17 μg/mL	1.00 (ref)	1.00 (ref)
0.17 to <0.33 μg/mL	0.88 (0.60, 1.29)	0.93 (0.61, 1.41)
≥0.33 μg/mL	0.84 (0.56, 1.27)	0.70 (0.44, 1.12)
P for trend	0.41	0.14
1–3 y		
<0.17 μg/mL	1.00 (ref)	1.00 (ref)
0.17 to <0.33 μg/mL	0.73 (0.47, 1.14)	0.62 (0.28, 1.00)
≥0.33 μg/mL	0.43 (0.26, 0.74)	0.34 (0.19, 0.61)
P for trend	0.002	0.0002
2–4 y		
<0.17 μg/mL	1.00 (ref)	1.00 (ref)
0.17 to <0.33 μg/mL	0.74 (0.46, 1.19)	0.61 (0.36, 1.04)
≥0.33 μg/mL	0.48 (0.27, 0.83)	0.40 (0.21, 0.74)
P for trend	0.008	0.003
3–5 y		
<0.17 μg/mL	1.00 (ref)	1.00 (ref)
0.17 to <0.33 μg/mL	0.82 (0.51, 1.32)	0.78 (0.46, 1.32)
≥0.33 μg/mL	0.58 (0.34, 0.99)	0.47 (0.25, 0.88)
P for trend	0.05	0.02
β-Cryptoxanthin		
Average		
<0.06 μg/mL	1.00 (ref)	1.00 (ref
0.06 to <0.10 μg/mL	1.05 (0.71, 1.57)	0.95 (0.61, 1.48)
≥0.10 μg/mL	1.13 (0.76, 1.68)	0.95 (0.61, 1.48)
P for trend	0.55	0.82
1–3 y		
<0.06 μg/mL	1.00 (ref)	1.00 (ref)
0.06 to <0.10 μg/mL	0.94 (0.58, 1.53)	0.84 (0.49, 1.44)
≥0.10 μg/mL	0.91 (0.57, 1.47)	0.87 (0.52, 1.46)
P for trend	0.71	0.59
2–4 y		
<0.06 μg/mL	1.00 (ref)	1.00 (ref)
0.06 to <0.10 μg/mL	1.04 (0.63, 1.74)	1.00 (0.57, 1.76)
≥0.10 μg/mL	0.99 (0.60, 1.64)	0.87 (0.49, 1.53)
P for trend	0.96	0.62
3–5 y		
<0.06 μg/mL	1.00 (ref)	1.00 (ref)
0.06 to <0.10 μg/mL	1.06 (0.64, 1.76)	1.07 (0.62, 1.86)
≥0.10 μg/mL	0.91 (0.55, 1.51)	0.71 (0.39, 1.28)
P for trend	0.72	0.27
Lycopene		
Average		
<0.30 μg/mL	1.00 (ref)	1.00 (ref)
0.30 to <0.47 μg/mL	1.10 (0.75, 1.61)	1.15 (0.75, 1.78)
≥0.47 μg/mL	1.19 (0.80, 1.78)	1.36 (0.87, 2.13)
P for trend	0.40	0.18
1–3 y		
<0.30 μg/mL	1.00 (ref)	1.00 (ref)
0.30 to <0.47 μg/mL	1.11 (0.71, 1.73)	1.17 (0.72, 1.92)
≥0.47 μg/mL	0.86 (0.52, 1.41)	0.96 (0.56, 1.64)
P for trend	0.59	0.92
2–4 y		
<0.30 μg/mL	1.00 (ref)	1.00 (ref)
0.30 to <0.47 μg/mL	1.00 (0.62, 1.61)	0.94 (0.55, 1.60)
≥0.47 μg/mL	0.88 (0.53, 1.47)	0.83 (0.47, 1.47)
P for trend	0.65	0.52
3–5 y		
<0.30 μg/mL	1.00 (ref)	1.00 (ref)
0.30 to <0.47 μg/mL	0.86 (0.52, 1.42)	0.81 (0.46, 1.43)
≥0.47 μg/mL	1.02 (0.63, 1.67)	1.04 (0.60, 1.80)
P for trend	0.95	0.91
Lutein + zeaxanthin		
Average		
<0.16 μg/mL	1.00 (ref)	1.00 (ref)
0.16 to <0.24 μg/mL	0.98 (0.67, 1.44)	0.99 (0.65, 1.51)
≥0.24 μg/mL	1.04 (0.69, 1.57)	0.87 (0.55, 1.39)
P for trend	0.85	0.57
1–3 y		
<0.16 μg/mL	1.00 (ref)	1.00 (ref)
0.16 to <0.24 μg/mL	0.88 (0.55, 1.40)	0.77 (0.47, 1.26)
≥0.24 μg/mL	0.83 (0.50, 1.36)	0.58 (0.33, 1.01)
P for trend	0.45	0.05

(Continued)

TABLE 5 (*Continued*)

Analyte	HR (95% CI) All cases (n = 190)	Invasive cases (n = 153)
2–4 y		
<0.16 μg/mL	1.00 (ref)	1.00 (ref)
0.16 to <0.24 μg/mL	0.81 (0.50, 1.33)	0.68 (0.39, 1.17)
≥0.24 μg/mL	0.79 (0.47, 1.32)	0.61 (0.34, 1.09)
P for trend	0.36	0.09
3–5 y		
<0.16 μg/mL	1.00 (ref)	1.00 (ref)
0.16 to <0.24 μg/mL	1.05 (0.64, 1.75)	0.96 (0.54, 1.69)
≥0.24 μg/mL	1.01 (0.60, 1.71)	0.95 (0.53, 1.71)
P for trend	0.98	0.85
α-Tocopherol		
Average		
<12.36 μg/mL	1.00 (ref)	1.00 (ref)
12.36 to <17.23 μg/mL	0.87 (0.58, 1.30)	0.82 (0.51, 1.29)
≥17.23 μg/mL	0.84 (0.56, 1.26)	0.84 (0.54, 1.33)
P for trend	0.41	0.52
1–3 y		
<12.36 μg/mL	1.00 (ref)	1.00 (ref)
12.36 to <17.23 μg/mL	1.09 (0.69, 1.74)	1.05 (0.63, 1.75)
≥17.23 μg/mL	0.62 (0.37, 1.04)	0.59 (0.33, 1.03)
P for trend	0.06	0.05
2–4 y		
<12.36 μg/mL	1.00 (ref)	1.00 (ref)
12.36 to <17.23 μg/mL	1.10 (0.67, 1.83)	1.16 (0.66, 2.06)
≥17.23 μg/mL	0.83 (0.49, 1.42)	0.82 (0.45, 1.51)
P for trend	0.46	0.47
3–5 y		
<12.36 μg/mL	1.00 (ref)	1.00 (ref)
12.36 to <17.23 μg/mL	0.93 (0.55, 1.56)	0.97 (0.54, 1.76)
≥17.23 μg/mL	0.93 (0.55, 1.55)	0.96 (0.54, 1.73)
P for trend	0.77	0.90
γ-Tocopherol		
Average		
<1.30 μg/mL	1.00 (ref)	1.00 (ref)
1.30 to <2.55 μg/mL	1.29 (0.89, 1.88)	1.21 (0.79, 1.85)
≥2.55 μg/mL	1.58 (1.03, 2.41)	1.71 (1.08, 2.73)
P for trend	0.03	0.03
1–3 y		
<1.30 μg/mL	1.00 (ref)	1.00 (ref)
1.30 to <2.55 μg/mL	1.22 (0.78, 1.91)	1.03 (0.62, 1.69)
≥2.55 μg/mL	1.30 (0.78, 2.16)	1.26 (0.73, 2.17)
P for trend	0.29	0.45
2–4 y		
<1.30 μg/mL	1.00 (ref)	1.00 (ref)
1.30 to <2.55 μg/mL	1.08 (0.67, 1.74)	1.09 (0.64, 1.86)
≥2.55 μg/mL	1.09 (0.64, 1.86)	1.07 (0.58, 1.95)
P for trend	0.74	0.81
3–5 y		
<1.30 μg/mL	1.00 (ref)	1.00 (ref)
1.30 to <2.55 μg/mL	1.31 (0.81, 2.12)	1.49 (0.86, 2.57)
≥2.55 μg/mL	1.27 (0.74, 2.18)	1.62 (0.88, 2.98)
P for trend	0.35	0.10

[1] Adjusted for the following variables: age (continuous), education (less than high school, high school graduate/some college, college graduate, or post-college), ethnicity (white, black, or other), BMI (in kg/m^2; <24.7, 24.7 to <28.1, 28.1 to <32.5, or ≥32.5), oral contraceptive use (ever or never), hormone therapy (ever or never), age at menarche (continuous), age at first birth (<20, 20–29, ≥30, or missing), age at menopause (<50 y, ≥50 y, or missing), alcohol (servings per week—continuous), family history of breast cancer (yes or no), history of breast biopsy (ever or never), physical activity (metabolic equivalent tasks), energy intake (continuous), and randomization status in hormone therapy, calcium plus vitamin D, and dietary modification trials. ref, reference.

showed no association with disease. Borderline inverse associations were observed for recent (1–3 y before diagnosis) measurements of lutein + zeaxanthin and of α-tocopherol, whereas baseline values of these compounds showed no association. Also, in contrast with the baseline results, in the time-dependent analyses there was no suggestion of any increased risk of invasive breast cancer associated with elevated serum lycopene. Finally, in the time-dependent analyses average γ-tocopherol concentrations were significantly associated with increased risk of all breast cancer and invasive breast cancer.

One might reason that if there were a real association with an analyte measured at baseline, this association might be expected to be even stronger when repeated measurements are used. A time-integrated measure based on multiple measurements over the follow-up period may improve exposure misclassification and precision, thereby increasing the power to detect an effect. Particularly, the average concentration might be expected to show a stronger association. Such a pattern may describe the results for γ-tocopherol, for which there was a small excess at baseline (HR: 1.34; 95% CI: 0.86, 2.07) for invasive cancer and for which the average concentration shows a significant association (HR: 1.71; 95% CI: 1.08, 2.73).

In the time-dependent analyses, both α- and β-carotene exhibited inverse associations with risk that were strongest for the period 1–3 y preceding diagnosis and were attenuated with increasing interval preceding diagnosis. The finding of a stronger association with more recent measurements may reflect a late-stage effect. This would be consistent with evidence that carotenoids and retinoids may play a role in the inhibition of cell proliferation (16–18), rather than in inhibition of cancer initiation. Alternatively, the association with more recent measurements may reflect reverse causality (ie, women who have preclinical breast cancer have reduced concentrations of α-carotene and β-carotene), although we attempted to counteract this possibility by excluding measurements made within 1 y preceding diagnosis. Particularly, for α-carotene, the pattern may be consistent with an effect of breast cancer on circulating concentrations.

Strengths of the present analysis included the availability of repeated measurements, the ability to adjust for a wide range of potential confounding factors (including dietary and nondietary factors and other serum carotenoids and micronutrients), and the completeness of follow-up in the WHI. The main limitations of this study are the relatively small number of cases and the large number of comparisons, which could be responsible for some chance associations. Had a Bonferroni correction been applied to take account of the fact that our time-dependent covariates analysis included 8 different compounds and measurements at 5 different points in time, only the result for β-carotene 1–3 y before diagnosis would have been statistically significant ($P < 0.00125$).

In conclusion, our findings add to the available evidence that relatively high serum concentrations of certain carotenoids (particularly α-carotene and β-carotene) are inversely associated with risk of breast cancer. The findings that baseline serum lycopene and average serum γ-tocopherol are positively associated with risk need to be confirmed by other studies.

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