Circulating Carotenoids, Mammographic Density, and Subsequent Risk of Breast Cancer

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Abstract

Mammographic density is one of the strongest predictors of breast cancer risk. Recently, it has been suggested that reactive oxygen species may influence breast cancer risk through its influence on mammographic density. In the current study, we addressed this hypothesis and also assessed if the association between carotenoids and breast cancer risk varies by mammographic density. We conducted a nested case-control study consisting of 604 breast cancer cases and 626 controls with prospectively measured circulating carotenoid levels and mammographic density in the Nurses' Health Study. Circulating levels of α-carotene, β-carotene, β-cryptoxanthin, lycopene, and lutein/zeaxanthin were measured. We used a computer-assisted thresholding method to measure percent mammographic density. We found no evidence that circulating carotenoids are inversely associated with mammographic density. However, mammographic density significantly modified the association between total circulating carotenoids and breast cancer (*P* heterogeneity = 0.008). Overall, circulating total carotenoids were inversely associated with breast cancer risk (*P* trend = 0.01). Among women in the highest tertile of mammographic density, total carotenoids were associated with a 50% reduction in breast cancer risk (odds ratio, 0.5; 95% confidence interval, 0.3–0.8). In contrast, there was no inverse association between carotenoids and breast cancer risk among women with low mammographic density. Similarly, among women in the highest tertile of mammographic density, high levels of circulating α-carotene, β-cryptoxanthin, lycopene, and lutein/zeaxanthin were associated with a significant 40% to 50% reduction in breast cancer risk (*P* trend < 0.05). Our results suggest that plasma levels of carotenoids may play a role in reducing breast cancer risk, particularly among women with high mammographic density. [Cancer Res 2009;69(24):9323–9]

Introduction

Mammographic density is one of the strongest predictors of breast cancer risk (1). The radiographic appearance of the breast on a mammogram varies depending on the composition of the breast. Fat is radiolucent and appears dark on a film screen mammogram. In contrast, epithelial cells and connective tissue are radiodense. They appear light on a mammogram and are considered to be "mammographically dense." Women whose breasts are composed of 75% or more of dense tissue are at a 4- to 6-fold greater risk of breast cancer than women with entirely fatty breasts (no measurable dense tissue; refs. 1, 2).

The biological mechanism by which mammographic density is associated with breast cancer risk is unclear. It has been hypothesized that mammographic density represents proliferation of epithelial and/or stromal cells (3), exposure of breast tissue to mutagens or mitogens (4), and the number of cells at risk (5). Although these hypotheses are not necessarily mutually exclusive, they provide different frameworks by which we may try to understand the relation between exposures, mammographic density, and breast cancer risk. If mammographic density represents the effect of an exposure in the breast, then one would expect to see a cross-sectional association between that factor and mammographic density. In addition, if the effect is mediated through mammographic density, then we would expect the association between that factor and breast cancer risk to be attenuated when adjusted for mammographic density. However, if mammographic density represents the number of cells at risk of developing breast cancer, and if this is established early in life as has been suggested (5), then in fact there may be no cross-sectional association between lifestyle factors and mammographic density. However, factors that are mitogenic or mutagenic may increase the risk of breast cancer among those with more cells at risk (i.e., increased mammographic density).

Antioxidants have been proposed to play a role in breast carcinogenesis. Oxidative stress has the potential to cause cellular DNA damage, lipid peroxidation, and membrane disruption (6). A few studies have reported increased oxidative DNA damage both in breast tumor tissue compared with normal tissue of the same women and when comparing normal adjacent tissue of women with breast cancer with tissue in women without breast cancer (7–9). Antioxidants can neutralize reactive oxygen species (10), which may reduce DNA damage. In addition, some carotenoids, including α-carotene, β-carotene, and β-cryptoxanthin, are metabolized to retinol (11, 12), which has no antioxidant function but is involved in cell differentiation (13). Only a few studies have prospectively assessed plasma carotenoids and breast cancer (14–21). Although most of these studies have observed an inverse association between carotenoids and breast cancer, there has been less consistency in the specific carotenoids involved. Recently, it has been suggested that reactive oxygen species may influence breast cancer risk through its influence on mammographic density (4). In the current study, we evaluated the relation between circulating carotenoids, mammographic density, and breast cancer risk.

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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Table 1. Age and age-adjusted characteristics at the time of mammography according to quartiles of mammographic density among postmenopausal controls (n = 626), Nurses' Health Study (1989–1998)

Quartile (range of % mammographic density)	Q1 (0% to <9.1%)	Q2 (9.2% to <21.6%)	Q3 (21.7% to <36.7%)	Q4 (36.8%+)
Median % mammographic density	4.1	14.6	28.0	49.2
n	159	154	154	159
Means				
Age at mammogram (y)	61.3	61.4	60.8	58.5
Age at first birth (y)	24.5	24.7	25.4	25.6
Age at menarche (y)	12.5	12.5	12.7	12.7
Age at menopause*	50.3	50.4	49.2	49.6
BMI (kg/m^2)	29.0	25.9	23.9	23.2
BMI at age 18 (kg/m^2)	22.8	21.4	20.5	20.3
Alcohol (g/d)	4.6	5.7	5.9	6.5
Parity†	3.4	3.5	3.1	3.0
Frequency (%)				
Family history of breast cancer	9.7	11.8	10.0	16.1
Benign breast disease	41.2	36.2	40.2	51.2
Nulliparous	8.1	5.7	9.1	9.7
Never PMH user	41.8	34.3	22.6	27.6
Current user of PMH	36.2	41.9	56.2	54.3
Never smoker	48.4	42.8	57.3	52.3
Current smoker	11.1	12.7	7.8	14.6

*Natural menopause.
†Among parous women only.

Materials and Methods

Study design and population. The Nurses' Health Study was initiated in 1976, when 121,700 U.S. registered nurses ages 30 to 55 y returned an initial questionnaire (22, 23). Information on body mass index (BMI), reproductive history, age at menopause, and postmenopausal hormone (PMH) use as well as diagnosis of cancer and other diseases is updated every 2 y through questionnaires. During 1989 and 1990, blood samples were collected from 32,826 women. Detailed information about blood collection methods has been published (24). In general, blood samples were returned within 26 h of blood draw; immediately centrifuged; aliquoted into plasma, RBCs, and buffy coat fractions; and stored in liquid nitrogen freezers. The follow-up rate among women who provided blood samples was 99% through 1998.

We conducted a nested case-control study among the subcohort of women who had no history of cancer at the time they provided a blood sample (25). There were 974 cases diagnosed after the 1989/1990 blood collection but before June 1, 1998 and 973 matched controls with circulating carotenoid levels. Controls were matched to cases on age, month, time of day, and fasting status at the time of blood collection.

Breast cancer cases were confirmed by medical record review. At the time of mammography collection, 910 (93.4%) cases and 952 (97.8%) controls were alive and eligible to receive letters for participation in this study. Of those that were eligible, 879 (96.6%) cases and 886 (93.1%) controls gave permission to obtain mammograms. Among the controls, 3.9% did not give permission and 3.1% reported not having a mammogram. Among the breast cancer cases, 3.0% did not give permission and 0.4% reported not having a mammogram. For all consenting women, we attempted to obtain the mammograms taken as close to the date of blood collection as possible. We successfully obtained film mammograms from 843 cases (95.9% of those consenting) and 839 controls (94.7% of those consenting). The median time between mammography and blood draw was 4 mo before blood collection (interquartile range, 24 mo before blood collection to 4 mo after blood collection). Women for whom we obtained mammograms were very similar to those for whom we were unable to get mammograms with respect to age, BMI, circulating hormone (26), and carotenoid levels. We excluded 18 cases and 1 control whose mammograms were not usable. Because menopausal status is strongly associated with mammographic density, and we were limited in the number of premenopausal women with both mammograms and circulating carotenoids measured, we restricted all analyses to women who were postmenopausal at the time of both mammography and blood collection (604 cases and 626 controls). This study was approved by the Committee on the Use of Human Subjects in Research at Brigham and Women's Hospital.

Laboratory analyses. Frozen plasma samples were sent to the Micronutrient Analysis Laboratory in the Department of Nutrition at the Harvard School of Public Health, where assays to determine concentrations of α-carotene, β-carotene, β-cryptoxanthin, lycopene, and lutein/zeaxanthin were conducted in four batches. Plasma samples for matched case-control sets were always placed next to each other, in random order, in boxes sent to the lab and were assessed in the same batch to minimize the effect of laboratory error due to batch drift. Quality control (QC) samples were also submitted with each batch and were randomly placed throughout the boxes. Laboratory technicians were blinded to case, control, or QC status of the samples. QC samples consisted of replicates of two pools of plasma. One QC sample was assayed per 10 study samples. Coefficients of variation were ≤8.0% for each of the carotenoids measured (17).

All five carotenoids were assessed using the same reversed-phase high-performance liquid chromatography methods described by El-Sohemy and colleagues (27). Detailed methods of the assay have been published previously (17). Lutein and zeaxanthin are isomers and are not separated by the method used; they were analyzed together as lutein/zeaxanthin. Total carotenoids in this analysis are the sum of individual concentrations of α-carotene, β-carotene, β-cryptoxanthin, lycopene, and lutein/zeaxanthin. Results were very similar when we examined a total carotenoid score, which summed the quintile value for each of the individual carotenoid quintiles.

Mammographic density measurements. To assess mammographic density, the craniocaudal views of both breasts were digitized at 261 μm/pixel with a Lumysis 85 laser film scanner, which covers a range of 0 to

4.0 absorbance. We used the Cumulus software for computer-assisted thresholding to measure percent and absolute mammographic density (28). The observer was blinded to case-control status when setting the thresholds. This measure of mammographic breast density was highly reproducible within this study. The within-person intraclass correlation coefficient was 0.93 (29). We used the average percent density of both breasts for this analysis. Previous studies have shown that breast density of the right and left breast is very highly correlated (28). We also evaluated the association between the absolute area of mammographic density and breast cancer risk, but because the pattern was similar and somewhat attenuated, we present the results of percent mammographic density only.

Covariate information. Menopausal status and use of PMHs at blood draw were assessed through a supplemental questionnaire administered at the time of blood collection. Women were considered postmenopausal if they reported no menstrual periods within the 12 mo before blood collection with natural menopause, bilateral oophorectomy, or hysterectomy with one or more ovaries retained and were ≥54 y if a smoker or ≥56 y if a nonsmoker. These are the ages at which 90% of the study participants who had a natural menopause were postmenopausal. Menopausal status and PMH use at the time of the mammogram were assessed using data from biennial questionnaires before the date of the mammogram. All other covariates were assessed from one or more biennial questionnaires.

Statistical analysis. There were differences in the distribution of carotenoid concentrations between laboratory batches. The QC samples included in each batch showed variability similar to that of the control samples, suggesting that these differences are due to batch-to-batch variability and are not true differences in concentrations. Therefore, we created tertiles and quintiles of circulating carotenoids based on batch-specific cutpoints among control subjects and adjusted for batch in all analyses with continuous carotenoid measures. Similarly, we created tertiles and quintiles of mammographic density based on the distribution among the controls.

Generalized linear models were used to determine the mean percentage of breast density per carotenoid quintile adjusted for potential confounders. We used unconditional logistic regression models adjusting for the matching variables and other potential confounders to determine the odds ratios (OR) as an estimate of the relative risks (RR) and 95% confidence intervals (95% CI). Covariates were considered potential confounders if there was a priori evidence in the published literature that the factor was related to either breast density or circulating carotenoids and breast cancer. The following covariates were included in multivariate models as potential confounders: BMI, parity/age at first birth, alcohol consumption, family history of breast cancer, age at menopause, age at menarche, and total duration of PMH use. Personal reported history of benign breast disease was not included in the final models, as often women with dense breasts may be told that they have a benign breast condition; thus, it may be a partial surrogate measure of breast density.

Tests for trend were conducted using square root transformation of the continuous measure for percent mammographic density. Transformation of these continuous variables improved the normality of their distributions. To determine if the effect of mammographic density and circulating carotenoids on breast cancer risk varied by level of the other factor, we created cross-classified variables using tertiles of both breast density and carotenoids. We evaluated the statistical interaction between mammographic density and carotenoids by conducting a likelihood ratio test comparing models with each tertile of mammographic density cross-classified with tertiles of carotenoids to the model with indicator variables for the main effects of both (30).

Results

This nested case-control study consisted of 604 breast cancer cases and 626 controls with prospectively measured circulating carotenoids and mammographic density. Among postmenopausal controls, women with higher mammographic density were more likely to be younger and leaner, consume more alcohol, and have lower parity than women with lower mammographic density (Table 1).

We observed a positive association between circulating carotenoids and mammographic density (Table 2). Women in the highest quintile of total carotenoids had 4.1 percentage points greater mammographic density than those in the lowest (P trend = 0.02). A similar association was observed for circulating α-carotene, β-cryptoxanthin, and lycopene; women in the highest quintile of each had between 3.2 and 5.7 percentage points greater mammographic density than those in the lowest (P trend ≤ 0.05). The positive association between these carotenoids and percent mammographic density persisted after additional adjustment for waist

Table 2. Mean percent mammographic density among postmenopausal (at both mammography and blood) controls according to quintiles of circulating carotenoids, Nurses' Heath Study

	Quintiles of circulating carotenoids					*P* trend
	1 (low)	2	3	4	5 (high)	
α-Carotene						
Age	18.4	23.4	25.3	28.6	29.5	<0.0001
Age + BMI*	22.8	24.7	24.9	26.7	26.3	0.06
MV†	22.2	24.5	25.3	27.0	26.4	0.03
n	127	115	128	130	126	
β-Carotene						
Age	19.4	22.4	27.7	28.0	28.8	<0.0001
Age + BMI*	24.3	24.3	26.6	25.2	25.4	0.56
MV†	23.7	24.3	26.6	25.5	25.6	0.38
n	134	130	114	122	126	
β-Cryptoxanthin						
Age	19.7	21.9	25.3	27.6	30.9	<0.0001
Age + BMI*	24.0	23.0	25.6	25.3	27.5	0.04
MV†	23.9	22.9	25.5	25.9	27.1	0.04
n	123	121	132	126	124	
Lycopene						
Age	18.7	26.4	27.3	23.5	29.4	0.0002
Age + BMI*	21.3	27.2	25.6	23.9	27.3	0.05
MV†	21.5	27.0	25.6	24.0	27.2	0.05
n	123	133	133	118	119	
Lutein/zeaxanthin						
Age	17.8	25.1	25.5	24.8	30.6	<0.0001
Age + BMI*	22.7	25.0	26.4	24.1	26.8	0.11
MV†	23.0	24.9	27.2	23.9	26.1	0.23
n	102	131	125	134	134	
Total carotenoids‡						
Age	17.2	23.6	27.6	27.6	28.2	<0.0001
Age + BMI*	22.4	24.5	27.0	25.4	26.6	0.02
MV†	22.4	24.3	27.1	25.5	26.5	0.02
n	129	130	114	135	118	

*Age (continuous), BMI (continuous).

†Age (continuous), BMI (continuous, kg/m^2), family history of breast cancer (yes or no), parity and age at birth of first child (nulliparous, parous with age at first birth of <25 y, parous with age at first birth of 25–29 y, parous with age at first birth of ≥30 y), alcohol consumption (0, <5, 5 to <15, ≥15 g/d), benign breast disease (yes/no), PMH use (never, past, current).

‡Total carotenoids are the sum of individual concentrations of α-carotene, β-carotene, β-cryptoxanthin, lycopene, and lutein/zeaxanthin.

to hip ratio. In secondary analyses, we examined the association between carotenoids and mammographic density in the subset of women whose blood and mammogram were taken within 6 months of each other (n = 407 controls). In general, a positive association remained, although the P trend was no longer significant. For example, among women whose blood draw and mammogram were within 6 months of each other, women in the highest quintile of total carotenoids had 3.2 percentage points greater mammographic density than those in the lowest (P trend = 0.24).

We observed an inverse association between circulating carotenoids and breast cancer risk in the current study population (Table 3), consistent with what was observed in our previous study (17). Women in the highest quintiles of total carotenoids had a 30% reduced risk of breast cancer (OR, 0.7; 95% CI, 0.5–1.0; P trend = 0.01) relative to women in the lowest. Overall, a similar 30% to 40% reduction in risk was observed for women in the highest quintiles of α-carotene, β-carotene, and lutein/zeaxanthin compared with women in the lowest quintile. Adjustment for percent mammographic density did not change these estimates.

As has been shown previously, percent mammographic density was a significant predictor of breast cancer risk in this study. Women in the highest quintile of breast density were at a 5-fold increased risk of breast cancer compared with women in the lowest quintile (OR, 5.1; 95% CI, 3.2–8.0). Additional adjustment for total carotenoids did not alter this association (OR, 5.4; 95% CI, 3.4–8.5). These results suggest that the associations between circulating carotenoids and breast cancer and that of mammographic density and breast cancer are independent of one another.

We examined if mammographic density modified the association between circulating carotenoids and breast cancer risk (Table 4). We found that mammographic density significantly modified the associations between circulating α-carotene, β-cryptoxanthin, and total carotenoids and breast cancer (P heterogeneity < 0.05). Women with high mammographic density and low circulating total carotenoids were at a 3-fold (OR, 3.1; 95% CI, 1.7–5.5) increased risk of breast cancer relative to women with low mammographic density and high circulating total carotenoid levels. Among women in the highest tertile of mammographic density, circulating total carotenoids were associated with 50% reduction in breast cancer risk (OR, 0.5; 95% CI, 0.3–0.8). In contrast, there was no inverse association between total carotenoids and breast cancer risk among women with low mammographic density (OR, 1.2; 95% CI, 0.6–2.3). Among women with high mammographic density, high levels of α-carotene, β-cryptoxanthin, lycopene, and lutein/zeaxanthin were associated with a 40% to 50% reduction of breast cancer risk (P trend < 0.05).

We conducted secondary analyses examining the association between carotenoids and absolute dense area on the mammogram. In general, results were in the same direction but attenuated in comparison with percent mammographic density (Supplementary Table S1). Only the positive trend between lycopene and absolute dense area was significant in multivariate models (P = 0.02). Additional adjustment for nondense area did not change these results. There was no interaction between absolute dense area and circulating carotenoids on breast cancer risk (Supplementary Table S2).

We also conducted secondary analyses among women who were not using PMHs at the time of blood collection and mammography

Table 3. RR of breast cancer according to circulating carotenoids among postmenopausal women at blood draw and mammogram

	Quintiles of circulating carotenoids					P trend
	1 (low)	2	3	4	5 (high)	
α-Carotene						
MV*	1.0 (reference)	1.1 (0.8-1.6)	1.1 (0.8–1.6)	0.8 (0.6–1.2)	0.7 (0.4–1.0)	0.004
MV* + MD†	1.0 (reference)	1.1 (0.7–1.6)	1.0 (0.7–1.5)	0.7 (0.5–1.1)	0.6 (0.4–0.9)	0.0008
β-Carotene						
MV*	1.0 (reference)	1.3 (0.9–1.8)	1.4 (1.0–2.1)	0.9 (0.6–1.4)	0.6 (0.4–1.0)	0.001
MV* + MD†	1.0 (reference)	1.3 (0.9–1.8)	1.3 (0.9–2.0)	0.9 (0.6–1.3)	0.6 (0.4–0.9)	0.0004
β-Cryptoxanthin						
MV*	1.0 (reference)	1.2 (0.8–1.7)	1.1 (0.8–1.6)	0.8 (0.5–1.2)	0.9 (0.6–1.3)	0.11
MV* + MD†	1.0 (reference)	1.2 (0.8–1.8)	1.1 (0.8–1.7)	0.8 (0.5–1.1)	0.8 (0.5–1.2)	0.04
Lycopene						
MV*	1.0 (reference)	1.2 (0.9–1.7)	1.0 (0.7–1.4)	1.0 (0.7–1.5)	1.0 (0.7–1.4)	0.56
MV* + MD†	1.0 (reference)	1.1 (0.7–1.5)	0.9 (0.6–1.3)	1.0 (0.7–1.4)	0.8 (0.6–1.2)	0.26
Lutein/zeaxanthin						
MV*	1.0 (reference)	0.7 (0.5–1.0)	0.7 (0.5–1.0)	0.6 (0.4–0.9)	0.6 (0.4–0.9)	0.01
MV* + MD†	1.0 (reference)	0.6 (0.4–0.9)	0.6 (0.4–0.9)	0.6 (0.4–0.8)	0.5 (0.4–0.8)	0.003
Total carotenoids‡						
MV*	1.0 (reference)	1.1 (0.8–1.5)	1.2 (0.8–1.7)	0.8 (0.5–1.1)	0.7 (0.5–1.0)	0.01
MV* + MD†	1.0 (reference)	1.0 (0.7–1.5)	1.1 (0.7–1.6)	0.7 (0.5–1.0)	0.6 (0.4–0.9)	0.002

*Age (continuous), BMI (continuous, kg/m^2), family history of breast cancer (yes or no), parity and age at birth of first child (nulliparous, parous with age at first birth of <25 y, parous with age at first birth of 25–29 y, parous with age at first birth of ≥30 y), alcohol consumption (0, <5, 5 to <15, ≥15 g/d), benign breast disease (yes/no), PMH use (never, past, current).

†Quintiles of percent mammographic density.

‡Total carotenoids are the sum of individual concentrations of α-carotene, β-carotene, β-cryptoxanthin, lycopene, and lutein/zeaxanthin.

Table 4. RR of breast cancer according to circulating carotenoids and mammographic density among postmenopausal women at blood draw and mammogram

	Mammographic density			*P* interaction
	Tertile 1 (low) RR (95% CI)	Tertile 2 RR (95% CI)	Tertile 3 (high) RR (95% CI)	
α-Carotene				0.02
Tertile 1	0.7 (0.4–1.2)	1.5 (0.8–2.7)	2.8 (1.6–5.0)	
Cases/controls	53/94	59/57	100/54	
Tertile 2	0.9 (0.5–1.6)	1.2 (0.7–2.2)	2.7 (1.6–4.6)	
Cases/controls	44/59	62/74	130/78	
Tertile 3	1.0 (reference)	0.8 (0.4–1.5)	1.3 (0.7–2.3)	
Cases/controls	35/47	41/68	80/95	
P trend	0.35	0.002	0.02	
β-Carotene				0.18
Tertile 1	0.8 (0.4–1.5)	1.8 (1.0–3.2)	2.9 (1.6–5.3)	
Cases/controls	54/96	61/63	97/62	
Tertile 2	1.4 (0.8–2.7)	1.9 (1.1–3.5)	3.5 (2.0–6.3)	
Cases/controls	49/57	65/62	130/76	
Tertile 3	1.0 (reference)	0.8 (0.4–1.5)	1.8 (1.0–3.3)	
Cases/controls	29/47	36/74	83/88	
P trend	0.86	0.007	0.01	
β-Cryptoxanthin				0.004
Tertile 1	0.6 (0.3–1.0)	1.0 (0.6–1.8)	2.3 (1.3–4.1)	
Cases/controls	55/84	57/61	110/59	
Tertile 2	0.5 (0.3–0.9)	1.1 (0.6–2.0)	1.6 (0.9–2.8)	
Cases/controls	39/76	63/63	101/74	
Tertile 3	1.0 (reference)	0.6 (0.3–1.1)	1.3 (0.7–2.2)	
Cases/controls	38/40	42/75	99/94	
P trend	0.17	0.07	0.01	
Lycopene				0.09
Tertile 1	0.8 (0.5–1.5)	1.1 (0.6–2.0)	3.4 (1.9–6.1)	
Cases/controls	54/87	45/67	108/57	
Tertile 2	1.0 (0.6–1.9)	1.6 (0.9–2.9)	2.2 (1.3–3.8)	
Cases/controls	45/62	62/62	107/90	
Tertile 3	1.0 (reference)	1.3 (0.7–2.3)	2.1 (1.2–3.6)	
Cases/controls	33/51	55/70	95/80	
P trend	0.94	0.78	0.02	
Lutein/zeaxanthin				0.25
Tertile 1	0.9 (0.5–1.7)	1.5 (0.8–2.8)	3.8 (2.2–6.9)	
Cases/controls	57/79	57/59	110/50	
Tertile 2	0.8 (0.5–1.5)	1.4 (0.8–2.8)	2.0 (1.2–3.6)	
Cases/controls	42/72	49/58	96/80	
Tertile 3	1.0 (reference)	1.1 (0.6–1.9)	1.9 (1.1–3.3)	
Cases/controls	33/49	56/82	104/97	
P trend	0.94	0.10	0.007	
Total carotenoids				0.008
Tertile 1	0.6 (0.4–1.1)	1.1 (0.6–2.0)	3.1 (1.7–5.5)	
Cases/controls	61/99	56/65	116/53	
Tertile 2	0.7 (0.4–1.4)	1.4 (0.8–2.5)	1.7 (1.0–3.0)	
Cases/controls	37/58	61/61	105/86	
Tertile 3	1.0 (reference)	0.8 (0.4–1.4)	1.5 (0.8–2.6)	
Cases/controls	34/43	45/73	89/88	
P trend	0.65	0.15	0.003	

NOTE: Models were adjusted for matching factors [age (<50, 50 to <55, 55 to <60, 60 to <65, 65+ y), month of blood draw (June 1989 to December 1989, January 1990 to June 1990, July 1990 to December 1990), fasting status (yes/no), and time of blood draw (1:00 a.m. to 6:59 a.m., 7:00 a.m. to 12:59 a.m., 1:00 p.m. to 6:59 p.m., 7:00 p.m. to 12:59 a.m.)], BMI (continuous, kg/m^2), family history of breast cancer (yes or no), parity and age at birth of first child (nulliparous, 1–4 children with age at first birth of <25 y, 1–4 children with age at first birth of 25–29 y, 1–4 children with age at first birth of ≥30 y, ≥5 children with age at first birth of <25 y, or ≥5 children with age at first birth of ≥25 y), alcohol consumption (0, <5, 5 to <15, ≥15 g/d), age at menarche (<12, 12, 13, or >13 y), age at menopause (<46, 46 to <50, 50 to <55, or ≥55 y), and total duration of PMH use (continuous).

(309 cases and 325 controls). Among these women, there was a significant 50% to 60% reduction in breast cancer risk associated with each of the carotenoids evaluated among women who had high breast density but not among those with low density. Although the interaction between carotenoids and mammographic density was not significant in these secondary analyses, the risk estimates are similar, suggesting that effects are the same and we may be underpowered to detect an interaction in this smaller subset of women.

Discussion

This is the first study to directly examine the association of circulating carotenoids, mammographic breast density, and breast cancer risk. Contrary to our initial hypothesis, we found a positive association between circulating α-carotene, β-cryptoxanthin, lycopene, and total carotenoids with mammographic density. Women in the highest quintile of circulating carotenoids had greater percent mammographic density than women in the lowest quintile (range, 1.9–5.7%). The magnitude of these differences is similar to increases seen for women initiating PMHs (31) and reductions observed for women beginning tamoxifen therapy (32).

Previous studies evaluating dietary intake of fruits and vegetables, carotenoids, and supplement use in relation to breast density have reported conflicting results. Two previous studies reported no association with carotene intake and breast density (33, 34). However, the only cross-sectional study examining specific carotenoid isomers reported a positive association between intake of β-cryptoxanthin, but not α-carotene or β-carotene, and percent mammographic density among Singaporean Chinese women (n = 380; ref. 35). Other nutrients with antioxidant properties have also been positively associated with mammographic density. In the Minnesota Breast Cancer Family Study, vitamin C and E intakes were positively associated with mammographic density among premenopausal (n = 283) but not among postmenopausal women (n = 1225; ref. 33). In addition, a recent study found that premenopausal women currently using multivitamin-multimineral supplements (n = 161) had higher breast density than nonusers (n = 362); this association was not observed in postmenopausal women (36). It is unclear by what mechanism carotenoids or antioxidants may increase breast density in these studies. Although it is possible that there may be residual confounding of this association, we did adjust for known predictors of mammographic density and controlled for BMI, the strongest confounder of the relation, continuously. Further adjustment for waist to hip ratio also did not materially alter these results.

We found that circulating carotenoids significantly modified the mammographic density breast cancer relationship. The mechanism by which mammographic density increases breast cancer risk remains unclear; however, several hypotheses have been proposed. The results of our current study are most consistent with the hypothesis that breast density represents the number of breast cancer cells at risk and that factors influencing proliferation (mitogens) or DNA damage (mutagens) will have the greatest effect on breast cancer risk among those with the greatest number of cells at risk. Under this hypothesis, one would expect that absolute area of dense tissue would be a stronger predictor of breast cancer risk than percent mammographic density. However, it has been consistently shown that percent density is a stronger predictor of risk than area of dense tissue. It is unclear if this difference is due to true biological variability or whether this reflects measurement error issues. Both measures are based on two-dimensional images of a three-dimensional organ; therefore, these are imperfect proxies for what is likely the more biologically relevant, dense volume.

One limitation of this study is that there is only one blood sample from which to assess carotenoid levels. There is evidence to suggest that a single sample is adequately representative of an individual's long-term exposure (14, 37). Toniolo and colleagues (14) reported intraclass correlations between a single measurement and average carotenoid concentrations over a 3-year period that ranged from 0.63 to 0.85. In addition, the nutrients assayed are lipid soluble and the long-term reproducibility from other studies is good, suggesting that these measures provide reasonable consistency over time. Variation that may occur will likely be random and would result in an attenuation of the true relationship (38). In addition, there is measurement error in the assays; however, the low coefficients of variation indicate high reliability.

The individual carotenoids examined in this study are correlated with one another. α-Carotene and β-carotene are the most highly correlated (Spearman correlation = 0.80), whereas lycopene and zeaxanthin/lutein are the least correlated (Spearman correlation = 0.26). Given the high degree of correlation, it is difficult to attribute any effects to a single carotenoid. The results we observed were consistent for a number of the individual carotenoids as well as total carotenoids, suggesting that the association we are observing may be due to general antioxidant effects and not specific to any one carotenoid. It also is possible that women with high carotenoid levels have different lifestyle behaviors than women with low carotenoid levels. However, we have adjusted for known predictors of breast cancer risk in our analyses, although we cannot rule out confounding by unknown risk factors.

The lower sensitivity of mammography in women with denser breasts has been well documented and is due to the fact that dense tissue can mask small lesions (39). In secondary analyses, we observed similar results after we excluded women diagnosed with breast cancer within 2 years of their mammogram. Therefore, it is unlikely that the observed association is caused by the masking of prevalent tumors.

Another potential limitation of the study is that we were unable to collect mammograms from all women in the nested case-control study and that there were some minor differences in success rates according to case-control status. However, carotenoid levels and breast cancer risk factors were similar between participants for whom we were and were not able to obtain mammograms. Thus, failure to obtain a mammogram was randomly distributed with respect to exposure and is unlikely to have resulted in any selection bias.

The results of this study support the hypothesis that oxidative stress is associated with breast cancer risk. Previous studies of dietary intake of antioxidants or fruits and vegetables have been null or inconsistent (33, 35, 40–42). Micronutrients, specifically carotenoids, exhibit a great deal of interindividual variation in their absorption, metabolism, and excretion (43, 44). Therefore, plasma levels of micronutrients may give a more accurate approximation of the amount available to target tissues than intake estimates. Although not entirely consistent, most prospective studies have observed inverse associations with circulating carotenoids and breast cancer risk (14, 15, 17–21). These results suggest that plasma levels of carotenoids may play a role in reducing breast cancer risk, particularly among women with high mammographic density.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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