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Plasma Carotenoids and the Risk of Premalignant Breast Disease in Women Aged 50 and Younger: A Nested Case-Control Study

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Abstract

Purpose—To examine the association of plasma carotenoids, micronutrients in fruits and vegetables, with risk of premalignant breast disease (PBD) in younger women.

Methods—Blood samples were collected at the Siteman Cancer Center between 2008 and 2012 from 3,537 women aged 50 or younger with no history of cancer or PBD. The analysis included 147 participants diagnosed with benign breast disease or breast carcinoma in situ during a 27-month followup and 293 controls. Cases and controls were matched on age, race/ethnicity, and date of and fasting status at blood draw. Plasma carotenoids were quantified. We used logistic regression to calculate odds ratios (ORs) and 95% confidence intervals (CIs) and linear regression to assess racial differences in plasma carotenoids.

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COMPLIANCE WITH ETHICAL STANDARDS:

Conflict of Interest:

KC, YL, JL, and GAC have no conflict of interest. CMA has a consulant/advisory role in Hologic and Siemens.

Ethical approval:

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in this study based on the protocol approved by the Institutional Review Board at the Washington University in St. Louis.

Author's contributions:

LY and GAC secured funding, conceived, and designed the study. KC and LY conducted statistical analyses with input from JL. KC wrote the first draft of the manuscript, which was critically revised by LY and GAC. All authors read and approved of the final manuscript.

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Results—The risk reduction between the highest and lowest tertiles varied by carotenoid, with β-cryptoxanthin having the greatest reduction (OR 0.62; 95% CI, 0.62–1.09; P_{trend}=0.056) and total carotenoids the least (OR 0.83; 95% CI, 0.48–1.44; P_{trend}=0.12). We observed an inverse association between plasma carotenoids and risk of PBD in obese women (BMI≥30 kg/m^2; 61 cases and 115 controls) but not lean women (BMI<25 kg/m^2; 54 cases and 79 controls), although the interaction was not statistically significant. Compared to white women, black women had lower levels of α and β-carotene and higher levels of β-cryptoxanthin and lutein/zeaxanthin.

Conclusions—We observed suggestive inverse associations between plasma carotenoids and risk of PBD in younger women , consistent with inverse associations reported for invasive breast cancer. Carotenoids may play a role early in breast cancer development.

Keywords

Carotenoids; Benign Breast Disease; Breast Cancer; African American; Prevention

INTRODUCTION

The incidence rates for breast cancer vary more than five-fold worldwide [1], suggesting that environmental, lifestyle, and dietary factors influence the etiology of breast cancer. Carotenoids, a group of over 600 fat-soluble pigments found in red, yellow, orange, and dark green fruits and vegetables, are hypothesized to possess anticarcinogenic properties. Of these, α-carotene, β-carotene, lutein/zeaxanthin, lycopene, and β-cryptoxanthin comprise over 95% of total plasma levels [2] and are used as a proxy for total circulating carotenoids.

Numerous studies have come to mixed conclusions regarding the relationship between plasma carotenoids and breast cancer risk. Varying degrees of protection for specific carotenoids have been reported [3–9], with circulating carotenoids more strongly associated with reduced breast cancer risk than dietary intake of carotenoids [10]. A pooled analysis of eight prospective studies by Eliassen et al.[4] observed significant inverse associations of circulating levels of α-carotene, β-carotene, lutein/zeaxanthin, lycopene, and total carotenoids with the risk of breast cancer. Certain carotenoids exhibited a stronger inverse association with estrogen receptor negative (ER-) than estrogen receptor positive (ER+) tumors [4], although this was not replicated in subsequent studies [7, 11].

While a few studies have investigated the relationship between serum carotenoid levels and breast cancer risk in premenopausal women [11, 12], the majority of studies have focused on postmenopausal women over the age of 50. With 24% of all breast cancer cases occurring in women under age 50 [13] and breast cancer accounting for 45% of all cancer diagnoses in women aged 25–49 [14], there is a tremendous need to understand modifiable exposures in younger women. Breast cancer progresses through histologically distinct stages, from normal terminal ductal-lobular units to premalignant hyperplasia, then carcinoma in situ, and finally to invasive carcinoma [15]. An analysis of carotenoid intake among adolescent girls demonstrated an inverse association between β-carotene consumption and risk of benign breast disease (BBD) [16].

In this study, we examined the association between plasma carotenoids and risk of premalignant breast disease in predominantly premenopausal women aged 50 and younger. Because black women under the age of 35 have twice the incidence rates of breast cancer and three times the mortality of their white counterparts [17], identifying risk factors that influence the racial disparity in younger women is a high priority. To the best of our knowledge, this study provides the first comparative analysis of carotenoid concentrations between predominantly premenopausal black and white women.

Methods

Study Design and Population

Between 2008 and 2012, 12,207 adult women provided blood samples to the ongoing Women Health Repository (WHR) within the Siteman Tissue Procurement Core at the Washington University School of Medicine in St. Louis. This study used prospective data from frozen plasma samples of 3,537 women who were age 50 or younger at the time of blood draw with no history of cancer or premalignant breast disease. Among them, 47 participants were later diagnosed with BBD, ductal carcinoma in situ (DCIS), or lobular carcinoma in situ (LCIS) by April 30th, 2016. For each eligible case, two controls were randomly selected from the WHR who were alive and free of cancer and any premalignant breast disease as of April 30th, 2016. Cases and controls were matched on: year of birth (99% within ± 2 years of deviation, 1% within ± 4 years of deviation), race/ethnicity, date of blood draw (98% within ± 2 months), and fasting status at blood draw (84.5% of matched case-control sets had information regarding fasting status). The Institutional Review Board at the Washington University in St. Louis approved the protocol and study.

Laboratory Assays for Carotenoids

Frozen plasma samples were retrieved from the Siteman Tissue Procurement Core and sent to the Micronutrient Analysis Laboratory in the Department of Nutrition at the Harvard T.H. Chan School of Public Health where assays to determine the concentrations of α-carotene, β-carotene, β-cryptoxanthin, lycopene, and lutein/zeaxanthin were conducted. Matched case-control sets were assessed in the same batch to minimize the impact of batch drift. Specimens were randomly placed in boxes that contained 40 samples each. Quality control (QC) samples were constructed by pooling plasmas from a minimum of 10 volunteers. Four QC samples, two positive and two negative controls, were added to each box. Four QC samples were used to determine the within-run variability, and one positive and one negative QC samples were randomly selected from each run to determine the between-run variability. Technicians were blinded to the case/control status of specimens.

All of the carotenoids were assessed using the same reversed phase, high-performance liquid chromatography described by El Sohemy et al [18]. In brief, 250μl aliquots of thawed plasma samples were deproteinized with alcohol and extracted with hexane to remove lipid analytes. Extracted samples were dried and reconstituted in a 250μl mixture of 3:1:1 acetonitrile:dioxane:ethanol. Because isomers cannot be separated with this method, lutein and zeaxanthin were analyzed together as lutein/zeaxanthin. Total carotenoid concentration

was the sum of the individual concentrations of α-carotene, β-carotene, β-cryptoxanthin, lycopene, and lutein/zeaxanthin.

Covariates

Breast cancer risk factors were collected at the time of mammography screening/blood draw. They included age at menarche, age at first birth, age at each subsequent births, age and type of menopause (natural or surgical), use of postmenopausal hormone therapy, current height and weight, recalled weight at age 18, current alcohol intake, current smoking behavior, and family history of breast cancer in a first or second degree relative. Body mass index (BMI) (current and recalled at age 18) was calculated as the ratio of weight in kilograms and height in meters squared (kg/m^2).

Ascertainment of Premalignant Breast Disease

All of the breast biopsies were performed at the Siteman Cancer Center in Washington University School of Medicine. The diagnosis of premalignant breast disease had been determined histologically by pathologists before the assays for carotenoids. Premalignant breast disease was defined as the following group of lesions: BBD which included nonproliferative (n=34), proliferative without atypia (n=86), and atypical hyperplasia (n=7) according to the criteria of Dupont and Page[19], ductal carcinoma in situ (DCIS, n=14) and lobular carcinoma in situ (LCIS, n=6).

Statistical Analysis

Statistical analyses were conducted using SAS (Version 9.3, SAS Institute Inc., Cary, NC) or SPSS (Version 23) and results were considered significant with a two sided P value <0.05. T tests and Chi-square tests were used to compare continuous and categorical characteristics of matched cases and controls. Due to a small sample size, carotenoids were divided into tertiles using cutpoints from the controls. Conditional logistic regression models were used to assess the odds ratios (ORs) and 95% confidence intervals (95% CIs) of premalignant breast disease. The multivariable logistic regression analyses were adjusted for variables which were associated with risk of premalignant breast disease in univariate analyses at P <0.10. Tests for the trend were conducted using a carotenoid component as a continuous independent variable and calculating the Wald statistic on the coefficient.

We conducted the stratified analysis by BMI at blood draw (<25 kg/m^2, 25–29 kg/m2, and ≥30 kg/m2) and race (non-Hispanic White and non-Hispanic Black). Due to a small number of cases in BMI strata and race strata, we calculated the ORs of premalignant breast disease for an increase in plasma carotenoid levels equivalent to a standard deviation of the mean level of a specific analyte in the controls. The interactions of plasma carotenoids with BMI and race were assessed by including cross-product terms in the multivariable-adjusted model.

We also fit multivariable linear regression models with carotenoid components as the dependent variables to assess the relationships between race and plasma carotenoid levels among the controls only. This analysis was restricted to non-Hispanic white and non-Hispanic black participants due to the limited number of cases with other races and

ethnicities. A square-root transformation was used to improve normality of plasma carotenoid levels. Generalized estimating equations were used to take into account the correlation between two controls matched for a case, with adjustment for age (continuous), current BMI (continuous), alcohol consumption (non drinker, ≤1 drink/week, >1 drink/week, missing), current smoking (yes, no, missing), and months between blood draw and assays (continuous).

Results

The mean age at blood draw was 44.7 years with a range from 24–50 years (Table 1). The study included 330 non-Hispanic White women (109 cases), 98 Black women (32 cases), and 12 women of other races and ethnicities (6 cases). Three hundred and sixty-three (82.5%) women were pre-menopausal, with slightly more cases (85.7%) than controls (80.9%). The mean time from blood draw to diagnosis of premalignant breast disease was 27.0 months. Cases were less likely to be a current smoker (11.2 vs 18.6%, P=0.04) and more likely to have a family history of breast cancer in a first or second degree relative (45.6 vs 37.2%, P=0.09) than controls. There were no significant differences between cases and controls for current weight, BMI at blood draw, BMI at age 18, weight change since age 18, age at first birth, age of menarche, alcohol consumption, and plasma levels of carotenoids. The univariate logistic regression analysis of covariates identified three variables that were associated with risk of premalignant breast disease with P <0.10 (data not shown). They included BMI at blood draw (continuous), family history of breast cancer in a first or second degree relative (yes, no), and current smoking status (yes, no, missing). These variables and age at blood draw (continuous) were taken into account in the multivariable analysis.

Table 2 shows the multivariable conditional logistic regression analyses. Compared with the age and BMI-adjusted ORs for all carotenoids, additional adjustments for family history of breast cancer and smoking status decreased the ORs for all carotenoids. The fully adjusted risk reduction varied by carotenoid components, but was at least 17% when comparing the highest to lowest tertiles. β-cryptoxanthin had the greatest reduction in risk (OR 0.62; 95% CI, 0.36–1.09), followed by α-carotene (OR 0.68; 95% CI, 0.37–1.27), β-carotene (OR 0.76; 95% CI, 0.43–1.34), lycopene (OR 0.81; 95% CI, 0.49–1.35), lutein/zeaxanthin (OR 0.82; 95% CI, 0.47–1.43), and total carotenoids (OR 0.83; 95% CI, 0.48–1.44). A suggestive inverse trend was observed for β-cryptoxanthin (P_{trend}=0.056), but not for other analytes.

In a secondary analysis of only cases of BBD (DCIS and LCIS cases were removed), there were no notable differences compared to the data presented in Table 2 (Supplemental Table 1). The ORs of BBD ranged from 0.62 (β-cryptoxanthin) to 0.80 (total carotenoids) between the highest and lowest tertiles. Fasting status at blood draw was not known for 23 case-control sets. The results were similar when we conducted a sensitivity analysis restricting to 372 women whose fasting status at blood draw was available (Supplemental Table 2).

We also conducted the analyses stratified by BMI at blood draw and race. In general, obese women (BMI≥30 kg/m^2) had an inverse association between carotenoid levels and risk of premalignant breast disease and lean women (BMI<25 kg/m^2) had slightly to moderately positive associations, although the interactions were not statistically significant (Table 3).

Among women with BMI<25 kg/m^2, the adjusted OR per standard deviation increase in carotenoids was 1.16 (95% CI, 0.71–1.91)for total carotenoids, 1.17 (95% CI, 0.74–1.85)for β-carotene, 1.06 (95% CI, 0.58–1.93)for lycopene, 1.81 (95% CI, 0.87–3.75)for lutein/zeaxanthin, and 1.07 (95% CI, 0.71–1.62)for β-cryptoxanthin. However, the adjusted OR per standard deviation increase in carotenoids among women with current BMI≥30 kg/m^2 was 0.63 (95% CI, 0.34–1.20) for total carotenoids, 0.86 (95% CI, 0.42–1.79) for β-carotene, 0.71 (95% CI, 0.43–1.18) for lycopene, 0.78 (95% CI, 0.45–1.37) for lutein/zeaxanthin, and 0.34 (95% CI, 0.14–0.83) for β-cryptoxanthin. There was an increase in OR for α-carotene in the women with BMI≥30 kg/m^2 (OR=1.49 per standard deviation increase; 95% CI, 0.53–4.21) and a decrease in OR in the women with BMI<25 kg/m^2 (OR=0.88 per standard deviation increase; 95% CI, 0.55–1.42). There was no statistically significant difference in the ORs between non-Hispanic white and non-Hispanic black women (data not shown).

To determine the association between race and plasma carotenoid levels, we first performed a square root transformation on the data to improve normality (Table 4). After adjusting for age, months between blood draw and lab assay, current BMI, alcohol consumption, and current smoking, black women had similar levels of total carotenoids (27.72 vs 28.01, P=0.72) and lycopene (20.03 vs 20.10, P=0.98) to white women. However, black women had lower levels of α-carotene (3.82 vs 5.53, P<0.0001) and β-carotene (10.49 vs 11.56, P=0.08) than white women. Conversely, black women had significantly higher levels of β-cryptoxanthin (8.91 vs 8.02, P<0.01) and lutein/zeaxanthin (11.77 vs 10.84, P<0.001).

Discussion

In this nested case-control study of predominantly premenopausal women, we observed a suggestive inverse association between plasma β-cryptoxanthin and the risk of premalignant breast disease, with the risk reduction of 38% in the hightest verse lowest tertiles. This was consistent with several, although not all, nested case-control studies in which β-cryptoxanthin was significantly associated with lower risk of invasive breast cancer [8, 9, 20, 21]. While not statistically significant, the risk reduction for other carotenoid components ranged from 17 to 32% in our study when comparing the highest versus lowest tertiles, which was similar to or greater than the associations reported for invasive breast cancer [4, 5, 8, 12]. For example, compared to the pooled analysis of Eliassen et al.[4] where the ORs for the highest vs lowest quintiles for α-carotene and total carotenoids were 0.87 and 0.81, we observed the ORs of 0.68 and 0.83 for the highest vs lowest tertiles, respectively.

Although plasma carotenoids have been consistently associated with a lower risk of invasive breast cancer [4, 8], data on the impact of plasma carotenoids on risk of premalignant breast disease are lacking. Dietary intake of carotenoids in adolescence has been associated with lower risk of BBD in young women [16]. Intake of fruits and vegetables has been associated with lower risk of BBD [22–26]. These results were consistent with our finding of suggestive inverse associations between plasma carotenoids and risk of premalignant breast disease in younger women. This warrants additional studies of how plasma carotenoids slow earlier stages of breast carcinognenesis.

The primary protective mechanism of carotenoids is thought to be through their antioxidant properties. By neutralizing reactive oxygen species and mitigating oxidative stress, carotenoids decrease DNA damage [27, 28]. Carotenoids also modulate estrogen receptor signaling [29], possess antiproliferative properties [30], and include provitamin A molecules (α-carotene, β-carotene, and β-cryptoxanthin) which can be metabolized to retinol, a compound involved in the regulation of cellular proliferation and differentiation [31]. Given these numerous protective mechanisms, it is plausible that carotenoids reduce the incidence of premalignant breast disease. However, it is conceivable that other bioactive compounds found in the same dietary sources as carotenoids provide the observed beneficial effects. To the best of our knowledge, no studies have reported the relationship between carotenoids and premalignant lesions in animal models of breast cancer.

While experimental studies have demonstrated that carotenoids decrease proliferation and inhibit tumor progression in ER- and ER+ breast cancer cells [32], the effects in vivo remain unclear. Multiple recent studies have demonstrated a stronger inverse association between carotenoid levels and breast cancer risk in ER- but not ER+ tumors [4, 33], while others found associations for ER + tumors only [7], or both [11]. Although we did not have information about the ER status of our participants, compared to Bakker et al.[33] and Eliassen et al.[4], our risk reduction appears to be less than that of ER- tumors but similar or greater than that of ER+. In biopsies of benign breast tissue, Tamimi et al.[34] reported that over 90% of the participants were ER+. This supports our data if the majority of our premalignant lesions are ER+.

Race/ethnicity has been shown to be a predictor for plasma carotenoid concentrations [35–37]. In an analysis of NHANES III data, Kant et al.[36] reported that non-Hispanic black had significantly lower levels of plasma lutein/zeaxanthin and β-cryptoxanthin than non-Hispanic white women, which is consistent with our data. We also observed lower levels of α-carotene and β-carotene in non-Hispanic blacks women, which although not found by Kant et al., was demonstrated in the UCLA Energetics Study [37]. The reasons for the racial/ethnic discrepancy in plasma carotenoid concentrations remain poorly elucidated. Plasma carotenoid concentrations had higher correlations with dietary consumption for whites than blacks [37]. Factors such as socioeconomic standing, genetic polymorphisms, and dietary differences may influence circulating carotenoid levels. It remains unknown about how the differences in carotenoid levels contribute to the observed racial disparity in breast cancer among young women.

In contrast to the findings of Eliassen et al.[4], we found slightly to moderately increased risk of premalignant breast disease associated with carotenoid concentrations in lean women (BMI<25 kg/m^2) and inverse associations among obese women (BMI≥30 kg/m^2), although the interaction was not statistically significant. These results are not completely unexpected, as obesity is known to permanently increase levels of oxidative stress [38]. Given the antioxidant properties of carotenoids, this may partially explain why we observed a potentially more beneficial effect in obese women. Additionally, the plasma carotenoid concentrations of participants in our study were much lower than those in the pooled analysis of eight prospective studies by Eliassen et al.[4] except for lycopene. In that analysis, all eight studies had median plasma levels of β-carotene (172–274μg/L), lutein/

zeaxanthin (181–380μg/L), and β-cryptoxanthin (68–180μg/L) higher than our sample (β-carotene 130μg/L, lutein/zeaxanthin 131μg/L, β-cryptoxanthin 67μg/L). Women in seven of eight studies included in that analysis had median plasma levels of α-carotene (37–89μg/L) and total carotenoids (886–1157μg/L) higher than our sample (α-carotene 28μg/L and total carotenoids 830μg/L). This disparity was unlikely due to inter-lab variabaility as our samples were processed by the same lab used by Eliassen. Our blood sample was processed and stored immediately after blood draw. High concentrations of carotenoids have been shown to exert pro-oxidative effects in mice [39]. If the circulating carotenoid concentrations of obese women in the Eliassen[4] study are notably higher than ours, it may partially explain why they found an increased risk associated with carotenoids in obese women.

There are several limitations to our study. First, our small sample size limited our ability to detect modest to moderate associations and interactions with BMI. Premalignant breast disease that we defined for this study is a heterogenous group of breast lesions, including BBD, DCIS, and LCIS, and represents different pathological stages in the development of breast cancer. Carotenoids may play important roles only at the certain stages of carcinogenesis. However, the result was similar when the cases of DCIS and LCIS were excluded from the analysis. Among BBD, only proliferative subtype is a well confirmed risk marker for invasive breast cancer [40], but our cases included both proliferative and nonproliferative BBD. Second, all of our analyses were based on a single measurement of carotenoids. The Nurses Health Study found that in postmenopausal women, plasma carotenoids had a high reproducibility over a three-year period (Intraclass correlation coefficients (ICCs)=0.73–0.88) [41] and a weak reproducility over a 10-year period (ICCs=0.39–0.54) [8]. Hence, a single specimen may be appropriate to represent three-year exposures. Our mean time from blood draw to diagnosis of premalignant breast disease is within this range at 27 months. Third, some breast cancer risk factors were unavailable, such as physical activity, in this study. Alcohol intake has been associated with risks of proliferative BBD and invasive breast cancer [42]. Alcohol intake was not taken into account due to a small sample size and its null association with premalignant breast disease in our sample. Finally, we had no information about multivitamin use and plasma cholesterol that might influence plasma levels of carotenoids. However, Eliassen et al[4] reported that adjustment for plasma cholesterol or restriction of the analysis to multivitamin nonusers generated a similar association between plasma carotenoids and risk of invasive breast cancer.

While not statistically significant, the observed inverse associations between plasma carotenoids and risk of premalignant breast disease are consistent with the inverse associations already reported for invasive breast cancer. It suggests that carotenoids may have a role early in breast cancer development. Further understanding the etiology of human breast cancer, especially in its early stages, is vital to develop new preventative measures against breast cancer. Additional work is needed to determine what, if any, clinical implications there are regarding the difference in α-carotene, β-carotene, β-cryptoxanthin, and lutein/zeaxanthin levels between white and black women.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Baseline characteristics of cases with premalignant breast disease and matched controls

	Cases n=147	Controls n=293	*P* value
Age (years)			
Mean (SD)	44.7 (4.4)	44.7 (4.3)	
<40, n (%)	15 (10.2%)	26 (8.9%)	
≥40, n (%)	132 (89.8%)	267 (91.1%)	0.86
Race and ethnicity, n (%)			0.47
Non-Hispanic African American	32 (21.8%)	66 (22.5%)	
Non-Hispanic White	109 (74.1%)	221 (75.4%)	
Other/Unknown	6 (4.1%)	6 (2.1%)	
Body mass index at blood draw (kg/m^2), mean (SD)	29.4 (7.7)	29.3 (7.0)	0.89
BMI at age 18 (kg/m^2), mean (SD)	21.5 (4.2)	21.8 (3.6)	0.50
Weight change since age 18 (lbs), mean (SD)	48.9 (42.8)	44.4 (37.7)	0.32
Parity and age at first pregnancy, n (%)			0.19
1–2 pregnancies and age at first birth <25	23 (15.6%)	32 (10.9%)	
1–2 pregnancies and age at first birth ≥25	28 (19%)	63 (21.5%)	
≥3 pregnancies and age at first birth <25	32 (21.8%)	66 (22.5%)	
≥3 pregnancies and age at first birth ≥25	26 (17.7%)	40 (13.7%)	
Nulliparous	27 (18.4%)	49 (16.7%)	
Missing	11 (7.5%)	43 (14.7%)	
Age at menarche (years)	12.5 (1.7)	12.7 (1.8)	0.14
Menopausal status, n (%)			0.54
Premenopausal	126 (85.7%)	237 (80.9%)	
Postmenopausal	21 (14.3%)	56 (19.1%)	
Alcohol intake†, n (%)			0.41
Never	53 (36.8%)	94 (36.2%)	
≤1 drink/week	47 (32.6%)	100 (38.5%)	
>1 drink/week	44 (30.6%)	66 (25.4%)	
Current smoking≠, n (%)			0.04
No	127 (88.8%)	210 (81.3%)	
Yes	16 (11.2%)	48 (18.6%)	
Family history of breast cancer in first or second degree relatives, n (%)			
No/unknown	80 (54.4%)	184 (62.8%)	0.09
Yes	67 (45.6%)	109 (37.2%)	
Months between blood draw and diagnosis of premalignant breast disease, mean (SD)	27.0 (20.0)	-	
Carotenoid levels (μg/L), median (10%, 90%)			
α-carotene	29.0 (7.4, 110.5)	27.8 (7.0, 103.4)	0.91
β-carotene	131.5 (40.4, 379.1)	130.1 (42.4, 380.1)	0.87
Lycopene	397.9 (208.7, 621.6)	410.8 (207.5, 689.9)	0.35
Lutein/Zeaxanthin	134.3 (68.5, 242.2)	130.5 (67.7, 216.3)	0.55

	Cases n=147	Controls n=293	*P* value
β-cryptoxanthin	61.2 (27.3, 142.0)	66.9 (32.9, 158.1)	0.26
Total Carotenoids	802.4 (436.9, 1353.6)	829.5 (476.0, 1440.0)	0.58

† 3 cases and 33 controls had no information on alcohol intake

‡ 4 cases and 35 controls had no information on smoking

Table 2

Odds ratios (ORs) and their 95% confidence intervals (CIs) of premalignant breast disease for tertiles of plasma carotenoid levels

	Cases	Controls	OR (95% CI)†	OR (95% CI)‡
Total Carotenoids, ug/L				
<666.415	45	97	1.00 (Reference)	1.00 (Reference)
666.415- <1004.133	57	98	1.26 (0.77, 2.08)	1.13 (0.67, 1.90)
≥1004.133	45	98	0.98 (0.58, 1.67)	0.83 (0.48, 1.44)
			P_{trend}=0.34	P_{trend}=0.12
α-carotene, ug/L				
<17.898	46	97	1.00 (Reference)	1.00 (Reference)
17.898- <43.438	56	98	1.22 (0.73, 2.04)	0.99 (0.58, 1.71)
≥43.438	45	98	0.97 (0.55, 1.72)	0.68 (0.37, 1.27)
			P_{trend}=0.51	P_{trend}=0.21
β-carotene, ug/L				
<95.529	50	97	1.00 (Reference)	1.00 (Reference)
95.529- <188.283	49	98	0.95 (0.59, 1.54)	0.95 (0.57, 1.57)
≥188.283	48	98	0.92 (0.54, 1.58)	0.76 (0.43, 1.34)
			P_{trend}=0.79	P_{trend}=0.44
β-cryptoxanthin, ug/L				
<49.226	53	97	1.00 (Reference)	1.00 (Reference)
49.226- <87.038	51	98	0.96 (0.59, 1.56)	0.92 (0.55, 1.55)
≥87.038	43	98	0.80 (0.48, 1.34)	0.62 (0.36, 1.09)
			P_{trend}=0.23	P_{trend}=0.056
Lycopene, ug/L				
<343.704	51	97	1.00 (Reference)	1.00 (Reference)
343.704- <480.980	53	98	1.03 (0.63, 1.69)	1.01 (0.61, 1.69)
≥480.980	43	98	0.85 (0.51, 1.39)	0.81 (0.49, 1.35)
			P_{trend}=0.16	P_{trend}=0.12
Lutzein/zeaxanthin, ug/L				
<104.963	47	97	1.00 (Reference)	1.00 (Reference)
104.963- <157.313	51	98	1.07 (0.64, 1.77)	0.95 (0.56, 1.60)
≥157.313	49	98	1.05 (0.63, 1.76)	0.82 (0.47, 1.43)
			P_{trend}=0.34	P_{trend}=0.76

†ORs were adjusted for age and body mass index at blood draw.

‡ORs were further adjusted for family history of breast cancer in a first or second degree relative and current smoking.

Table 3

Odds ratios (ORs) and their 95% confidence intervals (CIs) of premalignant breast disease for incremental increases of plasma carotenoid levels stratified by body mass index (BMI) at blood draw

	Cases	Controls	OR (95% CI)[†] [‡]	$P_{interaction}$
Total carotenoids				
BMI < 25kg/m^2	54	79	1.16 (0.71, 1.91)	
25kg/m^2 ≤ BMI < 30kg/m^2	32	99	0.71 (0.32, 1.58)	
BMI ≥ 30kg/m^2	61	115	0.63 (0.34, 1.20)	0.93
Alpha-carotene				
BMI < 25kg/m^2	54	79	0.88 (0.55, 1.42)	
25kg/m^2 ≤ BMI < 30kg/m^2	32	99	0.50 (0.16, 1.53)	
BMI ≥ 30kg/m^2	61	115	1.49 (0.53, 4.21)	0.42
Beta-carotene				
BMI < 25kg/m^2	54	79	1.17 (0.74, 1.85)	
25kg/m^2 ≤ BMI < 30kg/m^2	32	99	0.96 (0.40, 2.35)	
BMI ≥ 30kg/m^2	61	115	0.86 (0.42, 1.79)	0.97
Beta-cryptoxanthin				
BMI < 25kg/m^2	54	79	1.07 (0.71, 1.62)	
25kg/m^2 ≤ BMI < 30kg/m^2	32	99	0.52 (0.13, 2.05)	
BMI ≥ 30kg/m^2	61	115	0.34 (0.14, 0.83)	0.41
Lycopene				
BMI < 25kg/m^2	54	79	1.06 (0.58, 1.93)	
25kg/m^2 ≤ BMI < 30kg/m^2	32	99	0.73 (0.38, 1.43)	
BMI ≥ 30kg/m^2	61	115	0.71 (0.43, 1.18)	0.94
Lutein/zeaxanthin				
BMI < 25kg/m^2	54	79	1.81 (0.87, 3.75)	
25kg/m^2 ≤ BMI < 30kg/m^2	32	99	1.17 (0.45, 3.09)	
BMI ≥ 30kg/m^2	61	115	0.78 (0.45, 1.37)	0.71

[†]ORs were for an increase in plasma carotenoid levels equivalent to a standard deviation of the mean level of a specific analyte in the controls: ORs for each 411.7 μg/L increase in total carotenoids, for each 57.8 μg/L increase in alpha-carotene, for each 166.3 μg/L increase in beta-carotene, for each 63.0 μg/L increase in beta-cryptoxanthin, for each 214.3 μg/L increase in lycopene, and for each 66.3 μg/L increase in lutein/zeaxanthin

[‡]ORs were adjusted for age, family history of breast cancer in a first or second relative, and current smoking.

Table 4

Differences in least square means[†] of plasma carotenoids concentrations (square-root transformed) between non-Hispanic white women and non-Hispanic black women among the controls

	Black	White	Difference (95% CI)	*P*
N	66	219		
Total carotenoids	27.72	28.01	−0.29 (−1.84, 1.27)	0.72
Alpha-carotene	3.82	5.53	−1.71 (−2.35, −1.07)	<.0001
Beta-carotene	10.49	11.56	−1.07 (−2.26, 0.12)	0.08
Beta-cryptoxanthin	8.91	8.02	0.89 (0.20, 1.58)	0.01
Lycopene	20.03	20.10	−0.06 (−1.53, 1.40)	0.93
Lutein/zeaxanthin	11.77	10.84	0.93 (0.36, 1.49)	0.001

[†]adjusted for age, body mass index, alcohol consumption, and current smoking, all at blood draw, as well as months between blood draw and assays for carotenoids