Plasma Carotenoids, Retinol, and Tocopherols and Risk of Breast Cancer

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The roles of carotenoids, retinol, and tocopherols in breast cancer etiology have been inconclusive. The authors prospectively assessed the relations between plasma \( \alpha \)-carotene, \( \beta \)-carotene, \( \beta \)-cryptoxanthin, lycopene, lutein/zeaxanthin, retinol, \( \alpha \)-tocopherol, and \( \gamma \)-tocopherol and breast cancer risk by conducting a nested case-control study using plasma collected from women enrolled in the Nurses’ Health Study. A total of 969 cases of breast cancer diagnosed after blood draw and prior to June 1, 1998, were individually matched to controls. The multivariate risk of breast cancer was 25–35% less for women with the highest quintile compared with that for women with the lowest quintile of \( \alpha \)-carotene (odds ratio (OR) = 0.64, 95% confidence interval (CI): 0.47, 0.88; \( p \) trend = 0.01), \( \beta \)-carotene (OR = 0.73, 95% CI: 0.53, 1.02; \( p \) trend = 0.01), lutein/zeaxanthin (OR = 0.74, 95% CI: 0.55, 1.01; \( p \) trend = 0.04), and total carotenoids (OR = 0.76, 95% CI: 0.55, 1.05; \( p \) trend = 0.05). The inverse association observed with \( \alpha \)-carotene and breast cancer was greater for invasive cancers with nodal metastasis.

The authors conclude that some carotenoids are inversely associated with breast cancer. Although the association was strongest for \( \alpha \)-carotene, the high degree of collinearity among plasma carotenoids limits our ability to conclude that this association is specific to any individual carotenoid.

Incidence rates for breast cancer vary by more than five-fold worldwide, suggesting that environmental and lifestyle factors are important in the etiology of breast cancer (1). Migration studies indicate that offspring of women moving from countries of low incidence to countries of high incidence acquire the high breast cancer rates of the new country (2, 3). Such evidence has motivated research on diet and breast cancer.

The relation of vegetable consumption to risk of breast cancer has been investigated in numerous epidemiologic studies, with inconsistent results. The majority of case-control studies have found an inverse association (4–10), while cohort studies report more modest and null associations (11–16). Fruits and vegetables contain bioactive substances including carotenoids, which may exhibit anti-carcinogenic effects (17).

The primary mechanism by which carotenoids and tocopherols are proposed to prevent cancer is through their antioxidant properties. Oxidative stress has the potential to cause cellular DNA damage, lipid peroxidation, and membrane disruption (18). 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 (19–21). Antioxidants can neutralize reactive oxygen species (22).
which may reduce DNA damage. In addition, some carotenoids including α-carotene, β-carotene, and β-cryptoxanthin are metabolized to retinol (17, 23), which is involved in cell differentiation and has no antioxidant function (24). Only a few studies have prospectively assessed plasma carotenoids, retinol, or tocopherols and breast cancer (25–32). Earlier studies focused primarily on plasma β-carotene and retinol, with inconclusive results. In addition, these studies had limitations including small sample sizes, limited evaluation of nutritional factors, short duration of follow-up, and suboptimal handling/storage of specimens (31). We evaluated concentrations of eight plasma micronutrients and their relation to subsequent breast cancer risk in the Nurses’ Health Study cohort.

MATERIALS AND METHODS

Study design and population

The Nurses’ Health Study started in 1976, when 121,700 US registered nurses between the ages of 30 and 55 years returned an initial questionnaire. Every 2 years, information on reproductive variables, cigarette smoking, postmenopausal hormone use, and dietary information (in 1980, 1984, 1986, 1990, 1994, and 1998) was collected. Incident breast cancer cases were identified through self-report and were confirmed by medical record review. Histopathologic characteristics of breast tumors were obtained from medical records when available. Between 1989 and 1990, blood samples were collected from 32,826 women. Blood samples were returned within 26 hours of being drawn; immediately centrifuged; aliquoted into plasma, red blood cells, and buffy coat fractions; and stored in liquid nitrogen freezers maintained at –130°C or colder. Follow-up for this subcohort has been greater than 96 percent for all questionnaire cycles. This study was approved by the Committee on Human Subjects at Brigham and Women’s Hospital.

Eligible cases in this study consisted of women with pathologically confirmed, incident invasive and in situ breast cancer from the subcohort of women who returned a blood sample and were diagnosed by June 1, 1998. Cases were excluded if they had any other prior cancer diagnosis except for nonmelanoma skin cancer. Controls were randomly selected from the subcohort of women who returned a blood sample and never reported a diagnosis of cancer (except for nonmelanoma skin cancer) up to and including the 2-year interval during which the case was diagnosed. Controls were matched to cases on year of birth, menopausal status, postmenopausal hormone use, and time of day, month, and fasting status at the time of blood draw. Although blood draw characteristics are unlikely to confound the plasma micronutrient-breast cancer relation, matching on these characteristics was necessary for analyses involving other plasma biomarkers in this nested case-control study. There were 974 eligible cases and 973 controls with plasma micronutrient data. Because of the following laboratory issues, a total of nine samples were left unmatched and were dropped from the matched analyses: six lost during extraction, two not received by the laboratory, and one with invalid data possibly due to oxidation. This nested case-control study consists of 969 matched pairs for which plasma carotenoids, retinol, and tocopherols were prospectively collected.

Laboratory methods

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, lutein/zeaxanthin, retinol, α-tocopherol, and γ-tocopherol 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 impact of laboratory error due to batch drift. Quality control samples were also submitted with each batch and were randomly placed throughout the boxes. Laboratory technicians were blinded to case, control, or quality control status of the samples. Quality control samples consisted of replicates of two pools of plasma. One quality control sample was assayed per 10 study samples. Coefficients of variation, weighted by the proportion of samples on a batch- and pool-specific basis, were 7.1 percent for α-carotene, 8.0 percent for β-carotene, 7.5 percent for β-cryptoxanthin, 7.7 percent for lycopene, 7.2 percent for lutein/zeaxanthin, 11.0 percent for retinol, 7.3 percent for α-tocopherol, and 7.2 percent for γ-tocopherol.

All eight micronutrients were assessed using the same reversed-phase, high-performance liquid chromatography methods described by El-Sohemy et al. (33). Briefly, 250-µl aliquots of thawed plasma samples were deproteinized with alcohol and extracted with hexane to remove lipid analytes. Extracted samples were dried and reconstituted in 250 µl of a 3:1:1 mixture of acetonitrile:ethanol:dioxane. Batches 1 and 2 were analyzed using a Hitachi L6000 (isocratic pump) high-performance liquid chromatography system, and batches 3 and 4 were analyzed using a Hitachi L7000 (dual pump) system (Hitachi, El Sobrante, California). Lutein and zeaxanthin are isomers and are not separated by the method utilized; 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.

Total cholesterol was assayed from plasma using the enzymatic methods described by Allain et al. (34). Plasma folate levels were determined by radiolabeled assay kit (Bio-Rad, Richmond, California) at the Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging at Tufts University.

Statistical analysis

Paired t tests, Wilcoxon’s signed-rank tests, and McNemar’s tests were conducted to compare continuous and binary characteristics of matched cases and controls (35, 36). All p values are two sided. Weighted 10th, 50th, and 90th percentile values for each nutrient were calculated by weighting batch-specific values by the proportion of observations in each batch. Quintiles of micronutrients were created by using batch-specific cutpoints based on distributions among the controls.
in each batch. Quintiles were included in the models as indicator variables, with the lowest quintile serving as the referent. In separate models, tests for trend were conducted by assigning the weighted median value of each quintile among controls to both cases and controls in each category and using it as a continuous independent variable. Weighted median values for each quintile were calculated by weighting batch-specific medians by the proportion of observations in each batch, among the controls only.

Although blood draw characteristics are unlikely to confound this particular exposure-disease relation and although results did not differ when variables related to blood draw were excluded, all analyses were conducted taking matching into account. Conditional logistic regression models were used to assess odds ratios and 95 percent confidence intervals of plasma micronutrient levels and risk of breast cancer (37). Unconditional multivariate models included matching factors, allowing all controls to be included in models in which cases were restricted according to tumor characteristics.

In addition to matching factors, multivariate analyses controlled for the following potential confounders and breast cancer risk factors: body mass index at age 18 years (weight (kg)/height (m)², continuous), weight gain since age 18 years (<5, ≥5–<20, ≥20 kg), age at menarche (<12, 12, 13, >13 years), parity/age at first birth (nulliparous, 1–4 children/age at first birth of ≤24 years, 1–4 children/age at first birth of >24 years, ≥5 children/age at first birth of ≤24 years, ≥5 children/age at first birth of >24 years), family history of breast cancer (yes/no), history of benign breast disease (yes/no), and alcohol consumption (none, <3, 3–6, 7–13, >14 drinks/week). Analyses including postmenopausal women were also adjusted for age at menopause (≤45, 46–50, ≥51 years) and duration of postmenopausal hormone use (never, past use of <5 years, past use of ≥5 years, current use of <5 years, current use of ≥5 years).

Most plasma micronutrients were correlated with one another. For example, among controls, the Spearman correlation for α-carotene with β-carotene was 0.78, with lycopene was 0.31, with lutein/zeaxanthin was 0.35, and with α-tocopherol was 0.21. To assess the independent effects of specific micronutrients, it is important to control for other nutritional factors associated with breast cancer. Stepwise logistic regression with backwards elimination was conducted to determine micronutrients exhibiting independent effects on breast cancer risk, which should be included in multivariate models (38). Only plasma micronutrients for which the \( \beta_{\text{trend}} \) was less than 0.20 were retained in the final nutrient-adjusted multivariate model. With these criteria, α-carotene was the only independent predictor of breast cancer risk with a \( \beta_{\text{trend}} \) of 0.01.

To examine whether the association between nutritional factors and breast cancer was modified by breast cancer risk factors, we conducted two statistical tests of interaction. First, we utilized the likelihood ratio test (LRT) to assess the statistical significance of a linear interaction using an ordered scale for the antioxidant quintiles with potential effect modifiers, which we refer to as an ordinal LRT (LRT_{ord}). We also conducted a second LRT, which makes no assumption of linearity in either variable (LRT_{nom}). The nominal LRT compares models with each level of breast cancer risk factor cross-classified with quintiles of micronutrients to the model with indicator variables for the main effects (37). To determine if the relations of plasma micronutrients and invasive breast cancers with and without specific tumor characteristics (e.g., nodal metastasis) were statistically different, we conducted a likelihood ratio test from a case-case analysis (where the outcome is having the characteristic vs. not), comparing the model with the linear term for the micronutrient with the model without (39).

**RESULTS**

The mean age of women in this study was 57 (standard deviation, 7) years, with a range from 43 to 70 years. There were 418 (cases = 206) premenopausal and 1,329 (cases = 666) postmenopausal women at blood draw, with mean ages of 48.5 (standard deviation, 3) years and 61 (standard deviation, 5) years, respectively. Blood samples were collected from 4 months to 9 years prior to diagnosis (median = 4 years). Cases and controls had the same mean body mass index at blood draw (25 kg/m²). Compared with controls, cases had nonsignificantly earlier age at menarche (12.5 vs. 12.6 years), lower mean parity (2.89 vs. 2.97), and older mean age at first birth (25.1 vs. 24.9 years) among parous women. Cases had a later mean age of menopause (48.2 vs. 47.7 years, \( p = 0.01 \)), higher prevalence of family history of breast cancer (21.0 vs. 13.6 percent, \( p = 0.001 \)), and more frequent history of benign breast disease (64.6 vs. 52.5 percent, \( p = 0.001 \)) compared with controls.

The median values and range of plasma micronutrient concentrations are presented in table 1. For all of the micronutrients assayed, median concentrations were higher in the controls compared with the cases, although none of the differences was statistically different.

In conditional logistic regression analyses, significant inverse associations were observed between α-carotene (linear \( \beta_{\text{trend}} = 0.03 \)) and β-carotene (linear \( \beta_{\text{trend}} = 0.02 \)) and risk of breast cancer (table 2), although the inverse trends were primarily due to lower odds ratios in the highest quintiles (α-carotene: odds ratio (OR) = 0.75, 95 percent confidence interval (CI): 0.56, 1.00; β-carotene: OR = 0.74, 95 percent CI: 0.56, 1.00). After adjustment for breast cancer risk factors, these inverse trends remained significant. Women with the highest quintile of α-carotene had 35 percent lower risk of breast cancer compared with women with the lowest quintile (OR = 0.64, 95 percent CI: 0.47, 0.88). Women with the highest quintile of lutein/zeaxanthin (OR = 0.74, 95 percent CI: 0.55, 1.01; linear \( \beta_{\text{trend}} = 0.04 \)) and total carotenoids (OR = 0.76, 95 percent CI: 0.55, 1.05; linear \( \beta_{\text{trend}} = 0.05 \)) had an approximately 25 percent lower risk of breast cancer compared with women with the lowest quintile.

Upon adjustment for α-carotene, inverse associations with breast cancer observed with respect to the highest quintiles of β-carotene and lutein/zeaxanthin were attenuated, and the inverse trends were abolished. The odds ratio comparing the highest quintile of α-carotene with the lowest remained unchanged after mutual adjustment for all of the other nutri-
Plasma folate is considered a possible protective factor for breast cancer (40) and, thus, a potential confounder of the micronutrient-breast cancer relation. Plasma folate levels were available for cases diagnosed through June 1, 1996, and their matched controls. Adjustment for plasma folate levels resulted in no appreciable change in relative risks for the carotenoids and tocopherols. Because vitamin E and most carotenoids are transported in the blood by lipoproteins, accounting for cholesterol level in the analysis is thought to provide results unconfounded by blood lipid concentrations (41). Information on serum cholesterol levels was available on cases diagnosed through June 1, 1996, and their matched controls. Inclusion of total cholesterol in multivariate models resulted in no appreciable difference in odds ratios, and it was not included in the final models.

Studies of dietary intake of carotenoids, retinol, and tocopherol and breast cancer suggested that the effect of these nutritional factors on breast cancer risk differs according to menopausal status and may be more pronounced among premenopausal women (42). In this nested case-control study, there were only 102 premenopausal breast cancer cases, and we were underpowered to draw any conclusions regarding these micronutrients and breast cancer risk in these women. Multivariate comparisons of highest with lowest quintiles of plasma nutrients among only premenopausal women did not suggest a more pronounced effect on breast cancer risk. Statistical tests of interaction revealed that the associations between plasma nutrients and breast cancer were not statistically different for premenopausal women compared with postmenopausal women; therefore, the analyses are not stratified by menopausal status.

To assess if preclinical disease may have affected plasma micronutrient levels (23), we excluded 161 cases diagnosed within 2 years of the date of blood collection and their matched controls. Multivariate results were essentially unchanged (e.g., comparison of the top quintile with the bottom: \( \alpha \)-carotene: OR = 0.62, 95 percent CI: 0.43, 0.88; linear \( p_{\text{trend}} = 0.009 \); \( \beta \)-carotene: OR = 0.74, 95 percent CI: 0.52, 1.07; linear \( p_{\text{trend}} = 0.02 \)).

Plasma folate levels were associated with a significant decreased risk of breast cancer (40). Information on gender was available for cases diagnosed through June 1, 1996, and their matched controls. Multivariate results were essentially unchanged (e.g., comparison of the top quintile with the matched controls. Multivariate results were essentially unchanged (e.g., comparison of the top quintile with the

<table>
<thead>
<tr>
<th>Plasma nutrients</th>
<th>Controls ((n = 969))</th>
<th>Cases ((n = 969))</th>
<th>( p ) value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha )-Carotene ((\mu\text{mol/liter}))</td>
<td>0.11</td>
<td>0.05–0.26</td>
<td>0.10</td>
</tr>
<tr>
<td>( \beta )-Carotene ((\mu\text{mol/liter}))</td>
<td>0.45</td>
<td>0.18–1.15</td>
<td>0.44</td>
</tr>
<tr>
<td>( \beta )-Cryptoxanthin ((\mu\text{mol/liter}))</td>
<td>0.12</td>
<td>0.05–0.22</td>
<td>0.11</td>
</tr>
<tr>
<td>Lutein/zeaxanthin ((\mu\text{mol/liter}))</td>
<td>0.29</td>
<td>0.17–0.47</td>
<td>0.28</td>
</tr>
<tr>
<td>Lycopene ((\mu\text{mol/liter}))</td>
<td>0.97</td>
<td>0.39–1.63</td>
<td>0.96</td>
</tr>
<tr>
<td>Total carotenoids ((\mu\text{mol/liter}))</td>
<td>1.85</td>
<td>1.01–3.05</td>
<td>1.78</td>
</tr>
<tr>
<td>Retinol ((\mu\text{mol/liter}))</td>
<td>1.65</td>
<td>1.37–2.56</td>
<td>1.84</td>
</tr>
<tr>
<td>( \alpha )-Tocopherol ((\mu\text{mol/liter}))+</td>
<td>26.69</td>
<td>16.52–47.21</td>
<td>25.89</td>
</tr>
<tr>
<td>( \gamma )-Tocopherol ((\mu\text{mol/liter}))</td>
<td>4.39</td>
<td>1.89–7.70</td>
<td>4.32</td>
</tr>
</tbody>
</table>

* \( p \) values are from the Wilcoxon signed-rank test.
+ \( \alpha \)-Tocopherol values are based on 962 case-control pairs.

Exogenous factors that contribute to oxidative stress in populations include smoking (43) and alcohol consumption (18). Individuals exposed to high levels of oxidative stress may benefit to a greater extent by increased plasma levels of antioxidants. There was weak evidence that smoking may modify the risk of breast cancer associated with plasma \( \alpha \)-carotene (test for interaction: \( p = 0.10 \)). In multivariate analyses, \( \alpha \)-carotene was inversely associated with breast cancer among never smokers (OR = 0.5, 95 percent CI: 0.3, 0.8; linear \( p_{\text{trend}} = 0.01 \)) and past smokers (OR = 0.6, 95 percent CI: 0.3, 0.9; linear \( p_{\text{trend}} = 0.005 \)) but not among current smokers (OR = 0.9, 95 percent CI: 0.3, 2.6; linear \( p_{\text{trend}} = 0.30 \)).

An increased risk of breast cancer associated with drinking six or more alcoholic drinks per week tended to be restricted to women with the lowest quintiles of plasma micronutrients, although lutein/zeaxanthin was the only one exhibiting a significant inverse trend in risk among moderate drinkers (test for interaction: \( p = 0.06 \); linear \( p_{\text{trend}} = 0.03 \)). Among women with the lowest quintile of lutein/zeaxanthin, those who consumed six or more drinks per week had a 60 percent increased risk of developing breast cancer compared with women who drank less (OR = 1.6, 95 percent CI: 0.8, 3.1).

Some carotenoids and vitamin E may also inhibit proliferation and tumor progression (23), and oxidative stress may be associated with metastasis (44). \( \alpha \)-Carotene (linear \( p_{\text{trend}} = 0.002 \)) and \( \beta \)-carotene (linear \( p_{\text{trend}} = 0.002 \)), retinol (linear \( p_{\text{trend}} = 0.03 \)), and \( \alpha \)-tocopherol (linear \( p_{\text{trend}} = 0.01 \)) levels were associated with a significant decreased risk of metastasis (44).
TABLE 2. Odds ratios of breast cancer and 95% confidence intervals according to quintiles of plasma nutrients among 969 case-control pairs in the Nurses’ Health Study, 1989–1998

<table>
<thead>
<tr>
<th>Quintiles of plasma nutrients</th>
<th>1 (low)</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5 (high)</th>
<th>( \alpha_{\text{carotene}} ) a</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha)-Carotene (( \mu \text{mol/liter} ))†</td>
<td>0.05</td>
<td>0.08</td>
<td>0.11</td>
<td>0.16</td>
<td>0.26</td>
<td>0.08</td>
</tr>
<tr>
<td>( \beta)-Carotene (( \mu \text{mol/liter} ))†</td>
<td>0.18</td>
<td>0.31</td>
<td>0.45</td>
<td>0.67</td>
<td>1.15</td>
<td>0.20</td>
</tr>
<tr>
<td>( \beta)-Carotene‡</td>
<td>1.00</td>
<td>1.03</td>
<td>0.79</td>
<td>1.36</td>
<td>1.20</td>
<td>0.92</td>
</tr>
<tr>
<td>Lycopene (( \mu \text{mol/liter} ))†</td>
<td>0.17</td>
<td>0.24</td>
<td>0.29</td>
<td>0.36</td>
<td>0.43</td>
<td>0.18</td>
</tr>
<tr>
<td>Lycopene‡</td>
<td>1.00</td>
<td>1.36</td>
<td>1.03</td>
<td>1.80</td>
<td>1.22</td>
<td>0.91</td>
</tr>
<tr>
<td>Lutein/zeaxanthin (( \mu \text{mol/liter} ))†</td>
<td>1.00</td>
<td>1.43</td>
<td>1.06</td>
<td>1.92</td>
<td>1.23</td>
<td>0.90</td>
</tr>
<tr>
<td>Lutein/zeaxanthin‡</td>
<td>1.00</td>
<td>0.91</td>
<td>0.69</td>
<td>1.20</td>
<td>0.88</td>
<td>0.66</td>
</tr>
<tr>
<td>Lutein/zeaxanthin§</td>
<td>1.00</td>
<td>1.30</td>
<td>0.96</td>
<td>1.76</td>
<td>1.01</td>
<td>0.74</td>
</tr>
<tr>
<td>Total carotenoids (( \mu \text{mol/liter} ))†</td>
<td>1.01</td>
<td>1.48</td>
<td>1.85</td>
<td>2.27</td>
<td>3.05</td>
<td>1.02</td>
</tr>
<tr>
<td>Total carotenoids‡</td>
<td>1.00</td>
<td>1.09</td>
<td>0.83</td>
<td>1.44</td>
<td>1.11</td>
<td>0.83</td>
</tr>
<tr>
<td>Total carotenoids§</td>
<td>1.00</td>
<td>0.96</td>
<td>0.71</td>
<td>1.29</td>
<td>1.00</td>
<td>0.74</td>
</tr>
<tr>
<td>Retinol (( \mu \text{mol/liter} ))†</td>
<td>1.37</td>
<td>1.64</td>
<td>1.86</td>
<td>2.10</td>
<td>2.56</td>
<td>1.40</td>
</tr>
<tr>
<td>Retinol‡</td>
<td>1.00</td>
<td>0.88</td>
<td>0.67</td>
<td>1.17</td>
<td>0.85</td>
<td>0.63</td>
</tr>
<tr>
<td>Retinol§</td>
<td>1.00</td>
<td>0.86</td>
<td>0.64</td>
<td>1.15</td>
<td>0.82</td>
<td>0.60</td>
</tr>
<tr>
<td>( \alpha)-Tocopherol (( \mu \text{mol/liter} ))</td>
<td></td>
<td></td>
<td>16.42</td>
<td>21.83</td>
<td>26.56</td>
<td>32.10</td>
</tr>
<tr>
<td>( \alpha)-Tocopherol¶</td>
<td>1.00</td>
<td>0.93</td>
<td>0.70</td>
<td>1.23</td>
<td>0.85</td>
<td>0.64</td>
</tr>
<tr>
<td>( \alpha)-Tocopherol§</td>
<td>1.00</td>
<td>0.91</td>
<td>0.67</td>
<td>1.23</td>
<td>0.82</td>
<td>0.60</td>
</tr>
<tr>
<td>( \gamma)-Tocopherol (( \mu \text{mol/liter} ))†</td>
<td>1.90</td>
<td>3.32</td>
<td>4.37</td>
<td>5.47</td>
<td>7.22</td>
<td>1.90</td>
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<tr>
<td>( \gamma)-Tocopherol‡</td>
<td>1.00</td>
<td>1.04</td>
<td>0.79</td>
<td>1.38</td>
<td>0.88</td>
<td>0.66</td>
</tr>
<tr>
<td>( \gamma)-Tocopherol§</td>
<td>1.00</td>
<td>1.00</td>
<td>0.75</td>
<td>1.35</td>
<td>0.84</td>
<td>0.62</td>
</tr>
</tbody>
</table>

* \( \alpha_{\text{carotene}} \) based on values of the median of the quintiles.
† Conditional model adjusted for matching factors only (age, menopausal status, postmenopausal hormone use, month, time of day, and fasting status at blood collection).
‡ Conditional model additionally controlling for age at menopause, body mass index at age 18, weight gain since age 18 years, age at menarche, history of benign breast disease, history of breast cancer in a mother or sister, age at first birth/parity, postmenopausal hormone use, and alcohol consumption.
§ Conditional model additionally controlling for age at menopause, body mass index at age 18, weight gain since age 18 years, age at menarche, history of benign breast disease, history of breast cancer in a mother or sister, age at first birth/parity, postmenopausal hormone use, and alcohol consumption.
¶ \( \alpha\)-Tocopherol analysis is based on 962 case-control pairs.

breast cancer with nodal metastasis. In multivariate analyses, women with the highest quintile of \( \alpha\)-carotene (OR = 0.39, 95 percent CI: 0.22, 0.71) (table 3), \( \beta\)-carotene (OR = 0.45, 95 percent CI: 0.24, 0.82), and \( \alpha\)-tocopherol (OR = 0.53, 95 percent CI: 0.30, 0.93) were more than 50 percent less likely to have breast cancer with nodal metastases compared with women with the lowest. In comparison, the nutritional factors were not significantly associated with risk of invasive breast cancer with no nodal metastases. The associations between \( \alpha\)-carotene (LRT, \( p = 0.02 \)), \( \beta\)-carotene (LRT, \( p = 0.05 \)), and \( \alpha\)-tocopherol (LRT, \( p = 0.03 \)) and breast cancer risk were different for node-positive cancers compared with node-negative cancers. Odds ratios for the association between these micronutrients and invasive cancers involving metastasis were similar when the outcome was restricted to breast cancer metastasis with four or more nodes.

In addition, we investigated the relation of \( \alpha\)-carotene with pathohistologic characteristics of breast cancer tumors in multivariate analyses. \( \alpha\)-Carotene was not associated with tumors characterized as well differentiated, but it was marginally associated with moderately differentiated tumors and significantly associated with poorly differentiated tumors (table 3). The inverse association with \( \alpha\)-carotene was apparent for estrogen receptor-positive and -negative tumors (table 3).

In Western populations, the primary source of \( \alpha\)-carotene in the diet is carrots. In this study, carrot consumption was marginally associated with a decreased risk of breast cancer.
Women consuming carrots on average at least once a day had a 35 percent decreased risk of breast cancer compared with women who consumed carrots less than once a month (multivariate OR = 0.63, 95 percent CI: 0.34, 1.15; linear \( P_{\text{trend}} = 0.03 \)).

**DISCUSSION**

To date, this is the largest study to prospectively evaluate the major plasma carotenoids, tocopherols, and retinol with respect to breast cancer risk. We observed a 35 percent reduced risk of breast cancer for women with the highest quintile of \( \alpha \)-carotene. In addition, there was evidence that \( \alpha \)-carotene had a more pronounced inverse association with breast cancers with nodal metastasis.

Early studies, which focused on plasma \( \beta \)-carotene, retinol, and breast cancer, have been largely inconclusive (25–29). More recently, three studies have prospectively evaluated other carotenoids and tocopherols in relation to breast cancer risk (31, 32, 45). Dorgan et al. (45), reporting on 105 cases, found a significant inverse association with lycopene only. In contrast, Toniolo et al. (32), reporting on 270 cases, found no inverse relation with lycopene levels but found significant inverse associations for \( \alpha \)-carotene, \( \beta \)-carotene, \( \beta \)-cryptoxanthin, lutein, and total carotenoids. Sato et al. (31) reported results for two separate blood donation cohorts whose results were different from one another. In one cohort comprising 244 cases, \( \beta \)-carotene, lycopene, and total carotenoids were inversely associated with breast cancer, yet these associations were not observed in the second cohort comprising 115 cases.

This study was able to assess factors that may modify the relation between plasma micronutrients and breast cancer. Smoking and alcohol consumption are two environmental factors believed to contribute to oxidative stress. There was evidence that smoking status may modify the association between plasma \( \alpha \)-carotene and breast cancer. Contrary to our a priori hypothesis, the results suggest that the inverse association observed between \( \alpha \)-carotene and breast cancer is limited to former and never smokers.

There was also evidence that the lutein/zeaxanthin relation with breast cancer may differ according to alcohol consumption. Consumption of alcohol is considered a well-established, yet modest risk factor for breast cancer (46). Our data suggest that the observed increased risk of breast cancer associated with consuming high levels of alcohol may be limited to women with low levels of lutein/zeaxanthin. Zhang et al. (42) observed an interaction between alcohol consumption and lutein/zeaxanthin among premenopausal women in this cohort. A controlled feeding study in premenopausal women reported significantly lower plasma concentrations of lutein/zeaxanthin when participants consumed high levels of alcohol. In addition, they observed slightly increased levels of anhydrolutein, an oxidative metabolite of this carotenoid (47). Lutein/zeaxanthin may have antioxidant properties specific to reactive oxygen species induced by alcohol metabolism, and women consuming high levels of alcohol may therefore have higher requirements for lutein/zeaxanthin.

This is the first study to prospectively assess the relation between plasma carotenoids, retinol, and tocopherols and breast cancer nodal metastasis at diagnosis. Increased DNA damage associated with reactive oxygen species has been reported with metastatic breast cancer DNA compared with nonmetastatic tumor DNA, suggesting that oxidative stress enhances the cells’ ability to metastasize (48). Previously, in

**TABLE 3. Multivariate odds ratios of invasive breast cancer with and without specific tumor characteristics compared with breast cancer controls (n = 995) according to plasma \( \alpha \)-carotene levels in the Nurses’ Health Study, 1989–1998**

| Cases (no.) | Quintiles of plasma \( \alpha \)-carotene | Odds ratio | 95% confidence interval | Odds ratio | 95% confidence interval | Odds ratio | 95% confidence interval | Odds ratio | 95% confidence interval | Odds ratio | 95% confidence interval | \( P_{\text{trend}} \) | \( p \) value§
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<tr>
<td>Nodal metastasis</td>
<td>191</td>
<td>1 (low) (referent odds ratio)</td>
<td>1.00</td>
<td>0.77</td>
<td>0.48, 1.26</td>
<td>0.85</td>
<td>0.52, 1.40</td>
<td>0.74</td>
<td>0.45, 1.23</td>
<td>0.39</td>
<td>0.22, 0.71</td>
<td>0.002</td>
<td>0.02</td>
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<tr>
<td>No metastasis</td>
<td>588</td>
<td>1.00</td>
<td>1.02</td>
<td>0.72, 1.43</td>
<td>1.22</td>
<td>0.87, 1.71</td>
<td>1.00</td>
<td>0.70, 1.43</td>
<td>0.84</td>
<td>0.58, 1.20</td>
<td>0.18</td>
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<tr>
<td>ER§ negative</td>
<td>165</td>
<td>1.00</td>
<td>0.75</td>
<td>0.45, 1.25</td>
<td>0.64</td>
<td>0.37, 1.10</td>
<td>0.79</td>
<td>0.47, 1.34</td>
<td>0.50</td>
<td>0.28, 0.91</td>
<td>0.05</td>
<td>0.48</td>
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<tr>
<td>ER positive</td>
<td>564</td>
<td>1.00</td>
<td>0.95</td>
<td>0.67, 1.33</td>
<td>1.18</td>
<td>0.84, 1.66</td>
<td>0.92</td>
<td>0.65, 1.32</td>
<td>0.72</td>
<td>0.50, 1.04</td>
<td>0.03</td>
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‡ Comparing poorly differentiated tumors with well differentiated.

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<th>Differentiation</th>
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<tr>
<td>Poor</td>
<td>174</td>
<td>1.00</td>
<td>0.60</td>
<td>0.35, 1.03</td>
<td>1.06</td>
<td>0.65, 1.74</td>
<td>0.91</td>
<td>0.54, 1.53</td>
<td>0.40</td>
<td>0.21, 0.76</td>
<td>0.02</td>
<td></td>
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<tr>
<td>Moderate</td>
<td>248</td>
<td>1.00</td>
<td>1.22</td>
<td>0.77, 1.93</td>
<td>1.05</td>
<td>0.65, 1.71</td>
<td>0.93</td>
<td>0.57, 1.51</td>
<td>0.74</td>
<td>0.44, 1.24</td>
<td>0.07</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Well</td>
<td>110</td>
<td>1.00</td>
<td>1.13</td>
<td>0.53, 2.39</td>
<td>1.54</td>
<td>0.76, 3.09</td>
<td>1.26</td>
<td>0.60, 2.64</td>
<td>1.45</td>
<td>0.70, 3.00</td>
<td>0.41</td>
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§ ER, estrogen receptor.

a Unconditional logistic regression adjusted for matching factors, personal history of benign breast disease, family history of breast cancer, age at first birth, parity, age at menopause, age at menarche, alcohol consumption, and duration of postmenopausal hormone use.

\( \dagger \) \( P_{\text{trend}} \) based on values of the median of the quintile.

\( \ddagger \) \( p \) value is based on the likelihood ratio test of the case-case analysis comparing the model with the linear term for \( \alpha \)-carotene with the model without.

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vitro studies have demonstrated that carotenoids are capable of reducing proliferation in a number of cancer cell lines (23), including breast cancer lines (49). Results from this study suggest that α-carotene may be involved in the prevention of nodal metastases.

Previous analyses in the full cohort of the Nurses’ Health Study addressed the role of dietary intake of carotenoids and risk of breast cancer. Zhang et al. (42) reported inverse associations between the intake of carotenoids, primarily β-carotene and lutein/zeaxanthin, and risk of breast cancer in premenopausal women but not among postmenopausal women. In this nested case-control study, we had few premenopausal women (n of cases = 102), but there was no evidence that carotenoids were associated with a decreased risk of breast cancer.

In contrast, we observed an inverse association of carotenoids and breast cancer among postmenopausal women, while the intake data do not support such an association. Interestingly, in the full cohort of postmenopausal women, carrot consumption was inversely associated with breast cancer risk. The correlation between carrot consumption and α-carotene index is 0.9, suggesting that the discrepancy in the α-carotene index (based on quintiles) and carrot consumption analysis (based on servings) may be due to a washing out of the association when quintiles of dietary index are used as the exposure. If women with the very highest servings of carrot consumption are the individuals with the decreased risk of breast cancer, the inverse association may not be apparent when these women are forced into the same quintile category with women consuming less α-carotene.

One limitation of this study is that there is only one blood sample from which to assess micronutrient levels. There is evidence to suggest that a single sample is adequately representative of an individual’s long-term exposure. Toniolo et al. (32) reported intraclass correlations between a single measurement and average concentrations of carotenoids 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 relation (50).

With any observational study, there is potential for residual and unmeasured confounding. The analyses presented have controlled for all major breast cancer risk factors. In addition, we were able to adjust for the confounding effects of other plasma nutrients in an effort to ascertain independent nutrient effects. It is still possible that other nutritional factors yet unidentified or dietary patterns may be confounding this relation.

Breast cancer is an important public health concern. To date, there is little information about modifiable risk factors. Micronutrients, specifically carotenoids, exhibit a great deal of interindividual variation in their absorption, metabolism, and excretion (51, 52). Therefore, plasma levels of micronutrients may give a more accurate approximation of the amount available to target tissues than intake estimates. These results suggest that plasma levels of α- or β-carotene may play a role in reducing breast cancer risk although, because of the high degree of collinearity between the plasma carotenoids, we have limited ability to conclude that the observed association is specific for α-carotene. Further studies are necessary to confirm the inverse associations observed between α-carotene and breast cancer risk and nodal metastases and the potential interactions observed between plasma carotenoids and smoking and alcohol consumption.

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