Adolescent Carotenoid Intake and Benign Breast Disease

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METHODS: In 6593 adolescent girls in the prospective Growing Up Today Study cohort, intakes of a-carotene, b-carotene, b-cryptoxanthin, lutein/zeaxanthin, and lycopene were assessed by using the means from food-frequency questionnaires in 1996, 1997, and 1998. Girls reported biopsy-confirmed BBD on questionnaires in 2005, 2007, and 2010 (n = 122). We conducted logistic regression of energy-adjusted carotenoid intakes in relation to BBD, adjusted for age, family history of breast cancer or BBD, age at menarche, nulliparity, alcohol intake, BMI, and physical activity.

RESULTS: Mean (SD) age at baseline was 12.0 (1.6) years. b-Carotene intake was inversely associated with BBD; comparing the highest to lowest quartile, the multivariate-adjusted odds ratio was 0.58 (95% confidence interval: 0.34–1.00; P-trend = 0.3). a-Carotene and lutein/zeaxanthin were also inversely associated with BBD, but the associations were not statistically significant.

CONCLUSIONS: Adolescent carotenoid intake may be associated with lower BBD risk; these findings warrant further study. Pediatrics 2014;133:1–7
Carotenoids, a group of fat-soluble pigments that give red, orange, and yellow fruits and vegetables their colors, are hypothesized to reduce risk of breast cancer due to their antioxidative or antiproliferative properties. A recent pooled analysis that included 3055 cases of breast cancer reported significant inverse associations of circulating levels of α-carotene, β-carotene, lutein/zeaxanthin, lycopene, and total carotenoids with breast cancer risk, and a meta-analysis came to similar conclusions. For example, in the pooled analysis, women in the top versus bottom quintile of total circulating carotenoids had a 19% lower risk of breast cancer (relative risk: 0.81; 95% confidence interval [CI]: 0.68–0.96; P trend = .01). Studies of dietary carotenoid intake and breast cancer have been inconsistent, although overall, these studies suggest a protective role of α- and β-carotene. A pooled analysis of 18 prospective cohort studies found that women with higher intakes of α-carotene, β-carotene, and lutein/zeaxanthin had a lower risk of estrogen receptor (ER)–negative but not ER–positive breast cancer.

Carotenoids may also reduce risk of developing benign breast disease (BBD), a group of breast lesions that can develop during adolescence and young adulthood and are associated with higher risk of breast cancer. During adolescence, especially in the period between menarche and first birth, breast tissue may be particularly sensitive to environmental exposures because cells are not yet fully differentiated. In this period, carotenoids may be especially important in preventing hyperplasia, cyst development, or other proliferative conditions. Carotenoids absorb free radicals, protecting cells from oxidative damage, and may also inhibit cell growth and angiogenesis, increasing apoptosis and the immune response. To our knowledge, no previous prospective studies have assessed adolescent dietary carotenoid intake in relation to BBD risk in young women. We examined this association in the Growing Up Today Study (GUTS), a prospective cohort initiated in childhood. We hypothesized that higher intakes of carotenoids, especially α-carotene, β-carotene, and lutein/zeaxanthin, would be associated with a lower risk of BBD.

METHODS

Study Population

GUTS was initiated in 1996. The original cohort comprised 16 882 girls and boys aged 9 to 15 years living throughout the United States, as described previously. The participants are all children of women in the Nurses’ Health Study II (NHSII), a prospective cohort of adult women. Questionnaires primarily were completed by mail but also were available online beginning in 2001. Response rates were >80% in 1997–1998 and 72% in 2005, 68% in 2007, and 62% in 2010. Approximately 80% of participants answered at least 1 of the questionnaires inquiring about BBD status in 2005, 2007, and/or 2010.

Of the 9039 girls participating in the study, 8999 reported carotenoid intake in 1996. Eight girls with a history of childhood cancer were excluded, as well as participants who did not fill out at least 1 questionnaire in 2005, 2007, and 2010; those who reported BBD that was not confirmed by biopsy; and those missing data on covariates, which left 6593 participants in the analytic data set.

Dietary Assessment

Participants reported their habitual dietary intake in 1996, 1997, and 1998 on the Youth/Adolescent Food Frequency Questionnaire (YAQ) developed from the validated adult NHS semiquantitative food-frequency questionnaire. Participants described how often in the past year they ate specified quantities of foods, in 5 categories ranging from never/less than once per month to ≥1 times per day, depending on the food. Among 179 children of the NHSII aged 9 to 18 years, the reproducibility for nutrients ranged from 0.26 to 0.58; reproducibility was higher in girls than in boys and did not vary by age. In 261 children of the NHSII, the average correlation coefficient between the average of 2 YAQs and 3 24-hour recalls was 0.54 after correction for within-person error: Although the correlation was not available for the individual carotenoids examined in this study, the deattenuated correlation for total measured carotenoids was 0.45.

The questionnaires included carotenoid-rich foods such as raw and cooked carrots, yams/sweet potatoes, cantaloupe/melons, raw and cooked spinach, and kale/mustard/chard greens. Carotenoid intake was calculated on the basis of US Department of Agriculture data on carotenoid and retinol content in foods. Retinol activity equivalent (RAE) was calculated to represent vitamin A activity based on bioconversion from provitamin A carotenoids by using the following formula: RAE (µg) = (retinol, µg) + (β-carotene from food, µg/12) + (β-carotene from vitamins and supplemented foods, µg/2) + (α-carotene, µg/24) + (β-cryptoxanthin, µg/24). In a validation study in 162 adult women in the NHS, investigators assessed the correlation between plasma carotenoids and the mean carotenoid intake values from 2 food-frequency questionnaires similar to the YAQ and administered at least 1 year apart, after adjustment for age, BMI, and other known confounders. They found correlations of 0.48 for α-carotene, 0.27 for β-carotene and lutein, 0.32 for β-cryptoxanthin, and 0.21 for lycopene. Micronutrient intakes were adjusted for total energy intake by using a residual method, and mean intakes in 1996, 1997, and 1998 were calculated.
Ascertainment of BBD
In 2005, 2007, and 2010, participants reported whether they ever had been diagnosed with BBD by a health care provider; and whether the BBD was confirmed by biopsy. The questionnaires did not query exact date of diagnosis. Most BBD cases were likely diagnosed because participants (or their physicians) found a clinically palpable mass, which was then biopsied, because participants were too young to be undergoing routine mammography screening. The most common type of BBD occurring in adolescents and young women is fibroadenoma, which accounts for nearly 70% of benign breast lesions.21 The remaining types are primarily cysts and fibrocystic changes.21 In a validation study in 730 women reporting BBD in the NHSII, 95% of those with biopsy pathology material available for review were confirmed as valid proliferative or non-proliferative BBD cases.22

Covariate Assessment
Participant age at baseline was calculated by using date of the baseline questionnaire and date of birth. At baseline and on subsequent questionnaires, participants reported weight and height. BMI was calculated as weight in kilograms/(height in meters)2. Participants reported time per week spent engaging in 18 different moderate and vigorous activities (e.g., basketball, biking, and swimming) in 6 categories ranging from 0 to ≥10 hours/week in 1996, 1997, and 1998. We filled in missing data on BMI and physical activity by carrying forward the value from the previous questionnaire and adding the median change between the 2 questionnaires. Mean BMI and hours per week of physical activity were calculated in 1996, 1997, and 1998. Total energy intake was assessed on the YAQ. Girls reported alcohol intake in 1996, 1997, 1998, and 2001; mean intake during this time period was calculated.

Heavy smoking (defined as ≥100 cigarettes in their lifetime) was reported annually through 2003. Participants reported age at menarche through 2003, by which time 99.9% had undergone menarche, and whether they had ever been pregnant. The participants’ mothers (in the NHSII cohort) reported personal history of biopsy-confirmed BBD and personal and family history of breast cancer.23 Pregnancies and family history of BBD and breast cancer were included through report of BBD diagnosis or end of follow-up because these factors could lead to increased BBD detection.

Statistical Analysis
Micronutrient intake was divided into quartiles to allow for nonlinear associations and to minimize the influence of outliers. We used multivariable-adjusted logistic regression to assess carotenoid intake in relation to BBD by using generalized estimating equations to adjust for the nonindependence of sibling clusters. Models were adjusted for age at the baseline questionnaire, family history of breast cancer in mother or aunt (mother’s sister), mother’s history of BBD, age at menarche, BMI, nulliparity, physical activity, and alcohol intake. Adjusting for family income, region of the country, multivitamin use, height, total caloric intake, and smoking did not change the magnitude of the associations, so these variables were not included in the final models. Tests for trend were assessed by including the median value within each quartile as a continuous variable in the model and calculating the Wald statistic.

Carotenoid-rich foods including carrots also were examined in relation to BBD. Those models were adjusted for mean total energy intake in 1996, 1997, and 1998. In secondary analyses, we stratified by smoking status, family history of BBD, age at menarche, parity, BMI (≤20 vs >20), and alcohol consumption.

All analyses were conducted with SAS version 9.2 (SAS Institute, Cary, NC). All statistical tests were 2-sided, and P values <.05 were considered statistically significant. This protocol was approved by the Human Subjects Committees of the Harvard School of Public Health and the Brigham and Women’s Hospital.

RESULTS
Mean participant age at baseline was 12.0 (SD: 1.6; range: 9.0–15.9) years. Although some of these dietary data were collected before adolescence, and some as young as age 9 years, we shall hereafter refer to this as a study of adolescent diet. Mean (SD) carotenoid intakes were 491 (349) μg/day for α-carotene, 2417 (1467) μg/day for β-carotene, 153 (94) μg/day for β-cryptoxanthin, 2058 (1850) μg/day for lutein/zeaxanthin, 5418 (2073) μg/day for lycopene, and 1137 (571) μg/day for RAE of vitamin A. Girls with higher β-carotene intake were more physically active (12.9 hours/week of moderate to vigorous activity in the highest quartile compared with 11.1 hours/week in the lowest quartile; Table 1) and were more likely to have a family history of breast cancer (10.0% vs 7.5%) and BBD (26.0% vs 23.9%). One hundred twenty-two girls reported biopsy-confirmed BBD over the study follow-up period.

β-Carotene intake was highly correlated with α-carotene (r = 0.79) and lutein/zeaxanthin intake (r = 0.81; Table 2). β-Cryptoxanthin was moderately correlated with α-carotene, β-carotene, and lutein/zeaxanthin (r = 0.34–0.37). Lycopene had limited correlation with the other carotenoids (r ≤0.16).

In age- and energy-adjusted analyses, intakes of α-carotene, β-carotene, and lutein/zeaxanthin were inversely associated with BBD, but associations were not significant (Table 3). For example,
TABLE 1 Characteristics of Girls in GUTS, by Quartile of β-Carotene Intake

<table>
<thead>
<tr>
<th>Variable</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>1648</td>
<td>1648</td>
<td>1649</td>
<td>1648</td>
</tr>
<tr>
<td>β-Carotene, μg/d</td>
<td>1020</td>
<td>1778</td>
<td>2500</td>
<td>4371</td>
</tr>
<tr>
<td>α-Carotene, μg/d&lt;sup&gt;a&lt;/sup&gt;b</td>
<td>185</td>
<td>384</td>
<td>546</td>
<td>850</td>
</tr>
<tr>
<td>β-Cryptoxanthin, μg/d&lt;sup&gt;a&lt;/sup&gt;b</td>
<td>116</td>
<td>139</td>
<td>161</td>
<td>196</td>
</tr>
<tr>
<td>Lycopene, μg/d&lt;sup&gt;a&lt;/sup&gt;b</td>
<td>5051</td>
<td>5280</td>
<td>5498</td>
<td>5861</td>
</tr>
<tr>
<td>Lutein/Zeaxanthin, μg/d&lt;sup&gt;a&lt;/sup&gt;b</td>
<td>838</td>
<td>1319</td>
<td>1950</td>
<td>4124</td>
</tr>
<tr>
<td>Vitamin A, RAE&lt;sup&gt;a&lt;/sup&gt;b</td>
<td>902</td>
<td>1035</td>
<td>1152</td>
<td>1460</td>
</tr>
<tr>
<td>Age at baseline questionnaire, y</td>
<td>11.9</td>
<td>12.0</td>
<td>12.0</td>
<td>12.2</td>
</tr>
<tr>
<td>BMI&lt;sup&gt;a&lt;/sup&gt;b</td>
<td>19.8</td>
<td>19.5</td>
<td>19.8</td>
<td>19.9</td>
</tr>
<tr>
<td>Age at menarche, y</td>
<td>12.3</td>
<td>12.4</td>
<td>12.3</td>
<td>12.3</td>
</tr>
<tr>
<td>Alcohol intake, drinks/wk&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.3</td>
<td>0.4</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Total energy intake, kcal/d&lt;sup&gt;a&lt;/sup&gt;b</td>
<td>1957</td>
<td>2040</td>
<td>1994</td>
<td>1965</td>
</tr>
<tr>
<td>Moderate to vigorous physical activity, h/wk&lt;sup&gt;d&lt;/sup&gt;</td>
<td>11.1</td>
<td>11.4</td>
<td>12.2</td>
<td>12.9</td>
</tr>
<tr>
<td>Ever pregnant&lt;sup&gt;e&lt;/sup&gt;</td>
<td>%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother or aunt with breast cancer</td>
<td>%</td>
<td>7.5</td>
<td>9.5</td>
<td>8.7</td>
</tr>
<tr>
<td>Mother with BBD, %</td>
<td>23.9</td>
<td>23.4</td>
<td>27.1</td>
<td>26.0</td>
</tr>
</tbody>
</table>

Data are presented as means (SD) or percentages. N = 6593. Q, quartile.

<sup>a</sup> Nutrient intake was assessed by using the YAQ and adjusted for total energy intake by using the residual method.

<sup>b</sup> Data derived as means of participant values reported in 1996, 1997, and 1998.


<sup>d</sup> Through year of BBD report or end of follow-up (2010), whichever came first.

<sup>e</sup> Data as means of participant values reported in 1997, 1998, and 1999.

DISCUSSION

In this prospective cohort study in young women, higher adolescent β-carotene intake was associated with lower risk of BBD in young women. The highest intakes of α-carotene and lutein/zeaxanthin were suggestively inversely associated with BBD risk, although these associations did not reach statistical significance. Higher intakes of carrots appeared to be associated with lower risk of BBD, but associations were not statistically significant. The other carotenoids examined were not associated with BBD in young women, although the limited

TABLE 2 Spearman Correlations Between Mean Intakes of Energy-Adjusted Carotenoids in Girls in GUTS

<table>
<thead>
<tr>
<th>α-Carotene</th>
<th>β-Carotene</th>
<th>β-Cryptoxanthin</th>
<th>Lutein/Zeaxanthin</th>
<th>Lycopene</th>
<th>Vitamin A (RAE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Carotene</td>
<td>1.00</td>
<td>0.79</td>
<td>0.34</td>
<td>0.46</td>
<td>0.07</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>1.00</td>
<td>0.37</td>
<td>0.81</td>
<td>0.05</td>
<td>0.16</td>
</tr>
<tr>
<td>β-Cryptoxanthin</td>
<td>1.00</td>
<td>0.37</td>
<td>0.81</td>
<td>0.05</td>
<td>0.16</td>
</tr>
<tr>
<td>Lutein/Zeaxanthin</td>
<td>1.00</td>
<td>0.37</td>
<td>0.81</td>
<td>0.05</td>
<td>0.16</td>
</tr>
<tr>
<td>Lycopene</td>
<td>1.00</td>
<td>0.37</td>
<td>0.81</td>
<td>0.05</td>
<td>0.16</td>
</tr>
<tr>
<td>Vitamin A (RAE)</td>
<td>1.00</td>
<td>0.37</td>
<td>0.81</td>
<td>0.05</td>
<td>0.16</td>
</tr>
</tbody>
</table>

N = 6593.
sample size makes it difficult to rule out a modest association.

To our knowledge, this is the first prospective cohort study examining the relation of adolescent carotenoid intakes and BBD risk in young women. Our findings align with recent pooled studies and meta-analyses suggesting an inverse association between dietary intake/circulating levels of \( \beta \)-carotene and breast cancer risk in adult women.\(^1\)\(^{-5} \)

In the 2 existing pooled analyses, both blood levels and dietary intake of \( \alpha \)-carotene and lutein/zeaxanthin were also inversely associated with breast cancer risk, particularly risk of ER-negative breast cancer. The highest intakes (top quartile) of these nutrients were associated with lower risk of BBD in this study, although the associations did not reach statistical significance. However, \( \alpha \)-carotene and lutein/zeaxanthin intakes are highly correlated with \( \beta \)-carotene intake; therefore, the individual carotenoid(s) driving the associations cannot be isolated with certainty. Alternatively, in light of the fact that individual carotenoid supplementation trials have not shown benefits on cancer risk,\(^{24} \) it may be that the group of micronutrients found in carotenoid-rich foods is important, more so than any 1 individual nutrient.

In the pooled analysis of plasma carotenoids, the authors also observed an inverse association between plasma lycopene and breast cancer risk,\(^2 \) but in our study lycopene was not related to BBD risk.

Carotenoids may reduce risk of both BBD and breast cancer through several mechanisms. As antioxidants, carotenoids may reduce oxidative damage due to environmental exposures and by normal metabolic processes in the body.\(^9\)\(^{-11} \) Finally, these phytochemicals may enhance the cell-mediated immune response through increased production of major histocompatibility complex class II molecules, T-cell activation, and improved intracellular communication in gap junctions.\(^9\)\(^{11}\)\(^{12} \)

Our study is limited by the small number of biopsy-confirmed BBD cases. BBD cases were self-reported and date of diagnosis was not assessed, which may have caused some misclassification of the outcome and did not allow us to assess the timing of exposure and disease. However, any error in BBD report is unlikely to be differentially related to carotenoid intake, and in GUTS, lower adiposity, greater adolescent alcohol consumption, lower intakes of nuts and peanut butter, more rapid height growth, and greater adult height were associated with BBD,\(^{25}\)\(^{-28} \) as expected on the basis of known associations with breast cancer. These findings indicate sufficient power and that, overall, the BBD reports in our study represent true BBD. Because dietary intake was self-reported by girls, some misclassification of carotenoid
intake is likely, but any measurement error should not be differentially related to future BBD status. Therefore, misclassification of the exposure and outcome is likely to have attenuated the true association. Stronger associations with breast cancer have been observed with plasma carotenoids versus dietary carotenoid intake, which may be true with breast cancer have been observed with plasma carotenoids versus dietary carotenoid intake, which may be true with breast cancer have been observed with plasma carotenoids versus dietary carotenoid intake, which may be true with breast cancer have been observed with plasma carotenoids versus dietary carotenoid intake, which may be true with breast cancer have been observed with plasma carotenoids versus dietary carotenoid intake, which may be true with breast cancer have been observed with plasma carotenoids versus dietary carotenoid intake, which may be true with breast cancer have been observed with plasma carotenoids versus dietary carotenoid intake, which may be true with breast cancer have been observed with plasma carotenoids versus dietary carotenoid intake, which may be true with breast cancer have been observed with plasma carotenoids versus dietary carotenoid intake, which may be true with breast cancer have been observed with plasma carotenoids versus dietary carotenoid intake, which may be true with breast cancer have been observed with plasma carotenoids versus dietary carotenoid intake, which may be true.

The detailed data allowed us to carefully adjust for variables associated with both carotenoids and BBD.

CONCLUSIONS
Higher adolescent β-carotene intake was associated with lower BBD risk in young women in this prospective cohort study. The highest intakes of α-carotene and lutein/zeaxanthin also were inversely associated with BBD risk, but these findings were not statistically significant. Given the limited number of biopsy-confirmed BBD cases in this population, our findings should be evaluated later after considerably more cases have been diagnosed in this cohort and confirmed in other prospective studies. Given the potential harmful effects of β-carotene supplementation and that the association could be due to other phytonutrients correlated with β-carotene, supplementation is not recommended at this time. However, these data provide intriguing preliminary evidence that carrots and other carotenoid-rich vegetables may help to protect against BBD. These findings suggest that BBD prevention in young women may be 1 of many positive health effects of fruit and vegetable consumption.

ACKNOWLEDGMENTS
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