Dietary carotenoids and risk of breast cancer

Paul Terry, Meera Jain, Anthony B Miller, Geoffrey R Howe, and Thomas E Rohan

ABSTRACT

Background: Many studies of fruit and vegetable consumption showed inverse associations with breast cancer risk, suggesting the potential importance of carotenoids (and other phytochemicals) contained in these foods. To date, however, only one prospective cohort study has examined dietary carotenoids other than β-carotene in relation to breast cancer risk.

Objective: Our aim was to examine the relations between dietary intakes of β-carotene, α-carotene, β-cryptoxanthin, lycopene, and lutein + zeaxanthin and breast cancer risk in a large cohort study of Canadian women.

Design: A case-cohort analysis was undertaken in a cohort of 56,837 women who were enrolled in the Canadian National Breast Screening Study and who completed a self-administered dietary questionnaire. During follow-up to the end of 1993 a total of 1,589 women were diagnosed with biopsy-confirmed incident breast cancer. For comparison, a subcohort of 5,681 women was randomly selected. After exclusions for various reasons, the analyses were based on 1,452 cases and 5,239 noncases.

Results: We found no clear association between intakes of any of the studied carotenoids and breast cancer risk in the study population as a whole or in subgroups defined by smoking status; relative body weight (assessed by body mass index); intakes of total fat, energy, alcohol, or folic acid; family history of breast cancer; or menopausal status.

Conclusions: Our data do not support any association between dietary intakes of the studied carotenoids and breast cancer risk. However, prospective cohort studies of carotenoids in relation to breast cancer are scarce and further studies are warranted.

KEY WORDS Breast neoplasms, diet, carotenoids, β-carotene, cohort studies, women, Canadian National Breast Screening Study

INTRODUCTION

Two decades ago, evidence from observational studies in human populations, animal experiments, in vitro studies, and in vivo studies (1, 2) led to the initiation of several large-scale randomized trials of β-carotene supplementation in relation to cancer risk (3–5). The results of these trials showed no clear benefits, and indeed, in the β-carotene arm of these trials there was actually seen an increased risk of lung cancer (the explicit target of the interventions; 2). Nevertheless, interest in carotenoids as potential chemopreventive agents remains strong.

With respect to breast cancer, many studies showed inverse associations between fruit and vegetable consumption and risk (6, 7), suggesting the potential importance of carotenoids (and other phytochemicals) contained in these foods. However, not all studies found associations (6–9), which may reflect the fact that total fruit and vegetable consumption may not accurately rank individuals according to micronutrients that are abundant in some, but not all, fruit and vegetables (10).

The relative importance of the various dietary carotenoids in relation to cancer risk remains unclear. For example, it has been speculated that β-carotene might be less important than other carotenoids in cancer prevention (2, 11). Specifically, several studies do not provide support for the antioxidative properties of β-carotene and suggest that, isolated from the other carotenoids, β-carotene may even have prooxidative properties (2). These studies have prompted closer examination of the potential anticarcinogenic effects of other carotenoids that are predominant in the human diet, namely, α-carotene, β-cryptoxanthin, lycopene, and lutein. Moreover, total carotenoids might be more important than any single carotenoid studied in isolation because the various carotenoids appear to neutralize different free radicals at different locations within membranes (11–15), and therefore they may work synergistically to reduce cancer risk. Carotenoids might also have other antimutagenic and anticarcinogenic properties. For example, lutein and α-carotene may decrease the activity of cytochrome P450 1A2, an activator of procarcinogens (16), and β-cryptoxanthin might stimulate the expression of RB, a tumor-suppressor gene (17), and p73, a p53-related gene (11).

Information on the carotenoid content of various foods has become available in the past few years (17), and consequently the number of studies examining dietary carotenoids has increased. However, to date only one prospective cohort study examined dietary carotenoids other than β-carotene in relation to breast cancer risk (18). Therefore, in the present study, we examined the relations between dietary intakes of β-carotene, α-carotene,
β-cryptoxanthin, lycopene, and lutein + zeaxanthin and breast cancer risk.

SUBJECTS AND METHODS

Overview

We performed a case-cohort analysis with data from a cohort of 56,837 women in the Canadian National Breast Screening Study (NBSS) who completed a self-administered, quantitative food-frequency questionnaire (in addition to an epidemiologic questionnaire, which was completed by all NBSS participants). The NBSS is a multicenter randomized controlled trial of (primarily) mammographic screening for breast cancer in 89,835 women aged 40–59 y at recruitment (19, 20). Participants were recruited between 1980 and 1985 by various means, including personal invitation by letter, group mailings to employees of large institutions and to members of professional associations, advertisements in newspapers, and public service announcements on radio and television. The study was approved by the Human Subjects Review Committee of the University of Toronto.

Dietary and risk factor data

On enrollment in the NBSS, all participants completed a questionnaire that sought data on demographic characteristics, family history of breast cancer, menstrual and reproductive history, use of oral contraceptives and replacement estrogens, and cigarette smoking. Starting in 1982, a modified version of a previously validated self-administered quantitative food-frequency questionnaire (21) was distributed to all new attendees at all screening centers and to women returning to the screening centers for rescreening. By the time that the dietary questionnaire was introduced, some women had already been enrolled in the study and were not seen again at the screening centers. A total of 56,837 women returned completed dietary questionnaires, and these women constitute the cohort on which the study described here was based; these women were similar to the remainder of the cohort with respect to the variables included in the multivariate models.

The food-frequency questionnaire contained questions on the frequency of consumption and usual portion size of 86 food items, including 22 items related to fruit and vegetable consumption. Pho-

tographs of various portion sizes were included in the questionnaire to assist participants with quantification of intake. Data from the completed self-administered questionnaires were used to estimate daily intakes of carotenoids (β-carotene, α-carotene, β-cryptoxanthin, lycopene, and lutein + zeaxanthin), total energy, and various macro- and micronutrients, with the use of a nutrient database developed by modifying and extending food-composition tables from the US Department of Agriculture to include typically Canadian foods (22). The values for carotenoid intakes presented here are for intakes from dietary sources alone (17), because supplemental carotenoids were not in general use in Canada when the data were collected.

Case definition and ascertainment

Outcome (incident breast cancer or death) was ascertained by computerized record linkage to the National Mortality Database and to the Canadian Cancer Database (a composite of data collected by the provincial population-based cancer registries), both of which are maintained by Statistics Canada. Cases were women who were diagnosed during follow-up with incident in situ or invasive carcinoma of the breast. Diagnoses of breast cancer were confirmed by obtaining pathology reports from the relevant provincial cancer registry. There is good evidence from the NBSS and from other sources that the use of record linkage to ascertain incident cancer cases and deaths in Canada is both accurate and complete (23, 24). The linkages to the databases yielded data on mortality and cancer incidence to 31 December 1993. In all, 1589 cases were identified, of whom 144 were in the subcohort (see below). Of these 1589 women, 131 (3 of whom were in the subcohort) were excluded from the present study because their dietary questionnaires were not available.

Construction of the subcohort

A subcohort was constructed by selecting a stratified (by recruitment center) random sample of 5681 women from the dietary cohort. Of those selected, the 259 women whose dietary questionnaires were not available were excluded from the present analysis.

Statistical analysis

Incidence rate ratios for the associations between carotenoid intake and risk of breast cancer were estimated with a Poisson regression, and robust SEs were calculated (25), thereby yielding the appropriate CIs for the rate ratios, given the case-cohort sampling. Cases contributed person-time to the study from their date of enrollment until the date of diagnosis of their breast cancer, and noncases contributed person-time from their date of enrollment until the termination of follow-up (31 December 1993) or death, whichever was earlier. Nutrients were adjusted for total energy with the residual method (26), and the incidence rate ratios were adjusted for energy intake (fitted as a continuous variable) and for potential confounding by the following variables: age, age at menarche, number of live childbirths, self-reported menopausal status, family history of breast cancer in a first-degree relative, history of benign breast disease, duration of use of oral contraceptives and exogenous hormones, practice of breast self-examination, randomization group and study center, and dietary intakes of total fiber, calcium, folic acid, and alcohol. Individual carotenoids were analyzed in models with and without mutual adjustment.

Those subjects for whom the estimate of log-transformed total energy intake was more than 3 SDs from the mean were excluded from further consideration because their estimated energy intake was highly suggestive of incorrectly recorded intake. This resulted in the exclusion of 6 cases (none of whom was in the subcohort) and 63 noncases. Therefore, given the various exclusions described above, the analyses were based on 1452 cases and 5239 noncases.

In addition to analyzing risk in association with the individual carotenoids, we created a carotenoid index by summing the quintile scores for each of the 5 major carotenoids and categorized the resulting value by quartiles (quintile scores ranged from 1 to 5). For example, women who were in the lowest quintile for each of the 5 carotenoids had an index score of 5, and women who were in the highest quintile for each of the carotenoids had an index score of 25. For tests of trend in risk across successive levels of categorical variables, median values of each category were fitted in the risk models as successive integers. Tests for interaction were based on likelihood ratio tests comparing models with and without product terms representing the variables of interest. The likelihood ratio test that all of the interaction variables were zero was performed by referring the differences between the deviances of models with and without interaction terms to the chi-squared distribution on degrees of freedom equal to the number of interaction variables. Statistical analysis was performed with the STATA software (Stata Corp, College Station, TX). $P < 0.05$ indicated significance.
TABLE 1
Incidence rate ratios (IRRs) for the association between intakes of specific carotenoids and breast cancer risk

<table>
<thead>
<tr>
<th>Quintiles of energy-adjusted carotenoid intakes</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Carotene (µg/d)</td>
<td>2205 ± 610</td>
<td>3570 ± 355</td>
<td>4797 ± 385</td>
<td>6479 ± 647</td>
<td>9832 ± 3817</td>
<td>0.27</td>
</tr>
<tr>
<td>Breast cancer cases</td>
<td>300</td>
<td>299</td>
<td>260</td>
<td>312</td>
<td>281</td>
<td>0.27</td>
</tr>
<tr>
<td>Person-years</td>
<td>12723</td>
<td>13249</td>
<td>12415</td>
<td>12848</td>
<td>12802</td>
<td>0.27</td>
</tr>
<tr>
<td>Age-adjusted IRR (95% CI)</td>
<td>1.0</td>
<td>0.95 (0.7, 1.15)</td>
<td>0.86 (0.7, 1.04)</td>
<td>0.94 (0.7, 1.14)</td>
<td>0.93 (0.7, 1.16)</td>
<td>0.48</td>
</tr>
<tr>
<td>Multivariate IRR (95% CI)</td>
<td>1.0</td>
<td>0.91 (0.7, 1.12)</td>
<td>0.91 (0.7, 1.17)</td>
<td>1.01 (0.7, 1.33)</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>α-Carotene (µg/d)</td>
<td>344 ± 139</td>
<td>659 ± 91</td>
<td>979 ± 97</td>
<td>1394 ± 169</td>
<td>2441 ± 1186</td>
<td>0.57</td>
</tr>
<tr>
<td>Breast cancer cases</td>
<td>300</td>
<td>299</td>
<td>270</td>
<td>281</td>
<td>302</td>
<td>0.57</td>
</tr>
<tr>
<td>Person-years</td>
<td>12728</td>
<td>12587</td>
<td>12408</td>
<td>12487</td>
<td>13048</td>
<td>0.57</td>
</tr>
<tr>
<td>Age-adjusted IRR (95% CI)</td>
<td>1.0</td>
<td>1.01 (0.8, 1.21)</td>
<td>0.99 (0.7, 1.13)</td>
<td>0.94 (0.7, 1.16)</td>
<td>0.98 (0.8, 1.17)</td>
<td>0.57</td>
</tr>
<tr>
<td>Multivariate IRR (95% CI)</td>
<td>1.0</td>
<td>0.94 (0.7, 1.16)</td>
<td>0.91 (0.7, 1.13)</td>
<td>0.94 (0.7, 1.16)</td>
<td>1.01 (0.8, 1.25)</td>
<td>0.95</td>
</tr>
<tr>
<td>β-Cryptoxanthin (µg/d)</td>
<td>260 ± 15.8</td>
<td>72.5 ± 11.1</td>
<td>110.9 ± 12.7</td>
<td>163.8 ± 17.7</td>
<td>246.0 ± 82.5</td>
<td>0.57</td>
</tr>
<tr>
<td>Breast cancer cases</td>
<td>306</td>
<td>263</td>
<td>269</td>
<td>311</td>
<td>299</td>
<td>0.57</td>
</tr>
<tr>
<td>Person-years</td>
<td>12493</td>
<td>12357</td>
<td>12674</td>
<td>12822</td>
<td>12742</td>
<td>0.57</td>
</tr>
<tr>
<td>Age-adjusted IRR (95% CI)</td>
<td>1.0</td>
<td>0.85 (0.7, 1.02)</td>
<td>0.85 (0.7, 1.02)</td>
<td>0.95 (0.8, 1.14)</td>
<td>0.93 (0.7, 1.12)</td>
<td>0.86</td>
</tr>
<tr>
<td>Multivariate IRR (95% CI)</td>
<td>1.0</td>
<td>0.81 (0.6, 1.01)</td>
<td>0.77 (0.6, 0.96)</td>
<td>0.87 (0.6, 1.09)</td>
<td>0.88 (0.6, 1.13)</td>
<td>0.59</td>
</tr>
<tr>
<td>Lycopene (µg/d)</td>
<td>2283 ± 1070</td>
<td>5340 ± 843</td>
<td>8488 ± 1016</td>
<td>13039 ± 1814</td>
<td>23748 ± 17217</td>
<td>0.57</td>
</tr>
<tr>
<td>Breast cancer cases</td>
<td>283</td>
<td>326</td>
<td>264</td>
<td>278</td>
<td>300</td>
<td>0.57</td>
</tr>
<tr>
<td>Person-years</td>
<td>12460</td>
<td>12762</td>
<td>12357</td>
<td>12529</td>
<td>13044</td>
<td>0.57</td>
</tr>
<tr>
<td>Age-adjusted IRR (95% CI)</td>
<td>1.0</td>
<td>1.15 (0.9, 1.38)</td>
<td>0.93 (0.7, 1.13)</td>
<td>0.97 (0.8, 1.17)</td>
<td>1.01 (0.8, 1.22)</td>
<td>0.46</td>
</tr>
<tr>
<td>Multivariate IRR (95% CI)</td>
<td>1.0</td>
<td>1.18 (0.9, 1.46)</td>
<td>0.94 (0.7, 1.17)</td>
<td>0.96 (0.7, 1.19)</td>
<td>1.14 (0.9, 1.41)</td>
<td>0.85</td>
</tr>
<tr>
<td>Lutein + zeaxanthin (µg/d)</td>
<td>1219 ± 332</td>
<td>1961 ± 204</td>
<td>2721 ± 249</td>
<td>3783 ± 439</td>
<td>6838 ± 5490</td>
<td>0.57</td>
</tr>
<tr>
<td>Breast cancer cases</td>
<td>263</td>
<td>285</td>
<td>306</td>
<td>312</td>
<td>286</td>
<td>0.57</td>
</tr>
<tr>
<td>Person-years</td>
<td>12776</td>
<td>12578</td>
<td>12658</td>
<td>12719</td>
<td>12547</td>
<td>0.57</td>
</tr>
<tr>
<td>Age-adjusted IRR (95% CI)</td>
<td>1.0</td>
<td>1.11 (0.9, 1.34)</td>
<td>1.20 (1.0, 1.45)</td>
<td>1.21 (1.0, 1.46)</td>
<td>1.12 (0.9, 1.35)</td>
<td>0.15</td>
</tr>
<tr>
<td>Multivariate IRR (95% CI)</td>
<td>1.0</td>
<td>1.11 (0.9, 1.38)</td>
<td>1.25 (1.0, 1.56)</td>
<td>1.24 (0.9, 1.56)</td>
<td>1.17 (0.9, 1.53)</td>
<td>0.14</td>
</tr>
</tbody>
</table>

1Nutrients adjusted to 2065 kcal, which is the rounded value corresponding to the mean energy intake in the study group.
2Two-sided Wald tests.
3Median ± SD.
4Multivariate models included age (in 5-y age groups), screening center, allocation, smoking (never, past, or current), body mass index (in quintiles), hours of vigorous physical activity (in quintiles), education (3 categories), family history of breast cancer (yes or no), history of benign breast disease, age at menarche (quintiles), parity (quintiles), menopausal status (pre, peri, post), oral contraceptive use (never, duration in quintiles), hormone replacement therapy (never, duration in quintiles), practiced breast self-examination (yes or no), multivitamin use (yes or no), and intakes of total energy (continuous), alcohol, dietary fiber, folate, and calcium (quintiles).

RESULTS
The average follow-up period for the study was 9.5 y and the average age at breast cancer diagnosis was 55.2 y. Age, energy intake, and multivitamin use varied little over the quintiles of carotenoid intake (data not shown). Similarly, body mass index was not clearly associated with carotenoid intake, except for a weak positive association with β-cryptoxanthin, whereas smoking was inversely associated with intake of each of the studied carotenoids. Dietary fiber intake was positively associated with intakes of all of the studied carotenoids, most prominently with β-carotene (Pearson’s correlation \( R = 0.44 \)), β-cryptoxanthin (\( R = 0.44 \)), and lutein + zeaxanthin (\( R = 0.36 \)). Dietary folate was also positively associated with all of the studied carotenoids, most prominently with β-carotene (\( R = 0.51 \)) and lutein + zeaxanthin (\( R = 0.67 \)).

Dietary intakes of β-carotene, α-carotene, β-cryptoxanthin, lycopene, and lutein + zeaxanthin were not associated with breast cancer risk (Table 1). Examining deciles of the carotenoids also showed no association. Furthermore, the index of total carotenoid intake was also unrelated to risk: the multivariate-adjusted incidence rate ratios (95% CI) for increasing quartiles of the index, compared with the lowest, were 1.12 (0.94, 1.34), 1.10 (0.89, 1.36), and 1.10 (0.72, 1.65); \( P \) for trend = 0.34. Results were essentially unaltered when the carotenoids were mutually adjusted.

The associations between intake of carotenoids and risk did not vary according to strata of menopausal status, smoking status, alcohol consumption, family history of breast cancer, or intakes of energy, total fat, or folic acid (Table 2). On formal testing there was no evidence of interactions between dietary carotenoids and those factors in relation to breast cancer risk. For comparison with a previous prospective cohort study (18), we examined risk in relation to carotenoid intake among premenopausal women with either a family history of breast cancer in a first-degree relative or a previous prospective cohort study (18), we examined risk in relation to carotenoid intake among premenopausal women with either a family history of breast cancer in a first-degree relative or alcohol consumption of ≥ 15 g/d. We did not observe any association between carotenoid intake and breast cancer risk in these subgroups (data not shown).

The results described above were largely the same when the analyses were conducted separately in the screened and control arms of the NBSS (thereby restricting attention in each case to individuals screened to a comparable extent). The results were also largely unaltered after excluding in situ cases (\( n = 120 \)), after excluding folate intake from the multivariate model, and after excluding cases that occurred within the first year of follow-up, thereby (in the latter case) reducing the possibility that changes in carotenoid consumption caused by preclinical undiagnosed breast cancer might have influenced our results. Multivariate analyses in which dietary fiber intake (which was positively correlated with dietary carotenoids) was also positively associated with all of the studied carotenoids, most prominently with β-carotene (\( R = 0.51 \)) and lutein + zeaxanthin (\( R = 0.67 \)).
the full multivariate model. Finally, excluding multivitamin users (5.3% of the study population) did not alter the results.

**DISCUSSION**

We did not find any clear association between intake of any of the studied carotenoids and breast cancer risk in the study population as a whole or in subgroups defined by smoking status; relative body weight (as assessed by body mass index); intakes of total fat, energy, alcohol, and folic acid; family history of breast cancer; or menopausal status. Statistical adjustment for potentially confounding variables did not alter the results.

At least 20 previous studies addressed intake of carotenoids in relation to breast cancer risk (27). Most of these examined β-carotene but not other carotenoids. Of the studies that examined only β-carotene, case-control studies tended to show inverse associations, and the results of prospective cohort studies (which were fewer in number) were mixed (27). Only one previous cohort study examined dietary intakes of carotenoids other than β-carotene in relation to breast cancer risk (18). That study found weak inverse associations for dietary intakes of β-carotene and lutein + zeaxanthin in a cohort of female American nurses. Further analyses among various subgroups of that cohort revealed inverse associations for increasing quintiles of α-carotene, β-carotene, and lutein + zeaxanthin intakes among premenopausal women with a positive family history of breast cancer. The study also found an inverse association for increasing quintiles of β-carotene intake among premenopausal women who consumed ≥15 g alcohol/d. Our data from the present study do not provide any support for effect modification by a combination of menopausal status at recruitment and either a family history of breast cancer or high daily alcohol consumption, although the number of cases available for analyses in these particular subgroups was relatively low (n = 159 and 191, respectively). Furthermore, the number of women who were still premenopausal at the time of their breast cancer diagnosis was even smaller. Thus, the inverse associations between dietary intakes of certain carotenoids in particular subgroups of the population that were observed previously have yet to be confirmed.

Case-control studies, in contrast, have generally shown more consistent inverse associations between dietary carotenoids and breast cancer risk, particularly with β-carotene (27). Of the 3 case-control studies that examined other carotenoids in addition to β-carotene (29–31), one study in premenopausal women showed an inverse association with intakes of β-carotene and of lutein + zeaxanthin but not with intake of α-carotene (28); one study showed an inverse association with lycopene intake but not with intakes of α-carotene, lutein + zeaxanthin, or...
β-cryptoxanthin in women of all ages (29); and one study in young women showed no association between breast cancer and intakes of α- and β-carotene, lutein, lycopene, or β-cryptoxanthin (30). However, interpretation of these studies must include the possibilities of recall bias and selection bias, which are generally not issues in the prospective cohort studies.

Serum carotenoid concentrations have been found to reflect long-term dietary carotenoid intake (31). Four previous prospective cohort studies examined serum carotenoids in relation to breast cancer risk (32–35). Two of these studies found no association, either with total serum carotenoids (35) or with specific carotenoids (33). However, these analyses were based on 11 and 30 breast cancer cases, respectively, and the latter study was restricted to postmenopausal women. Another cohort study, with 105 cases, found a statistically significant inverse association only for serum lycopene, nonsignificant inverse associations for serum β-cryptoxanthin and lutein + zeaxanthin, and no association between β-carotene or α-carotene and breast cancer risk (34). The most recent of these 4 cohort studies, with 270 cases, found inverse associations with serum β-carotene, α-carotene, β-cryptoxanthin, and lutein (32). Thus, prospective cohort studies of serum carotenoids are few in number and their results have been mixed.

Among the strengths of the present study was the large sample size of our cohort of women and the relatively long-term follow-up. The completeness of follow-up of the cohort (23, 24) reduces the likelihood that our results reflect bias caused by differential follow-up of exposed compared with unexposed women. It is also unlikely that undiagnosed early stages of breast cancer altered carotenoid intake because we also observed no association after excluding cases that occurred during the first year of follow-up. Moreover, the large number of cases in our study allowed us to examine associations among subgroups of our study population with reasonable statistical power.

Our data were limited, however, by the likelihood of nondifferential measurement error with respect to carotenoid intake (36). Nondifferential misclassification would tend to attenuate true associations and can stem from measurement error in the assessment of diet caused by inaccurate recall of past diet (36), changes in diet over time (36), or degradation of nutrients during cooking and storage of fruit and vegetables (13, 36). Finally, although we adjusted our estimates for a wide range of potentially confounding variables, uncontrolled confounding from dietary (or other) factors cannot be excluded.

In conclusion, our data do not support any association between dietary intakes of the studied carotenoids and breast cancer risk. Although our null results are consistent with the main results of a previous cohort study of dietary carotenoid intake among American nurses (18), we were unable to confirm the inverse associations observed among premenopausal women who either had a family history of breast cancer or consumed relatively high amounts of alcohol daily. However, prospective cohort studies of dietary carotenoids in relation to breast cancer are scarce and further studies are warranted.

REFERENCES


