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ORIGINAL CONTRIBUTIONS

Serum Carotenoids and Breast Cancer

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The consumption of vegetables and fruit may protect against many types of cancer, but research evidence is not compelling for breast cancer. Carotenoids are pigments that are present in most plants and have known antioxidant properties. Blood concentrations of carotenoids have been proposed as integrated biochemical markers of vegetable, fruit, and synthetic supplements consumed. In a case-control study (270 cases, 270 controls) nested within a cohort in New York during 1985–1994, the carotenoids lutein, zeaxanthin, β-cryptoxanthin, lycopene, α-carotene, and β-carotene were measured in archived serum samples using liquid chromatography. There was an evident increase in the risk of breast cancer for decreasing β-carotene, lutein, α-carotene, and β-cryptoxanthin. The risk of breast cancer approximately doubled among subjects with blood levels of β-carotene at the lowest quartile, as compared with those at the highest quartile (odds ratio = 2.21; 95% confidence interval (CI): 1.29, 3.79). The risk associated with the other carotenoids was similar, varying between 2.08 (95% CI: 1.11, 3.90) for lutein and 1.68 (95% CI: 0.99, 2.86) for β-cryptoxanthin. The odds ratio for the lower quartile of total carotenoids was 2.31 (95% CI: 1.35, 3.96). These observations offer evidence that a low intake of carotenoids, through poor diet and/or lack of vitamin supplementation, may be associated with increased risk of breast cancer and may have public health relevance for people with markedly low intakes. *Am J Epidemiol* 2001;153:1142–7.

breast neoplasms; carotenoids; case-control studies; cohort studies; diet; nutrition; risk factors; vitamin A

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Western lifestyle and diet have long been associated with increased risk of breast cancer. As compared with Asia and Africa where breast cancer occurs severalfold less frequently (1), the diets of people living in the United States and Western Europe are dense in energy, rich in refined carbohydrates, fats, and animal proteins, and poor in vegetables, fruit, and fiber (2). Epidemiologic research on the topic has focused largely on excessive body weight and on the intake of animal fat, while the protective role of plant food has attracted only limited research attention. Only two prospective cohort studies reported on the association between vegetable intake and breast cancer, one suggesting a protective effect (3) and one reporting no association (4).

We investigated the consumption of vegetables and fruit in the etiology of breast cancer by examining biochemical markers in serum that may be related to their habitual consumption. Of interest were carotenoids, natural pigments which have an important role in the initiation of photosynthesis in plants and are present in appreciable concentrations also in some birds, insects, and marine animals (5). Carotenoids are important antioxidants. By counteracting oxidative processes that could damage macromolecules, such as proteins and DNA, and that could interfere with the normal functioning of cells, carotenoids may contribute to the prevention of cancer and other degenerative diseases (6).

MATERIALS AND METHODS

Study subjects

The New York University Women's Health Study is a prospective cohort study on hormones, diet, and metabolism in the etiology of cancer in women (7, 8). Cohort members were 14,275 women, aged 35–65 years at recruitment, who had volunteered for the study in 1985–1991 while undergoing mammography at a screening clinic in New York City.

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Abbreviation: CI, confidence interval.

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At enrollment, study subjects completed self-administered questionnaires reporting information on personal characteristics, lifestyle, reproductive experience, and medical history and answered a short self-administered dietary questionnaire concerning usual food consumption during the previous year. All cohort members had 30 ml of nonfasting peripheral venous blood drawn at baseline. Whole blood was kept over the counter for approximately 60–90 minutes before being spun (600 rpm for 15 minutes) to harvest supernatant serum, which was quickly partitioned into 1-ml aliquots for storage at −80°C. Additional blood samples were drawn from approximately one half of the participants at subsequent screening visits.

Study subjects were followed up to identify all cases of cancer that occurred during the study period. Follow-up consisted of periodic direct contacts with participants by mail and telephone and record linkages with both the statewide tumor registries of New York, New Jersey, Connecticut, and Florida and mortality databases. Approximately 80 percent of reported cancer cases were confirmed through an internal review of clinical and pathologic documents, and the remaining 20 percent were confirmed through record linkage with a tumor registry. We estimate follow-up to be over 97 percent complete for breast cancer cases.

Subjects diagnosed with breast cancer prior to 1995 were included in a case-control study nested within the cohort. To remove subjects who may have been symptomatic at baseline screening, cases were excluded if cancer diagnosis had been reached within 6 months or less after cohort enrollment. Controls were cohort members free of cancer randomly selected among those who matched a case by age at recruitment (±3 months), menopausal status at baseline (pre- or postmenopausal), date of baseline blood sampling (±3 months), and number of blood samplings prior to a case's date of diagnosis. If premenopausal, controls were matched also by phase and day of the menstrual cycle at the time of baseline blood collection (counting back from the first day of the next cycle). One control was chosen for each case.

The study was reviewed and approved annually by the Institutional Review Board of New York University School of Medicine in accord with an assurance filed and approved by the US Department of Health and Human Services. Written, informed consent was obtained from all human subjects.

Laboratory methods

For each study subject, one aliquot of serum never previously defrosted was retrieved from storage and shipped in dry ice to the laboratory of the Unit of Nutrition, International Agency for Research on Cancer, Lyon, France. On the day of analysis, samples were thawed at room temperature (about 25°C), protected from light. Cases and controls pertaining to the same matched set were analyzed on the same batch and on the same day. Samples from two standard pools were included every day as quality controls. Laboratory technicians could not identify samples of cases, controls, and quality controls. Samples were analyzed according to the method of Steghens et al. (9) adapted for a Hewlett-Packard model 1100 high performance liquid chromatography system with binary pump, automatic injector, column oven, and diode array detector with simultaneous tungsten and deuterium light sources, controlled by Chemstation A.04 software (Hewlett-Packard, Les Ulis, France). Serum (200 μl) was deproteinated with ethanol, extracted with hexane, and evaporated under vacuum, and the extract was then redissolved in a methanol/ethanol/hexane mixture (88/10/2, v/v/v). Two internal standards were included before extraction. The extract was injected onto a reversed-phase Adsorbosphere HS C18 column (Alltech Associates, Inc., Templemars, France) of 250 mm × 4.6 mm (a 100-mm and a 150-mm column combined with a direct connect coupler) and pumped with two mobile phases using a step gradient. A sample was injected every 24 minutes onto the column. Three chromatograms were detected simultaneously (325 nm for retinol, 450 and 473 nm for carotenoids). Peaks were integrated automatically, but all chromatograms were controlled manually by the same technician. Peak areas were transformed into quantities expressed as mg/liter or μmol/liter by comparison with calibration curves using internal standard correction.

Seven carotenoids were measured: lutein, zeaxanthin, canthaxanthin, β-cryptoxanthin, lycopene, α-carotene, and β-carotene. Canthaxanthin concentrations were close to or below the limit of detection for most samples and were not used in the present report. All-*trans* isomers of β-carotene were identified separately from *cis* isomers. For lycopene, *cis* and *trans* isomers were summed up. In addition to carotenoids, total cholesterol, triglycerides, and phospholipids (expressed as mmol/liter) were measured using an enzymatic method with colorimetric detection on a Hitachi model 747 automated system (Hitachi Instruments, Inc., Les Ulis, France). The between-batch coefficients of variation ranged from 4.5 percent for β-cryptoxanthin to 6.8 percent for β-carotene, except for zeaxanthin that had a coefficient of 11.4 percent.

Statistical analyses

In statistical analyses, the paired *t* test was used to compare carotenoid means after $\log_e$ transformation to reduce departures from the normal distribution. Values were subsequently categorized into quartiles using the frequency distribution of the cases and controls combined. Odds ratios were computed relative to the highest quartile using conditional logistic regression (10). Adjustment for serum lipids was achieved by including cholesterol in all models. To compute linear trends, we performed analyses with exposure variables introduced as continuous. Likelihood ratio tests were used to assess the statistical significance of overall associations, linear trends, and deviations from linearity. Statistical significance was estimated using 95 percent confidence intervals. All *p* values were two sided. The effect of known potential confounders was examined in conditional logistic regression models. We considered age at menarche, age at first full-term pregnancy, parity, history of breast cancer in first-degree relatives, history of treatment for benign breast conditions, and the Quetelet index (body weight (kg)/height

$(m)^2$). Only covariates that altered the relevant regression coefficients by at least 15 percent were retained. Of the possible confounders, only age at first full-term pregnancy, family history, and previous treatment for benign breast conditions were kept in final models (in addition to serum cholesterol). The influence of menopausal status at blood sampling and of time delay between blood draw and cancer diagnosis was examined by introducing interaction terms into final models, but no appreciable effects were observed.

RESULTS

The nested case-control study included 270 cases and 270 controls (125 pre- and 145 postmenopausal in each group). Characteristics of study subjects are summarized in table 1. There were no appreciable differences between cases and controls in age at menarche, age at first full-term pregnancy, parity, age at menopause, and Quetelet index. A family history of breast cancer and a history of treatment for benign breast conditions were reported considerably more frequently by the cases than by the controls. The time delay between the dates of blood sample and diagnosis among the 270 cases ranged between 6 months and 11.2 years (mean, 4.0 (standard deviation, 2.4) years; median, 3.7 years).

Table 2 describes the arithmetic means of carotenoids and retinol. Compared with controls, breast cancer cases had lower mean serum concentrations of lutein (–11 percent), β-cryptoxanthin (–10 percent), α-carotene (–21 percent), β-carotene (–18 percent), and total carotenoids (–11 percent). The mean concentrations of the remaining analytes were not remarkably different between the two groups.

Table 3 shows the odds ratio for the association between breast cancer and serum levels of each antioxidant of interest. There was a progressive increase in the risk of breast cancer for decreasing serum concentrations of α-carotene and β-carotene. The odds ratios for the lowest versus the highest quartile were 1.99 (95 percent confidence interval (CI): 1.18, 3.34) and 2.21 (95 percent CI: 1.29, 3.79), respectively. There was an evident increase in risk for decreasing concentrations of lutein (low quartile odds ratio = 2.08; 95 percent CI: 1.11, 3.90) and β-cryptoxanthin (odds ratio = 1.68; 95 percent CI: 0.99, 2.86). The odds ratios for zeaxanthin and lycopene, and for retinol, albeit in the same direction, were unremarkable. The odds ratio for the lowest versus the highest quartile of total carotenoids was 2.31 (95 percent CI: 1.35, 3.96).

DISCUSSION

In this prospective cohort study, we observed an evident increase in the risk of breast cancer with decreasing serum concentrations of α-carotene, β-carotene, β-cryptoxanthin, and lutein. Subjects with the lowest concentration of these carotenoids at cohort baseline (between 6 months and 11 years before cancer diagnosis) experienced a 1.6- to 2.2-fold increase in the risk of breast cancer compared with women with the highest concentrations. Those with the lowest concentrations of total carotenoids had approximately a 2.3-fold increase in risk.

Only a few epidemiologic studies have investigated the association between breast cancer and blood levels of carotenoids (6). Three hospital-based case-control studies in the United States (11), Turkey (12), and India (13) reported protective associations of breast cancer with increasing blood concentrations of β-carotene. Three much larger similar studies, in Europe and in the United States, did not show differences in β-carotene measured in blood (14, 15) or in depot fat (16). In traditional case-control studies, however, blood concentrations of many metabolites could be affected by the presence of cancer or by changes in dietary habits induced by the disease (6). Case-control studies nested within prospective cohorts might be of preferable design

TABLE 1. Selected characteristics of study subjects at cohort baseline, New York University Women's Health Study, 1985–1994

	Age (years) at blood sampling (mean (SD*))	Age (years) at menarche (mean (SD))	Age (years) at first pregnancy (mean (SD))†	Nulliparous (%)	Age (years) at menopause (mean (SD))‡	Quetelet index (kg/m^2)	Family history of breast cancer (%)	History of benign breast disease (%)
Cases (n = 270)	52.0 (8.5)	12.4 (1.4)	26.3 (5.7)	34.4	49.1 (5.9)	25.4 (4.4)	26.3	28.2
Controls (n = 270)	51.9 (8.5)	12.6 (1.5)	25.6 (4.8)	29.3	48.8 (6.0)	25.3 (5.1)	21.9	18.2

* SD, standard deviation.
† Among parous subjects.
‡ Among postmenopausal subjects.

TABLE 2. Serum concentrations (μg/dl) of carotenoids and retinol at cohort baseline, New York University Women's Health Study, 1985–1994

	Lutein (mean (SD*))	Zeaxanthin (mean (SD))	β-Cryptoxanthin (mean (SD))	Lycopene (mean (SD))	α-Carotene (mean (SD))	β-Carotene (mean (SD))	Total carotenoids (mean (SD))	Retinol (mean (SD))
Cases (n = 270)	25.0 (13.5)	9.4 (9.4)	11.3 (7.1)	42.7 (20.7)	11.2 (8.2)	26.0 (21.4)	125.7 (47.3)	56.9 (13.5)
Controls (n = 270)	28.0 (16.2)	9.7 (9.7)	12.5 (8.2)	45.1 (22.3)	14.2 (12.4)	31.8 (25.2)	141.3 (58.1)	56.0 (13.8)

* SD, standard deviation.

TABLE 3. Odds ratios of breast cancer for quartiles of serum concentrations of carotenoids and retinol, New York University Women's Health Study, 1985–1994

	Lutein		Zeaxanthin		β-Cryptoxanthin		Lycopene		α-Carotene		β-Carotene		Total carotenoids		Retinol	
	OR*,†	95% CI*	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Quartile																
4 (high)	1.0		1.0		1.0		1.0		1.0		1.0		1.0		1.0	
3	1.43	0.85, 2.41	1.05	0.54, 2.04	1.08	0.66, 1.76	1.75	1.06, 2.88	1.28	0.76, 2.14	1.29	0.78, 2.14	1.92	1.14, 3.21	1.01	0.61, 1.68
2	1.22	0.76, 1.97	0.88	0.47, 1.66	1.39	0.86, 2.22	1.62	0.97, 2.70	1.41	0.86, 2.31	1.49	0.88, 2.50	1.57	0.96, 2.56	0.98	0.58, 1.64
1 (low)	2.08	1.11, 3.90	1.12	0.59, 2.13	1.68	0.99, 2.86	1.50	0.88, 2.54	1.99	1.18, 3.34	2.21	1.29, 3.79	2.31	1.35, 3.96	0.78	0.45, 1.35
p trend		0.01		0.54		0.05		0.15		0.0006		0.006		0.0008		0.50

* OR, odds ratio; CI, confidence interval.
† Odds ratios adjusted for age at first full-term pregnancy, family history of breast cancer, history of benign breast disease, and total cholesterol.

because blood samples are obtained in advance of clinical detection of cancer. To our knowledge, five such studies, in the United States and in Europe, investigated the association between blood carotenoids and breast cancer (17–21) with results that, overall, are not supportive of important associations. Four of these studies (17–20) included between 14 and 67 cases and, thus, might have been too small to be sufficiently informative. In addition, measurements were usually limited to total carotenoids (18), β-carotene (17, 19), or, at best, β-carotene and lycopene (20). Only the most recent published study (21) was of relatively adequate sample size (105 cases) and analyzed five different carotenoids. This study suggested that β-cryptoxanthin, lycopene, and lutein/zeaxanthin may protect against breast cancer.

It is uncertain whether blood levels of carotenoids are meaningful biologic markers of exposures to carotenoids. First, there is a legitimate concern about the possible degradation of carotenoids during storage (22–24). This might be an especially serious problem when samples are maintained at temperatures warmer than −70°C (25, 26) and/or are subjected to repeated defrosting and refreezing. In our study, cases and controls were tightly matched by duration of time in storage, and samples were kept at temperatures below −80°C and never thawed until the day of laboratory analyses. Second, one must assume that a relation exists between levels of individual carotenoids in circulation and their concentrations in breast tissue, but there are no observations supporting such an assumption. Third, blood concentrations at a single point in time are likely to reflect more recent intake rather than average intakes over many years or decades (27). As part of a preliminary phase of our study, we compared serum concentrations of the seven carotenoids of interest among cohort members who had donated blood samples on two different occasions, 3 years apart. Concentrations at a single point in time were extremely good estimators of average concentrations over a 3-year period, with (intraclass) correlations ranging between 0.63 and 0.85. These observations suggest that, in our study population, most people tended to maintain a relatively stable nutritional and supplemental intake of carotenoids for some years. Nevertheless, it would be unrealistic to conclude that blood concentrations would remain as stable during the many years that precede the occurrence and clinical detection of breast cancer.

As in all observational studies, the association between blood carotenoids and breast cancer may be the result of confounding. Factors that have been reported to be associated with blood carotenoids include current tobacco smoking, intake of alcohol, exposure to ultraviolet light, and the use of oral contraceptives (24, 28–32). It is unlikely, however, that tobacco smoking (33) and oral contraceptives (34) play an important etiologic role in breast cancer, whereas substantial evidence supports an association with alcohol (2, 35). Unfortunately, we did not have adequate lifetime information on oral contraceptives, tobacco, and alcohol use in our cohort, so that an effect of confounding could not be excluded. It should be noted that carotenoids in the diet might be related to the consumption of other nutrients, such as phytoestrogens, vitamins, and minerals, that may be asso-

ciated with a protective effect on breast cancer and are by and large contained in the same foods. Thus, one cannot exclude that the reported associations are simply the result of the intake of vegetables and fruits, rather than the expression of a protective effect of the intake of specific carotenoids.

Our findings of a protective association between breast cancer and α-carotene, β-carotene, β-cryptoxanthin, and lutein are only partially consistent with a protective effect for β-cryptoxanthin, but not for α- and β-carotene, reported recently by Dorgan et al. (21), the only other sufficiently large study published to date. These authors also reported a protective effect for lycopene and for lutein/zeaxanthin, which we did not observe in our data. Our study and the one by Dorgan et al. (21) are the only ones that have included carotenoids other than β-carotene. The emerging results suggest that a number of carotenoids, in addition to β-carotene, may play a preventive role in breast cancer. Alternatively, our results seem to add substance to the theory that the abundant consumption of vegetables and fruit is protective against breast cancer in women.

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