Fruits, Vegetables, and Micronutrients in Relation to Breast Cancer Modified by Menopause and Hormone Receptor Status

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Abstract

Whether fruit, vegetable, and antioxidant micronutrient consumption is associated with a reduction in breast cancer incidence remains unresolved. To address this issue, we analyzed data from a large population-based case-control study, with consideration given to whether the associations varied with menopausal status or with clinical characteristics of the cases' disease. Study participants completed a modified Block food frequency questionnaire, which included assessment of the frequency and portion sizes of 13 fruits and fruit juices and 16 vegetables and the use of multiple and single vitamin supplements. Statistical analyses were done on 1,463 cases and 1,500 controls. Among postmenopausal women, reduced odds ratios [OR; 95% confidence intervals (95% CI)] were noted for the highest fifth, as compared with the lowest fifth, of intake of any vegetables [0.63 (0.46–0.86); *P* for trend < 0.01] and leafy vegetables [0.66 (0.50–0.86); *P* for trend = 0.03] after controlling for age and energy intake. Adjusted ORs (95% CIs) were also decreased for postmenopausal breast cancer in relation to high intake of carotenoids, α-carotene, β-carotene, lutein, and particularly lycopene [0.66 (0.48–0.90); *P* for trend = 0.03]. Inverse associations for fruits and vegetables were stronger for postmenopausal women with estrogen receptor (ER)+ tumors (OR, 0.65; 95% CI, 0.51–0.82) than ER− tumors (OR, 0.92; 95% CI, 0.64–1.32), but results were less consistent for micronutrients. No similarly reduced associations were observed among premenopausal women. ORs did not appreciably differ by *in situ* or invasive breast cancer or by whether cases had begun chemotherapy. Our results support an inverse association for fruit and vegetable intake among postmenopausal but not premenopausal breast cancer, which may be more pronounced among women with ER+ tumors. (Cancer Epidemiol Biomarkers Prev 2004;13(9):1485–94)

Introduction

Whether the consumption of fruits, vegetables, and antioxidant micronutrients is associated with a reduction in the incidence of breast cancer remains unresolved, with even the recent reviews or meta-analyses reaching conflicting conclusions (1-6). Several issues have been put forth as possibly contributing to the variability in results across studies, including whether the subjects were drawn from case-control or cohort studies (7); whether portion size (8), subgroups of fruits and vegetables (9), or vitamin supplementation (9) was considered in ascertaining dietary intake; whether subjects were categorized by menopausal status (1-6); whether the clinical characteristics of the cases' disease were considered, such as stage of disease and hormone receptor status (10); or whether chemotherapy had been initiated by the time of the subject's interview (11).

The analysis reported here, which uses data from a large population-based case-control study, was undertaken to address some of these issues. A food frequency questionnaire (FFQ) was used to assess diet, which inquired about portion size, as well as frequency of fruit and vegetable intake, and fruit juice consumption, along with vitamin supplement use. The large sample size permitted consideration of whether the association between intake of fruits, vegetables, and antioxidants varied with menopausal status, or with clinical characteristics of the cases' disease, including a joint measure of estrogen receptor (ER) and progesterone receptor (PR) status.

Methods

The Long Island Breast Cancer Study Project (LIBCSP) was a population-based case-control study that was conducted among adult female residents of Nassau and Suffolk counties, Long Island, NY (12). Institutional review board approval was obtained from all partici pating institutions, and signed informed consent was

Received 10/30/03; revised 3/22/04; accepted 4/16/04.

Grant support: National Cancer Institute and National Institutes of Environmental Health and Sciences grants UO1CA/ES66572, UO1CA66572, CA55283, and P30ES10126 and Department of Defense grants BC021366 and BC990191.

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obtained for all respondents. In brief, English-speaking cases were newly diagnosed with a first primary *in situ* or invasive breast cancer between August 1, 1996 and July 31, 1997. Study personnel identified cases through rapid case ascertainment via active contact with local pathology departments. The case's physician was contacted to verify details of her diagnosis and permission to contact her. Of the 2,030 eligible cases, physician's consent was obtained for 1,837 (90.5%). Physician refusal was commonly due to illness of the patient.

English-speaking controls, who were residents of the same two counties as the cases but did not have a history of breast cancer, were randomly selected by random digit dialing for women ages <65 years at reference and by Health Care Finance Administration rosters for women ages ≥65 years. Controls were frequency matched to the expected distribution of cases by 5-year age group.

In-home interviews, conducted by trained interviewers, were completed by 1,508 (82.1%) of eligible case women (235 with *in situ* breast cancer and 1,273 with invasive breast cancer) and 1,556 (62.7%) of eligible controls. The interview covered such topics as reproductive history, physical activity, height, weight, alcohol use, active and passive cigarette smoke exposure, and exogenous hormone use. The medical records of case women were abstracted for ER and PR status. Among women in the dietary analysis, ER/PR data were available for 965 (66.0%) women. As reported previously (12), odds ratios (OR) for breast cancer were elevated in relation to late age at first birth, lower parity, little to no breast-feeding, and family history of breast cancer.

A total of 98.2% (n = 1,481) of cases and 97.6% (n = 1,518) control participants completed a scannable self-administered FFQ. Completion of the FFQ took an average of 36 minutes, which did not vary by age of the participants (12). The modified Block FFQ (13) included questions regarding usual frequency and portion size of ~100 foods and beverages over the 12 months prior to interview. Intake of 13 fruits and fruit juices and 16 vegetables (excluding French fries) and use of multiple vitamins (i.e., one-a-day, therapeutic, and Stresstab type) and single supplements of vitamin A, β-carotene, vitamin E, calcium or Dolomite, vitamin C, and iron were also included in the FFQ. For food items and supplements, eight options of frequency ranged from <1 per month to 2+ per day. For food items, responses for portion sizes were relative to a stated average serving size with options of less, average, or more.

The frequency and portion sizes data were translated to daily intakes of nutrients from both dietary and supplement sources using the National Cancer Institute's DietSys version 3. Frequency and portion size of each food item were converted to a common denominator of 0.5 cup servings per week. Individual fruits and vegetables (see Appendix 1) were categorized into nine food groups: any fruits, fruit juices, and vegetables; any fruits and fruit juices; any fruits; fruit juices; citrus fruits; any vegetables; leafy vegetables; yellow vegetables; and cruciferous vegetables. These food groups were chosen based on the current breast cancer literature (13, 14). We also examined the dietary plus supplemental sources of β-carotene, vitamin C, and vitamin E and the dietary sources of α-carotene, β-carotene, cryptoxanthin, lutein plus zeaxanthin (further referred as lutein), lycopene, vitamin C, and vitamin E. For the main effect of fruits and vegetables, the cumulative frequencies of each food group and antioxidant nutrients were categorized based on quintiles of the control distribution (both premenopausal and postmenopausal women). The quintiles for premenopausal controls were slightly lower than for postmenopausal controls (data not shown); however, the ORs for the menopause-specific cut points (data not shown) did not differ from the ORs using cut points for all control women. Therefore, the latter cut points were used to facilitate comparison between premenopausal and postmenopausal women. To gain precision in stratified analyses, we created dichotomous variables by collapsing the lowest three fifths and the highest two fifths based on the similarity of the ORs in each of those groups.

To facilitate the results of our comparison with other studies, participants with daily energy intakes above or below 3 SDs of the log-transformed mean (16 cases and 22 controls) were dropped from the analyses (1). The case-control distribution of established risk factors for breast cancer among 1,463 cases and 1,500 controls included in these diet analyses (data not shown) were not substantially different from those identified among all LIBCSP participants (12).

Unconditional logistic regression (15) was used to estimate ORs and 95% confidence intervals (95% CI) for the association of selected fruit and vegetable groups and the antioxidants and breast cancer incidence. Age at reference date (defined as date of diagnosis for cases and date of identification for controls) and dietary energy intake (continuous) were included in all models. Factors considered as potential effect modifiers included physical activity from menarche to reference date, body mass index [weight (kg) / height $(m)^2$] at reference, passive/active cigarette smoking, lifetime alcohol intake, family history of cancer, and linoleic acid. Factors considered as potential confounders included menopausal status, active and passive smoking, lifetime alcohol intake, menopausal status, physical activity from menarche to reference date, family history of cancer, season of interview, education, use of oral contraceptives, use of hormone replacement therapy, lactation, race, age at first birth, parity, body mass index at reference, body mass index at age 20 years, and single and multiple vitamin supplements.

Effect modification on the multiplicative scale was evaluated using the log likelihood ratio test to compare logistic models with and without the interaction term. The interaction term for menopausal status [which was defined based on information provided by the subject on her date of last menstrual period, prior surgical information on hysterectomy or oophorectomies, cigarette smoking status, and use of hormone replacement (12)] was statistically significant (P < 0.05). Confounding was evaluated separately for premenopausal and postmenopausal women, starting with a full model and using backward elimination. None of the potential confounders altered the estimates of either the combined fruit and vegetable intake or the micronutrient intake by >10% among premenopausal or postmenopausal women (data not shown). To test for potential confounding among the exposures of interest, final models were further adjusted for intake of fruits, vegetables, and antioxidants and use

Table 1. Age- and energy-adjusted ORs and 95% CIs stratified by menopausal status for the association between fruit and vegetable intake in relation to breast cancer incidence, LIBCSP, 1996–1997

	Premenopausal			Postmenopausal		
	Cases (*n*)	Controls (*n*)	OR (95% CI)	Cases (*n*)	Controls (*n*)	OR (95% CI)
Any fruits, fruit juices, and vegetables*						
0–18	111	135	1.00	195	169	1.00
19–26	94	113	1.02 (0.70–1.49)	211	173	1.06 (0.79–1.41)
27–34	100	92	1.35 (0.91–1.99)	198	179	0.93 (0.69–1.25)
35–46	78	80	1.23 (0.80–1.88)	195	229	0.71 (0.53–0.96)
47+	74	67	1.36 (0.86–2.15)	178	203	0.72 (0.53–0.99)
P for trend†			0.33			<0.01
Any fruits and fruit juices*						
0–7	142	143	1.00	177	170	1.00
8–12	88	107	0.79 (0.55–1.15)	219	190	1.09 (0.82–1.45)
13–17	96	103	0.92 (0.63–1.34)	194	187	0.96 (0.72–1.29)
18–24	75	73	1.01 (0.67–1.53)	197	184	1.00 (0.74–1.34)
25+	56	61	0.85 (0.54–1.35)	190	221	0.79 (0.59–1.07)
P for trend†			0.64			0.10
Citrus fruits*						
0–1	138	136	1.00	199	193	1.00
2–4	103	130	0.77 (0.54–1.09)	205	204	0.98 (0.74–1.29)
5–7	82	84	0.95 (0.64–1.40)	204	184	1.06 (0.79–1.40)
8–11	89	78	1.07 (0.72–1.59)	204	204	0.94 (0.71–1.25)
12+	45	59	0.73 (0.45–1.17)	155	160	0.93 (0.68–1.26)
P for trend†			0.87			0.80
Any vegetables*						
0–9	108	127	1.00	244	196	1.00
10–13	92	98	1.11 (0.75–1.65)	189	164	0.92 (0.69–1.21)
14–17	72	95	0.93 (0.62–1.41)	174	175	0.78 (0.59–1.05)
18–24	96	85	1.32 (0.87–1.98)	206	216	0.76 (0.57–1.10)
25+	88	76	1.41 (0.92–2.17)	151	188	0.63 (0.46–0.86)
P for trend†			0.09			<0.01
Leafy vegetables*						
0–2	161	169	1.00	345	298	1.00
3–4	73	100	0.79 (0.54–1.14)	212	184	1.00 (0.77–1.28)
5–6	71	81	0.91 (0.62–1.34)	144	140	0.92 (0.69–1.21)
7–8	62	53	1.26 (0.82–1.95)	120	129	0.81 (0.60–1.09)
9+	89	78	1.16 (0.79–1.70)	140	187	0.66 (0.50–0.86)
P for trend†			0.58			0.03
Yellow vegetables*						
0–5	143	150	1.00	268	246	1.00
6–7	53	85	0.64 (0.42–0.97)	162	113	1.32 (0.97–1.78)
8–11	99	103	0.98 (0.68–1.43)	228	196	1.08 (0.83–1.40)
12–16	88	76	1.20 (0.81–1.79)	151	201	0.68 (0.52–0.91)
17+	74	73	1.06 (0.70–1.62)	168	197	0.78 (0.58–1.03)
P for trend†			0.28			<0.01
Cruciferous vegetables*						
0–1	128	154	1.00	305	291	1.00
2	115	121	1.18 (0.83–1.68)	233	196	1.15 (0.90–1.48)
3	48	53	1.06 (0.66–1.67)	109	107	0.99 (0.72–1.36)
4–5	63	81	0.93 (0.61–1.40)	167	162	0.99 (0.75–1.30)
6+	102	72	1.76 (1.18–2.61)	147	181	0.80 (0.60–1.05)
P for trend†			0.03			0.12

*In 0.5 cup servings per week.
†*P* for trend for the continuous variable.

of antioxidant vitamin supplements; estimates from these models were not substantially different from excluding these covariates (data not shown). Tests of trend were conducted using the continuous values for nutrients and food groups. To test for potential heterogeneity by ER/PR status and stage of disease (invasive versus *in situ* cancer), stratified analyses were done for premenopausal and postmenopausal women. To assess for potential recall bias among case women who have already begun chemotherapy, as described by Potischman et al. (11), cases were stratified by whether chemotherapy had been initiated by the time of interview and by the interval of time from diagnosis to interview (above and below the mean of 96 days for cases).

Results

As shown in Table 1, among postmenopausal women, breast cancer risk was decreased in relation to intake of fruits, fruit juices, and vegetables. For example, the ORs (95% CIs) were reduced by more than a third among postmenopausal women in the highest fifth of intake of any vegetables [0.63 (0.48-0.86)] or leafy vegetables [0.66 (0.50–0.85)] compared with women in the lowest fifth. Weaker inverse associations were found for yellow vegetables, cruciferous vegetables, and any intake of fruits and fruit juices. Citrus fruits were not related to breast cancer among premenopausal or postmenopausal women. In contrast, among premenopausal women,

Table 2. Age- and energy-adjusted ORs and 95% CIs stratified by menopausal status for the association between dietary and supplemental sources of micronutrients in relation to breast cancer incidence, LIBCSP, 1996–1997

	Premenopausal			Postmenopausal		
	Cases (n)	Controls (n)	OR (95% CI)	Cases (n)	Controls (n)	OR (95% CI)
Dietary α-carotene, μg/d						
0–79.9	102	115	1.00	210	173	1.00
79.9–159.2	84	86	1.10 (0.73–1.65)	194	198	0.80 (0.60–1.06)
159.2–266.7	99	98	1.09 (0.73–1.63)	206	189	0.89 (0.67–1.19)
266.6–413.4	91	106	0.91 (0.61–1.36)	181	185	0.81 (0.60–1.09)
413.4+	81	82	1.09 (0.71–1.67)	186	208	0.73 (0.54–0.99)
P for trend*			0.56			0.06
Dietary β-carotene, μg/d						
0–1,198.7	111	105	1.00	219	182	1.00
1,198.7–1,821.5	88	119	0.69 (0.47–1.02)	191	164	0.96 (0.72–1.28)
1,821.5–2,647.2	90	99	0.82 (0.55–1.23)	194	194	0.85 (0.63–1.13)
2,647.4–3,964.4	96	91	0.94 (0.62–1.43)	178	196	0.74 (0.55–1.00)
3,964.4+	72	73	0.93 (0.59–1.47)	195	217	0.74 (0.55–1.01)
P for trend*			0.97			0.24
Dietary cryptoxanthin, μg/d						
0–31.0	100	105	1.00	189	184	1.00
31.0–57.5	105	108	1.03 (0.70–1.53)	167	175	0.94 (0.70–1.27)
57.5–90.6	83	92	0.97 (0.64–1.46)	220	196	1.10 (0.83–1.46)
90.6–134.8	93	90	1.04 (0.69–1.59)	195	197	0.97 (0.72–1.30)
134.8+	76	92	0.86 (0.55–1.33)	206	201	1.00 (0.74–1.35)
P for trend*			0.97			0.24
Dietary lutein, μg/d						
0–750.7	90	101	1.00	245	187	1.00
750.8–1,301.1	101	102	1.11 (0.74–1.66)	205	191	0.84 (0.63–1.11)
1,301.1–1,937.3	91	99	1.01 (0.67–1.51)	167	183	0.71 (0.53–0.95)
1,937.3–3,107.3	80	87	1.02 (0.66–1.56)	177	201	0.69 (0.52–0.92)
3,107.3+	95	98	1.08 (0.71–1.64)	183	191	0.77 (0.58–1.04)
P for trend*			0.91			0.20
Dietary lycopene, μg/d						
0–601.8	54	68	1.00	258	224	1.00
601.9–1,121.9	78	92	1.15 (0.72–1.85)	212	200	0.92 (0.71–1.20)
1,121.9–1,602.7	104	123	1.18 (0.74–1.87)	177	165	0.96 (0.72–1.28)
1,602.7–2,483.9	109	95	1.66 (1.02–2.68)	200	188	0.95 (0.72–1.26)
2,483.9+	112	109	1.53 (0.92–2.52)	130	176	0.66 (0.48–0.90)
P for trend*			0.42			0.03
Dietary vitamin C, mg/d						
0–65.6	103	109	1.00	202	177	1.00
65.6–95.7	102	103	1.04 (0.70–1.54)	174	189	0.82 (0.61–1.11)
95.7–131.0	78	90	0.91 (0.59–1.39)	240	189	1.12 (0.84–1.50)
131.1–179.2	91	87	1.05 (0.68–1.62)	182	208	0.77 (0.57–1.05)
179.3+	83	98	0.87 (0.56–1.36)	179	190	0.83 (0.60–1.14)
P for trend*			0.76			0.13
Dietary vitamin E, a-te/d						
0–4.6	75	87	1.00	239	203	1.00
4.6–6.1	86	99	1.03 (0.66–1.60)	195	188	0.87 (0.65–1.17)
6.1–7.8	105	93	1.41 (0.88–2.27)	199	194	0.87 (0.64–1.20)
7.9–10.7	93	98	1.27 (0.75–2.14)	161	189	0.73 (0.52–1.03)
10.7+	98	110	1.23 (0.70–2.17)	183	179	0.87 (0.59–1.27)
P for trend*			0.70			0.44

*P for trend for continuous variable.

Table 3. Age- and energy-adjusted ORs and 95% CIs stratified by ER/PR status for the association between fruit and vegetable intake in relation to breast cancer incidence among postmenopausal women, LIBCSP, 1996–1997

	Controls (n = 953)	ER+PR+		ER+PR−		ER−PR+		ER−PR−	
		Cases (n = 378)	OR (95% CI)	Cases (n = 117)	OR (95% CI)	Cases (n = 29)	OR (95% CI)	Cases (n = 128)	OR (95% CI)
Any fruits, fruit juices, and vegetables*									
0–34	521	244	1.00	72	1.00	15	1.00	77	1.00
35+	432	134	0.64 (0.49–0.83)	45	0.68 (0.45–1.04)	14	0.99 (0.46–2.14)	51	0.91 (0.61–1.53)
P for trend†			0.02		0.11		0.32		0.63
Any fruits and fruit juices*									
0–17	547	236	1.00	66	1.00	15	1.00	87	1.00
18+	405	142	0.79 (0.61–1.02)	51	0.96 (0.64–1.45)	14	1.16 (0.54–2.51)	50	0.96 (0.65–1.43)
P for trend†			0.09		0.47		0.24		0.85
Citrus fruits*									
0–7	581	233	1.00	70	1.00	18	1.00	86	1.00
8+	364	142	0.94 (0.73–1.22)	45	0.97 (0.64–1.45)	10	0.89 (0.40–1.97)	41	0.80 (0.53–1.20)
P for trend†			0.66		0.47		0.53		0.27
Any vegetables*									
0–17	535	237	1.00	77	1.00	16	1.00	81	1.00
18+	404	136	0.79 (0.61–1.03)	38	0.65 (0.42–1.01)	13	0.91 (0.42–1.99)	46	0.86 (0.57–1.29)
P for trend†			<0.01		0.06		0.68		0.46
Leafy vegetables*									
0–6	622	274	1.00	88	1.00	20	1.00	97	1.00
7+	316	97	0.70 (0.53–0.92)	26	0.57 (0.36–0.91)	9	0.83 (0.37–1.85)	30	0.65 (0.42–1.00)
P for trend†			0.03		0.06		0.31		0.05
Yellow vegetables*									
0–11	555	258	1.00	79	1.00	17	1.00	82	1.00
12+	398	120	0.64 (0.49–0.84)	38	0.64 (0.42–0.98)	12	0.87 (0.40–1.88)	46	0.87 (0.58–1.30)
P for trend†			0.02		0.06		0.61		0.50
Cruciferous vegetables*									
0–3	594	249	1.00	78	1.00	19	1.00	78	1.00
4+	343	122	0.87 (0.67–1.13)	37	0.85 (0.56–1.30)	10	0.85 (0.38–1.86)	48	1.17 (0.79–1.73)
P for trend†			0.21		0.27		0.60		0.44

*In 0.5 cup servings per week.
†P for trend for continuous variable.

there was a suggestion of increase found for intake of any fruits, juices, and vegetables and any vegetables that seemed driven by the association with cruciferous vegetables (highest fifth of intake: OR, 1.76; 95% CI, 1.18–2.61). Consumption of any fruits and fruit juices, citrus fruits, any vegetables, leafy vegetables, and yellow vegetables was not consistently associated with premenopausal breast cancer.

There was no clear or consistent association between antioxidant nutrients and premenopausal breast cancer, as shown in Table 2. Among postmenopausal women, however, there was a consistent inverse association for most carotenoids (α-carotene, β-carotene, and lutein), particularly for lycopene (highest fifth of intake: OR, 0.66; 95% CI, 0.48–0.90; P for trend = 0.03). Postmenopausal women consuming ≥413.4 μg/d of dietary α-carotene had a 27% reduction in breast cancer incidence (OR, 0.73; 95% CI, 0.54–0.99) compared with women in the lowest fifth. Results for dietary intakes of vitamin C and vitamin E did not show a consistent inverse pattern.

Most cases, 89.2% of postmenopausal women and 82.0% of premenopausal women, were interviewed prior to the initiation of chemotherapy. The menopause-specific ORs for breast cancer in relation to fruit and vegetable intake did not vary substantially when cases were stratified by chemotherapy status (data not shown) or when cases were stratified by the timing of the interview relative to diagnosis (data not shown) compared with all controls.

When breast cancer cases were stratified by whether they were diagnosed with *in situ* or invasive disease, ORs in relation to the intake of fruits, vegetables, and micronutrients did not differ substantially from those for all women combined (data not shown). Among case women with information on diet and hormone receptor status, 562 (59.2%) were diagnosed with an ER+PR+ tumor, 136 (14.3%) had an ER+PR− tumor, 49 (5.2%) had an ER−PR+ tumor, and 220 (23.2%) had an ER−PR− tumor. The OR estimates among premenopausal women are not shown because they are based on limited numbers and did not allow for meaningful interpretation. Among postmenopausal women, the inverse associations between intake of fruits and vegetables were more pronounced among women with either ER+PR+ or ER+PR− tumors (Table 3), although this pattern was not evident for micronutrient intake (Table 4). For example, high intake of fruits and vegetables was associated with a 36% decrease in the OR (95% CI) among women with ER+PR+ tumors [0.64 (0.48-0.83)], whereas no relationship was found for women with ER−PR− tumors.

Further, our results based on categorizing the case women based only on the ER status of their tumor (regardless of PR status) illustrate that the reduced risk is more pronounced among women with ER+ tumors. For example, the OR (95% CI) for intake of any fruits, fruit juices, and vegetables among women with ER+ tumors was 0.65 (0.51–0.82); among women with ER− tumors, the corresponding OR (95% CI) was 0.92 (0.64–1.32; ratio of the OR, 0.71; 95% CI, 0.48–1.05).

Fifty percent of controls used an antioxidant vitamin supplement in the previous 10 to 15 years. Estimates for combined supplemental and dietary sources of β-carotene, vitamin C, and vitamin E (data not shown) did not appreciably differ from those results presented in Table 2, which were based on dietary intake of these nutrients only. Looking at supplements alone, use of any antioxidant vitamin supplement was not associated with the incidence of breast cancer (OR, 0.93; 95% CI, 0.78–1.11) among all women; however, there was a suggestion of a decrease in breast cancer among multiple vitamin supplement users (OR, 0.80; 95% CI, 0.62–1.04) compared with nonusers. Multiple vitamin supplements, particularly one-a-day–type supplements, were the most common supplement among users. Results stratified by menopausal status were similar. Neither single antioxidant supplements, carotenoids, vitamin C, and vitamin E nor use of multiple and single supplements were associated with breast cancer (data not shown).

Discussion

Inverse associations for intake of fruits and vegetables and consumption of antioxidant nutrients were found among postmenopausal but not premenopausal women. Among postmenopausal women, the inverse relationship was particularly strong for high consumers of all vegetables and leafy vegetables, α-carotene, β-carotene,

Table 4. Age- and energy-adjusted ORs and 95% CIs stratified by ER/PR status for the association between dietary and supplemental sources of micronutrients in relation to breast cancer incidence among postmenopausal women, LIBCSP, 1996–1997

	Controls (n = 953)	ER+PR+		ER+PR−		ER−PR+		ER−PR−	
		Cases (n = 378)	OR (95% CI)	Cases (n = 117)	OR (95% CI)	Cases (n = 29)	OR (95% CI)	Cases (n = 128)	OR (95% CI)
Dietary α-carotene, μg/d									
0–267.7	560	236	1.00	65	1.00	19	1.00	83	1.00
267.8+	393	142	0.89 (0.69–1.15)	52	1.16 (0.77–1.75)	10	0.63 (0.28–1.41)	45	0.86 (0.57–1.30)
P for trend*			0.23		0.42		0.46		0.18
Dietary β-carotene, μg/d									
0–2,647.4	540	240	1.00	75	1.00	13	1.00	87	1.00
2,647.5+	413	138	0.76 (0.58–0.99)	42	0.71 (0.46–1.09)	16	1.50 (0.68–3.33)	41	0.67 (0.44–1.02)
P for trend*			0.02		0.14		0.17		0.01
Dietary cryptoxanthin, μg/d									
0–90.7	555	223	1.00	68	1.00	15	1.00	79	1.00
90.8+	398	155	0.96 (0.74–1.23)	49	0.94 (0.62–1.42)	14	1.19 (0.55–2.57)	49	0.94 (0.64–1.42)
P for trend*			0.52		0.78		0.46		0.40
Dietary lutein, μg/d									
0–1,937.3	561	248	1.00	68	1.00	15	1.00	86	1.00
1,937.3+	392	130	0.80 (0.62–1.03)	49	1.13 (0.75–1.71)	14	1.19 (0.55–2.56)	42	0.78 (0.52–1.17)
P for trend*			0.05		0.56		0.46		0.10
Dietary lycopene, μg/d									
0–1,602.7	589	254	1.00	81	1.00	18	1.00	93	1.00
1,602.8+	394	124	0.84 (0.64–1.10)	36	0.74 (0.48–1.14)	11	0.77 (0.34–1.74)	35	0.68 (0.45–1.05)
P for trend*			0.12		0.20		0.83		0.03
Dietary vitamin C, mg/d									
0–131.0	555	245	1.00	72	1.00	18	1.00	81	1.00
131.1+	398	133	0.75 (0.58–0.98)	45	0.84 (0.55–1.29)	11	0.73 (0.33–1.63)	47	0.93 (0.61–1.41)
P for trend*			0.02		0.47		0.72		0.27
Dietary vitamin E, a-te/d									
0–7.8	585	245	1.00	70	1.00	18	1.00	91	1.00
7.9+	368	133	0.96 (0.72–1.30)	47	1.23 (0.77–1.97)	11	0.62 (0.24–1.55)	37	0.75 (0.47–1.20)
P for trend*			0.43		0.44		0.84		0.04

*P for trend for continuous variable.

lutein, and lycopene. In addition, postmenopausal women consuming the highest levels of lycopene had 34% lower incidence of breast cancer than women with the lowest intakes.

To the best of our knowledge, this is the first study to examine the influence of diet on breast cancer stratified by a joint measure of ER/PR status. The overall inverse association of fruit and vegetable intake was found among postmenopausal women, which was more pronounced among women with either ER+PR+ or ER+PR− tumors.

Potter et al. (10) has hypothesized that ER/PR status describes subtypes of breast cancer that may have different etiologies. Further, in a comparison of Danish women to Japanese women, Yasui and Potter (16) found that among Danish women the incidence of breast cancer for women with ER+PR+ tumor continues to increase with age after menopause, whereas the incidence for women with other tumor subtypes declines after menopause; in contrast, the incidence of breast cancer among Japanese women declines after menopause regardless of their ER/PR status. Several studies (17-24) have examined intake of fruit, vegetable, and micronutrients, stratified by ER status with inconsistent results. However, most studies were based on small numbers of subjects, which would limit their ability to detect a subtle difference between breast tumor subtypes, and none examined whether there was variation with ER and PR status concurrently. In our study, the inverse associations for fruits, vegetables, and micronutrients were stronger for those with ER+PR+ and ER+PR− tumors than they were for the other tumor types. Although our study is based on a large number of cases, the cell sizes for ER+PR− and ER−PR+ cases were still small.

Vitamin C, vitamin E, and the carotenoids, α-carotene, β-carotene, cryptoxanthin, lutein, and lycopene, act as antioxidants, which protect DNA from oxidative damage (25). In addition, each has other chemopreventive actions (25). α-Carotene, β-carotene, and cryptoxanthin, found in orange vegetables and fruits, may inhibit cell proliferation and inhibit cellular growth via conversion into retinols in the intestine (26, 27). A pooled analysis of 12 early case-control studies found that women in the highest fifth of β-carotene consumption had a 19% reduction in breast cancer incidence among postmenopausal women (1). A meta-analysis of literature from 1982 to 1997 found a similar result (5). However, only one of four recent cohort studies (28) found an inverse relationship for dietary β-carotene and only among premenopausal women (29). We found inverse associations for both β-carotene and α-carotene among postmenopausal women only.

Other carotenoids have been understudied in epidemiologic research of breast cancer, which may be due to difficulties associated with obtaining a comprehensive assessment with a FFQ. A large case-cohort study of dietary lycopene consumption (30) did not find an association with breast cancer even after stratification by menopausal status. Similarly, a review of observation studies of tomatoes and tomato-based products (31) did not support an association with breast cancer, although not all studies stratified by menopausal status. However, some but not all studies of high serum concentrations of lycopene have found inverse associations (32), although levels of lycopene in breast tissue were found in one study to be poorly correlated with dietary intake (31). Both α-carotene and lutein have been inversely associated with breast cancer in serum studies (32) and among premenopausal women in one prospective study of dietary intake (29).

In addition to its antioxidant potential, vitamin C is also critical for immune function (25). A meta-analysis of case-control studies found a 37% reduction in breast cancer risk among postmenopausal women in the highest fifth of consumption of vitamin C; the OR for premenopausal women was closer to the null (0.84, P = 0.26). A review (3) of five prospective studies found no association with breast cancer among both premenopausal and postmenopausal women. We found an inverse association, albeit not statistically significant, for the highest fifth of vitamin C intake, which was unchanged after the addition of supplemental sources of vitamin C.

In animal models, vitamin E has been found to inhibit tumors and reduce cell proliferation (25); however, available epidemiologic data do not support this effect (3). Our results also support a null association between dietary and supplemental sources of vitamin E and breast cancer.

We observed an inverse association for vegetables but not for fruits among postmenopausal but not premenopausal women. Other studies, but not all, have found results consistent with ours (1-6). A combined analysis of Italian breast cancer studies (4) and a meta-analysis (5) of 23 studies concluded that vegetable intake reduced breast cancer by 20% to 25%, although there was no association with fruit intake. However, in a pooled analysis of seven North American and European cohorts, neither vegetable nor fruit consumption was found to be associated with the risk of breast cancer regardless of menopausal status [for 100 g/d intake increments: relative risk (RR), 1.00; 95% CI, 0.97–1.02 and RR, 0.99; 95% CI, 0.89–1.00, respectively (7)].

On further analysis of individual vegetables, Smith-Warner et al. (7) found that broccoli and Brussels sprout consumption was associated with a 14% reduction in risk (for 0.5 cup increments: RR, 0.86; 95% CI, 0.72–1.02 and RR, 0.67; 95% CI, 0.35–1.27, respectively), which is consistent with our result for cruciferous vegetables among postmenopausal women. In addition, Smith-Warner et al. (7) found that increasing consumption of spinach (0.5 cup cooked increments: RR, 0.61; 95% CI, 0.33–1.15) was associated with decreasing risk of breast cancer. We found a 34% decrease in the OR with intake of nine or more 0.5 cup servings per week among postmenopausal women.

Each fruit or vegetable contains its own combination of numerous plausible nutritive and nonnutritive compounds that exert chemopreventive effects through stimulation of cell differentiation, cessation of cell division, antioxidant potential, antiestrogenic effects, induction of metabolic detoxification, and enhancement of immune function (25). The high correlation between antioxidant nutrients and their food sources makes it difficult to decipher whether antioxidants and/or other compounds are the preventive agent in fruits and vegetables. In our study, the inverse association between

Appendix 1: Composition of food groups

Fruits	Fruit juices	Any fruits and fruit juices	Citrus fruits	Any vegetables	Any fruits and vegetables, including juices and excluding french fries	Leafy vegetables	Yellow vegetables (and Fruits)	Cruciferous vegetables
Apples, applesauce, pears	Orange juice or grapefruit juice	All items in fruit and fruit juices	Oranges	String beans, green beans	All items in fruits and fruit juices and vegetables	Spinach (raw)	Peaches, apricots, nectarines (fresh in season)	Coleslaw, cabbage, sauerkraut
Bananas	Fruit drinks with added vitamin C, such as Hi-C		Grapefruit	Green peas		Spinach (cooked)	Peaches, apricots (canned, frozen, or dried)	Broccoli
Peaches, apricots, nectarines (in season)	Tomatoes, tomato juice, V8 juice*		Orange juice or grapefruit juice	Corn, including corn on the cob		Mustard greens, turnip greens, collards, kale	Cantaloupe (in season)	Cauliflower or Brussels sprouts
Peaches, apricots (canned, frozen, or dried)				Winter squash, baked squash		Green salad	Watermelon (in season)	Mustard greens, turnip greens, collards, kale
Cantaloupe (in season)				Broccoli			Winter squash, baked squash	
Watermelon (in season)				Cauliflower or Brussels sprouts			Broccoli	
Strawberries (in season)				Spinach (raw)			Spinach (raw)	
Cherries (in season)				Spinach (cooked)			Spinach (cooked)	
Oranges				Mustard greens, turnip greens, collards, kale			Mustard greens, turnip greens, collards, kale	
Grapefruit				Cole slaw, cabbage, sauerkraut			Carrots, or mixed vegetables containing carrots	
Tomatoes, tomato juice, V8 juice*				Carrots, or mixed vegetables containing carrots			Sweet potatoes, yams	
				Red or green peppers			Tomatoes, tomato juice, V8 juice	
				Green salad			Red or green peppers	
				Alfalfa sprouts				
				Sweet potatoes, yams				
				Other potatoes, including boiled, baked, mashed, and potato salad				

*A women's intake of was split between the categories of any fruit and fruit juice.

fruits and vegetables and breast cancer, even after adjustment for each antioxidant under study and supplement use, suggests that there may be additional chemopreventive agents in fruits and vegetables that may perform outside the oxidative stress pathway. However, our results of a pronounced reduction of ER+ breast cancer associated with fruits and vegetables among postmenopausal women seem to support the hypothesis that endogenous estrogen levels may contribute to oxidative stress (33). The biological mechanism for the effect measure modification by menopausal status observed in our data and by others is not clear. Given the hypothesized chemopreventive effects of fruits and vegetables, consumption of them would be expected to be protective for both postmenopausal and premenopausal women, which makes our findings for the later group particularly perplexing. Further research based on large numbers of both premenopausal and postmenopausal women is needed to help clarify this issue.

There may be noncausal explanations for our results. Measurement error in a FFQ, such as our inability to accurately capture intake of all fruits and vegetables, is an issue for all study designs. FFQs have been shown to rank individual's relative intake of micronutrients (8, 33). In addition, validation (8), intervention (34), and feeding studies (35) have shown that the FFQ may be an adequate, albeit not perfect, instrument for measuring relative fruit and vegetable intake. However, the nondifferential misclassification errors observed in the FFQ likely attenuate the estimated RR toward the null; consequently, true RRs are probably greater than they seem (8, 36). In our data, use of alternate cut points based on menopausal status did not alter our estimates; this suggests that our categorization of women and our results are robust.

Case-control studies have additional biases related to the potential for differential recall, which may be due to the cases' cancer diagnosis. In addition, recall bias may also be related to use of chemotherapy among the cases. Potischman et al. (11, 13) found that breast cancer cases, who currently reported that chemotherapy had been initiated by the time of the interview, had higher intakes of macronutrients and calories than did cases not receiving chemotherapy. In our study, great effort was taken to interview cases prior to chemotherapy. The distribution of intake of total energy and micronutrients did not vary substantially by chemotherapy status at the time of interview or by number of days from diagnosis to interview.

Residual or uncontrolled confounding may bias the association between consumption of fruits and vegetables and risk of breast cancer. Many lifestyle factors that may potentially confound the relation between breast cancer and fruit and vegetable intake are highly correlated with the exposure of interest (37, 38), making it difficult to firmly establish independent effects of fruits and vegetables on cancer risk (39). Our questionnaire had extensive assessment of exposures over the life course including recreational physical activity from menarche to age at diagnosis, lifetime active and passive smoking exposure, lifetime alcohol consumption, and vitamin supplement use. However, when we controlled mutually or individually for these factors, our results were not substantially altered.

Our study supports an inverse association between intake of fruit, vegetable, and carotenoids and breast cancer among postmenopausal women that is more pronounced among women with ER+PR+ tumors. Increasing intakes of leafy vegetables were associated with decreasing incidence of breast cancer regardless of ER/PR status. A modest inverse association was found between high intakes of lycopene and postmenopausal breast cancer. Despite the conflicting results from epidemiologic studies, the public health significance and the biological plausibility for a beneficial effect of fruits and vegetable suggest that further research should be conducted, particularly with respect to breast cancer, for which few modifiable risk factors have been identified. Future dietary studies, particularly those with large numbers of premenopausal women, should incorporate gene-diet interactions and stratify by tumor characteristics.

Acknowledgments

We thank the following for their valuable contributions to the LIBCSP: members of the Long Island Breast Cancer Network; the 31 participating institutions on Long Island and New York City, NY; our NIH collaborators, Gwen Colman, Ph.D., National Institutes of Environmental Health Sciences; G. Iris Obrams, M.D., Ph.D., formerly of the National Cancer Institute; members of the External Advisory Committee to the population-based case-control study: Leslie Bernstein, Ph.D. (Committee Chair); Gerald Akland, M.S.; Barbara Balaban, M.S.W.; Blake Cady, M.D.; Dale Sandler, Ph.D.; Roy Shore, Ph.D.; and Gerald Wogan, Ph.D.; as well as other collaborators who assisted with various aspects of our data collection efforts, including Regina Santella, Ph.D.; Gail Garbowski, M.P.H.; H. Leon Bradlow, Ph.D.; David Camann, B.S.; Martin Trent, B.S.; Ruby Senie, Ph.D.; Carla Maffeo, Ph.D.; Pat Montalvan; Gertrud Berkowitz, Ph.D.; Margaret Kemeny, M.D.; Mark Citron, M.D.; Freya Schnabel, M.D.; Allen Schuss, M.D.; Steven Hajdu, M.D.; and Vincent Vinceguerra, M.D.

References

1. Howe GR, Hirohata T, Hislop TG, et al. Dietary factors and risk of breast cancer: combined analysis of 12 case-control studies. J Natl Cancer Inst 1990;82:561–9.
2. American Institute for Cancer Research. Chapter 4.11 breast. In: Food, nutrition and the prevention of cancer: a global perspective. Washington (DC): American Institute for Cancer Research; 1997:252–87.
3. Willett WC. Diet and breast cancer. J Intern Med 2001;249:395–411.
4. La Vecchia C, Altieri A, Tavani A. Vegetables, fruit, antioxidants and cancer: a review of Italian studies. Eur J Nutr 2001 Dec;40: 261–7.
5. Gandini S, Merzenich H, Robertson C, Boyle P. Meta-analysis of studies on breast cancer risk and diet: the role of fruit and vegetable consumption and the intake of associated micronutrients. Eur J Cancer 2001;36:636–46.
6. Riboli E, Norat T. Epidemiologic evidence of the protective effect of fruit and vegetables on cancer risk. Am J Clin Nutr 2003;78:559S–69S.
7. Smith-Warner SA, Spiegelman D, Yaun SS, et al. Intake of fruits and vegetables and risk of breast cancer: a pooled analysis of cohort studies. JAMA 2001 Feb 14;285:769–76.
8. Subar AF, Thompson FE, Kipnis V, et al. Comparative validation of the Block, Willettt, and National Cancer Institute food frequency questionnaires: the Eating at America's Table Study. Am J Epidemiol 2001;154:1089–99.
9. Willett WC. Diet and cancer: one view at the start of the millennium. Cancer Epidemiol Biomarkers Prev 2001;10:3–8.
10. Potter JD, Cerhan JR, Sellers TA, et al. Progesterone and estrogen receptors and mammary neoplasia in the Iowa Women's Health Study: how many kinds of breast cancer are there? Cancer Epidemiol Biomarkers Prev 1995;4:319–26.
11. Potischman N, Swanson CA, Coates RJ, et al. Dietary relationships

with early onset (under age 45) breast cancer in a case-control study in the United States: influences of chemotherapy treatment. Cancer Causes Control 1997;8:713–21.
12. Gammon MD, Neugut AI, Santella RM, et al. The Long Island Breast Cancer Study Project: description of a multi-institutional collaboration to identify environmental risk factors for breast cancer. Breast Cancer Res Treat 2002;74:235–54.
13. Potischman N, Swanson CA, Coates RJ, et al. Intake of food groups and associated micronutrients in relation to risk of early-stage breast cancer. Int J Cancer 1999;82:315–21.
14. Freudenheim JL, Marshall JR, Vena JE, et al. Premenopausal breast cancer risk and intake of vegetables, fruits, and related nutrients. J Natl Cancer Inst 1996 Mar 20;88:340–8.
15. Hosmer DW, Lemeshow S. Applied logistic regression. New York (NY): John Wiley & Sons, Inc; 1989.
16. Yasui Y, Potter JD. The shape of age-incidence curves of female breast cancer by hormone-status. Cancer Causes Control 1999; 10:431–7.
17. Olsen A, Tjonneland A, Thomsen BL, et al. Fruits and vegetables intake differentially affects estrogen receptor negative and positive breast cancer incidence rates. J Nutr 2003;133:2342–7.
18. Jakovljevic J, Touillaud MS, Bondy ML, Singletary SE, Pillow PC, Chang S. Dietary intake of selected fatty acids, cholesterol and carotenoids and estrogen receptor status in premenopausal breast cancer patients. Breast Cancer Res Treat 2002;75:5–14.
19. Sellers TA, Vierkant RA, Cerhan JR, et al. Interaction of dietary folate intake, alcohol, and risk of hormone receptor-defined breast cancer in a prospective study of postmenopausal women. Cancer Epidemiol Biomarkers Prev 2002;11:1104–7.
20. Rock CL, Saxe GA, Ruffin MT, August DA, Schottenfeld D. Carotenoids, vitamin A, and estrogen receptor status in breast cancer. Nutr Cancer 1996;25:281–96.
21. Cooper JA, Rohan TE, McKCant EL, Horsfall DJ, Tilley WD. Risk factors for breast cancer by estrogen receptor status: a population-based case-control study. Br J Cancer 1989;59:119–25.
22. Holm LE, Callmer E, Hjalmar ML, Lidbrink E, Nilsson B, Skoog L. Dietary habits and prognostic factors in breast cancer. J Natl Cancer Inst 1989;81:1218–23.
23. Hislop TG, Kan L, Coldman AJ, Band PR, Brauer G. Influence of estrogen receptor status on dietary risk factors for breast cancer. CMAJ 1988;138:424–30.
24. Ingram DM, Roberts A, Nottage EM. Host factors and breast cancer growth characteristics. Eur J Cancer 1992;27A:1153–61.
25. Rock CL, Lampe JW, Patterson RE. Nutrition, genetics, and risks of cancer. Annu Rev Public Health 2000;21:47–64.
26. Arab L, Steck-Scott S, Bowen P. Participation of lycopene and β-carotene in carcinogensis: defenders, aggressors, or passive bystanders? Epidemiol Rev 2001;23:211–30.
27. Mayne S. β-carotene, carotenoids, and disease prevention in humans. FASEB J 1996;10:690–701.
28. Fairfield KM, Fletcher RH. Vitamins for chronic disease prevention in adults. JAMA 2002;287:3116–26.
29. Zhang S, Hunter DJ, Forman MR, et al. Dietary carotenoids and vitamins A, C, and E and risk of breast cancer. J Natl Cancer Inst 1999;91:547–56.
30. Terry P, Jain M, Miller AB, Howe GR, Rohan TE. Dietary carotenoids and risk of breast cancer. Am J Clin Nutr 2002;76:883–8.
31. Giovannucci E. Tomatoes, tomato-based products, lycopene, and cancer: review of the epidemiologic literature. J Natl Cancer Inst 1999;91:317–31.
32. Sato R, Helzlsouer KJ, Alberg AJ, Hoffman SC, Norkus EP, Comstock GW. Prospective study of carotenoids, tocopherols, and retenoid concentrations and the risk of breast cancer. Cancer Epidemiol Biomarkers Prev 2002;11:451–7.
33. Yager JD. Endogenous estrogens as carcinogens through metabolic activation. J Natl Cancer Inst Monogr 2000;(No.27):67–74.
34. Block G, Norkus E, Hudes M, Mandel S, Helzlsouer K. Which plasma antioxidants are most related to fruit and vegetable consumption? Am J Epidemiol 2001;154:1113–8.
35. McEligot AJ, Rock CL, Flatt SW, Newman V, Faerber S, Pierce JP. Plasma carotenoids are biomarkers of long-term high vegetable intake in women with breast cancer. J Nutr 1999;129:2258–63.
36. Kipnis V, Midthune D, Freedman LS, et al. Empirical evidence of correlated biases in dietary assessment instruments and its implications. Am J Epidemiol 2001;153:394–403.
37. Trudeau E, Kristal AR, Li S, Patterson RE. Demographic and psychosocial predictors of fruit and vegetable intakes differ: implications for dietary interventions. J Am Diet Assoc 1998; 98:1412–7.
38. Thompson B, Demark-Wahnefried W, Taylor G, et al. Baseline fruit and vegetable intake among adults in seven 5 a day study centers located in diverse geographic areas. J Am Diet Assoc 1999;99:1241–8.
39. Subar AF, Heimendinger J, Patterson BH, Krebs-Smith SM, Pivonka E, Kessler R. Fruit and vegetable intake in the United States: the baseline survey of the Five a Day for Better Health Program. Am J Health Promot 1995;9:352–60.