Breast Cancer Res Treat (2013) 138:925–930
DOI 10.1007/s10549-013-2484-3

EPIDEMIOLOGY

Intake of specific fruits and vegetables in relation to risk of estrogen receptor-negative breast cancer among postmenopausal women

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Received: 9 March 2013 / Accepted: 12 March 2013 / Published online: 27 March 2013

Abstract In previous studies of postmenopausal women, overall intake of fruits and vegetables groups has been inversely associated with estrogen receptor-negative (ER−) breast cancer. In this analysis, we prospectively examined the associations of specific fruits and vegetables with risk of ER− postmenopausal breast cancer among 75,929 women aged 38–63 years at baseline and followed for up to 24 years. Dietary data were collected seven times during this period. Cox proportional hazard models were used, adjusting for potential confounders, including a modified Alternate Mediterranean Diet score. We ascertained 792 incident cases of ER− postmenopausal breast cancer. The multivariate relative risk (RR) for every 2 servings/week consumption for total berries was 0.82 (95 % CI = 0.71–0.96, $p = 0.01$), and the RR for women who consumed at least one serving of blueberries a week was 0.69 (95 % CI = 0.50–0.95, $p = 0.02$) compared with non-consumers. Also, the RR for consuming at least 2 servings of peaches/nectarines per week was 0.59 (95 % CI = 0.37–0.93, $p = 0.02$). Risk of ER− breast cancer was not associated with intakes of other specific fruits or vegetables. In conclusion, higher intake of berries and peaches was associated with lower risk of ER− breast cancer among postmenopausal women. These results are considered exploratory and need to be confirmed in further studies.

Keywords Breast cancer · Diet · Estrogen receptor · Incidence · Risk

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Introduction

The potential for fruits and vegetables in breast cancer prevention has been studied frequently. In a recent pooled analysis, higher intakes of total fruits and vegetables, and vegetables, but not fruits, were associated with a lower risk of estrogen receptor negative (ER−) but not ER+ breast cancer [1]. In an earlier analysis among postmenopausal women in the Nurses' Health Study (NHS), we found that the association with fruits and vegetables was also limited to ER− breast cancer [2] and this was confirmed in the Black Women's Health Study [3]. In recent pooled analyses of dietary carotenoids [4] or blood carotenoid measurements [5], inverse association were also much stronger with or limited to ER− breast cancer. However, specific fruits and vegetables vary greatly in their composition and are not likely to have identical relationships with breast cancer. Although the pooled analysis did examine individual fruits and vegetables and observed inverse association with peaches and strawberries, the number of specific fruits and vegetables was limited to items measured in multiple cohorts. Berries, in particular blueberries, are rich in antioxidants and polyphenols [6] and may influence

breast cancer risk, yet were not examined in the pooled analysis. Thus, we prospectively examined associations between specific fruits and vegetables and risk of ER− breast cancer among postmenopausal women in the NHS. Our detailed food frequency questionnaire (FFQ) allowed us to examine 29 different fruits and vegetables, including blueberries.

Subjects and methods

Study population

The NHS is a cohort study of 121,700 female nurses aged 30–55 years living in 11 US states established in 1976. Questionnaires are sent biennially to collect medical, lifestyle, and other health-related information [7]. In 1980, participants completed a 61-item FFQ. This was expanded to 116 items in 1984 and similar FFQs were sent in 1986, 1990, 1994, 1998, 2002, and 2006.

For this analysis, we used 1984 as baseline as the expanded FFQ provides more detailed information on consumption of fruits and vegetables. We included women who completed the 1984 FFQ with plausible total energy intake (calculated from the FFQ, between 500 and 3,500 kcal/day) [8]. After excluding those with a history of cancer (except non-melanoma skin cancer) at baseline, we included 75,929 postmenopausal women with followup from 1984 through 2008. This study was approved by the Institutional Review Board of the Brigham and Women's Hospital, Boston, MA.

Dietary assessment

Self-administered semi-quantitative FFQs were designed to assess average food intake over the preceding year. A standard portion size and nine possible consumption frequency categories, from "never, or <1/month" to "6+ times per day" were given for each food. Total energy and nutrient intake were calculated by summing the contributions from all foods. Previous validation studies in the NHS revealed reasonably good correlations between energy-adjusted nutrients and foods assessed by the FFQ and multiple food records completed over the preceding year [9]. The corrected correlation coefficients for fruits and vegetables between diet records and FFQ ranged from 0.16 for winter squash to 0.74 for apples. The corrected correlation coefficients for most fruits and vegetables were above 0.40.

Breast cancer ascertainment

Incident breast cancer was ascertained from 1984 to 2008, a followup of up to 24 years. In each biennial questionnaire, participants self-report any diagnosis of breast cancer in the previous 2 years. We then obtained permission to review medical records for confirmation, and we confirmed 99 % of the cases for which records were available. ER and progesterone receptor (PR) status was obtained from pathology reports and each receptor was classified as positive, negative, or uncertain. Deaths were reported by the postal service, family members, or by searching the National Death Index. In this study, as in our previous report, we included only postmenopausal breast cancer cases to reduce potential etiologic heterogeneity.

Measurement of lifestyle and health factors

Body mass index (BMI) was calculated from weight reported on each biennial questionnaire and height reported on the first questionnaire. Smoking, history of hypertension, aspirin use, multivitamin intake, menopausal status and use of postmenopausal hormone therapy, history of benign breast disease, parity, and age at first birth were assessed every 2 years. Family history of breast cancer was assessed six times during followup. Leisure-time physical activity was measured with validated questions on 10 common activities beginning in 1986 [10]. In 1984, we collected hours of vigorous physical activity.

Statistical analysis

We used Cox proportional hazard models to assess the association between intake of specific fruits and vegetables and risk of postmenopausal ER− breast cancer between 1984 and 2008. To reduce random within-person variation and to best represent long-term dietary intake, we calculated cumulative averages of intake from our repeated FFQs [11]. Potatoes were not included as a vegetable in any of our analysis. Because legumes are sources of protein and not generally viewed as vegetables in meal planning, we combined all other vegetables without legumes as low protein vegetables. Groups of fruits and vegetable were classified into quintiles of intake with the first quintile as a reference. For specific fruits and vegetables, we computed the relative risk (RR) for every 2 servings/week increase of intake.

In multivariate analysis, we adjusted for age, energy intake (quintiles), alcohol (4 categories), multivitamin use (yes/no), BMI at age 18 (5 categories), weight change since age 18 (7 categories), family history of breast cancer (yes/no), history of benign breast disease (yes/no), physical activity in METs (quintiles), and age at menopause and postmenopausal hormone use (11 categories). We also adjusted for a modified Alternative Mediterranean Diet score in which we excluded fruits and vegetables to avoid redundancy with our primary variables [2]. Statistical

adjustment was updated for covariates measured more than once during followup.

Results

Women with high intakes of fruits and vegetables were more physically active, and were less likely to be current smokers, but no apparent trend was observed with BMI (Table 1). These women also consumed less alcohol and saturated fat but more fiber and folate.

During 24 years of followup, we documented 792 cases of ER− postmenopausal breast cancer. Intakes of total vegetables was only marginally associated with lower risk of ER− breast cancer (Table 2). On the other hand, there was no association with total fruits intake. When we explored the associations with individual fruits and vegetables, we noted a RR of 0.82 (95 % CI = 0.71–0.96, $p = 0.01$) for an increment of 2 servings/week of berries (Fig. 1). When we separately examined strawberries and blueberries, the two berries item in our FFQ, the RR for every 2 servings/week for strawberries was 0.80 (95 % CI = 0.65–0.99, $p = 0.04$), and for blueberries was 0.67 (95 % CI = 0.49–0.94, $p = 0.02$). Intakes of blueberries and strawberries were moderately correlated (Spearman correlation coefficient = 0.47, $p < 0.0001$), when both were in the model, the RRs were slightly attenuated and did not reach statistical significance (data not shown). Consumption of peaches and nectarines was also associated with a lower risk for ER− tumors (for every 2 servings/week consumption RR = 0.82, 95 % CI = 0.70–0.97, $p = 0.02$). Among vegetables, winter squash was the only vegetable that showed a marginal inverse association (RR for every 2 servings/week = 0.70, 95 % CI = 0.49–1.00) (Fig. 2).

Table 1 Age standardized baseline (1984) characteristics according to quintile of total fruits and vegetables intake

Median intake (servings/day)	Q1 2.4	Q2 3.9	Q3 5.1	Q4 6.5	Q5 9.8
BMI	24.6	24.7	24.6	24.8	24.7
Current smokers (%)	38	30	25	22	18
Physical activity (h/week)	2.5	2.7	2.8	2.9	3.1
Family history (%)	9	9	9	9	10
Alcohol (g/day)	8	7	7	7	6
Polyunsaturated fat (g/day)	12	12	12	12	11
Monounsaturated fat (g/day)	24	23	22	22	20
Saturated fat (g/day)	24	23	22	21	19
Fiber (g/day)	13	15	17	18	22
Folic acid (μg/day)	315	364	390	426	486
Glycemic load	97	98	99	100	104

Dietary factors are energy adjusted except for alcohol

When we examined categories of intakes for foods that were significant in continuous analyses, we observed the clearest trends with blueberries and peaches (Table 3). Compared to women who did not consume blueberries, those with greater than 1/week intake, the RR for postmenopausal ER− breast cancer was 0.69 (95 % CI = 0.50–0.95, $p = 0.02$). For peaches, women who consumed at least 2 servings of peaches at week had an RR of 0.59 (95 % CI = 0.37–0.93, $p = 0.02$).

Discussion

Because of previous suggestions that intakes of fruits and vegetables may be inversely associated with risk of ER− breast cancer, we conducted an exploratory analysis to identify specific foods that might account for this relationship. We observed inverse associations between intakes of blueberries, strawberries, and peaches/nectarines.

In an earlier meta-analysis, a weak inverse association was seen between intake of fruits and vegetables combined and overall breast cancer risk but this analysis did not differentiate cases by hormone receptor status [12]. In a recent pooled analysis that included hormone receptor status, greater intake of total vegetables, excluding potatoes and beans, was significantly associated with a lower risk of ER− breast cancer [1]. Although not statistically significant, in our analysis, we found a similar RR for the group of foods. In addition, in two recent pooled analyses both dietary [4] and circulating [5] carotenoids were inversely associated primarily with risks of ER− tumors. Collectively, these findings strongly suggest that higher intakes of fruits and vegetables reduce the risk of ER− breast cancer.

In an examination of specific foods within the recent pooled analysis of prospective studies, which included the NHS [1], intakes of apples/pears, peaches/nectarines/apricots, and strawberries, carrots, and lettuce/salad were associated with lower incidence of ER− breast cancer. Our results for peaches and strawberries agreed with the pooled analysis [1], and showed similar magnitude of risk reduction. Blueberries were not included in the pooled analysis because few other studies collected data on consumption, and to our knowledge, this is the first human report on blueberries and breast cancer. The greater power of the pooled analysis likely accounted for the statistically significant inverse associations with apples/pears, carrots, and lettuce that were not seen in this analysis. In our previous report, consumption of yellow/orange vegetables was associated with lower risk of ER− tumors [2]. In the Shanghai Breast Cancer Study, which was not included in the Pooling Project, higher intakes of citrus was significantly and rosaceae fruits (e.g., apples and peaches) were associated with a lower risk of ER−/PR− tumors [13].

Table 2 RRs (95 % CI) of ER− breast cancer by quintiles of fruits and vegetables intakes

	Q1	Q2	Q3	Q4	Q5	p_{trend}
Total fruits and vegetables						
Median (servings/day)	3.1	4.3	5.5	6.8	9.1	
Number of cases	158	149	184	146	155	
Age and energy adjusted	1	0.87 (0.69, 1.09)	1.01 (0.81, 1.26)	0.77 (0.61, 0.98)	0.78 (0.60, 0.99)	0.06
Multivariate adjusted[a]	1	0.86 (0.69, 1.10)	1.00 (0.80, 1.27)	0.80 (0.62, 1.03)	0.82 (0.62, 1.08)	0.13
Total vegetables[b]						
Servings/day	1.7	2.6	3.3	4.1	5.6	
Number of cases	154	166	172	158	142	
Age and energy adjusted	1	1.01 (0.81, 1.26)	1.00 (0.80, 1.25)	0.89 (0.71, 1.13)	0.77 (0.60, 0.98)	0.047
Multivariate adjusted[a]	1	1.01 (0.80, 1.26)	1.02 (0.83, 1.28)	0.91 (0.71, 1.17)	0.81 (0.61, 1.06)	0.06
Total low protein vegetables[c]						
Servings/day	1.6	2.4	3.1	3.9	5.3	
Number of cases	160	164	167	157	144	
Age and energy adjusted	1	0.97 (0.78, 1.11)	0.94 (0.75, 1.17)	0.86 (0.68, 1.08)	0.76 (0.59, 0.96)	0.049
Multivariate adjusted[a]	1	0.96 (0.77, 1.21)	0.95 (0.75, 1.19)	0.87 (0.68, 1.11)	0.79 (0.60, 1.03)	0.05
Total fruits						
Servings/day	0.9	1.6	2.2	2.8	3.9	
Number of cases	145	142	170	182	153	
Age and energy adjusted	1	0.91 (0.72, 1.15)	1.05 (0.83, 1.32)	1.07 (0.85, 1.34)	0.86 (0.67, 1.11)	0.30
Multivariate adjusted[a]	1	0.91 (0.72, 1.15)	1.06 (0.83, 1.34)	1.11 (0.87, 1.41)	0.93 (0.71, 1.21)	0.85

[a] Adjusted for age, energy intake, smoking, alcohol, weight change since age 18, height, postmenopausal hormone use, physical activity, BMI at age 18, family history, history of benign breast disease, modified Alternate Mediterranean Diet score

[b] Exclude potatoes

[c] Excludes legumes and potatoes

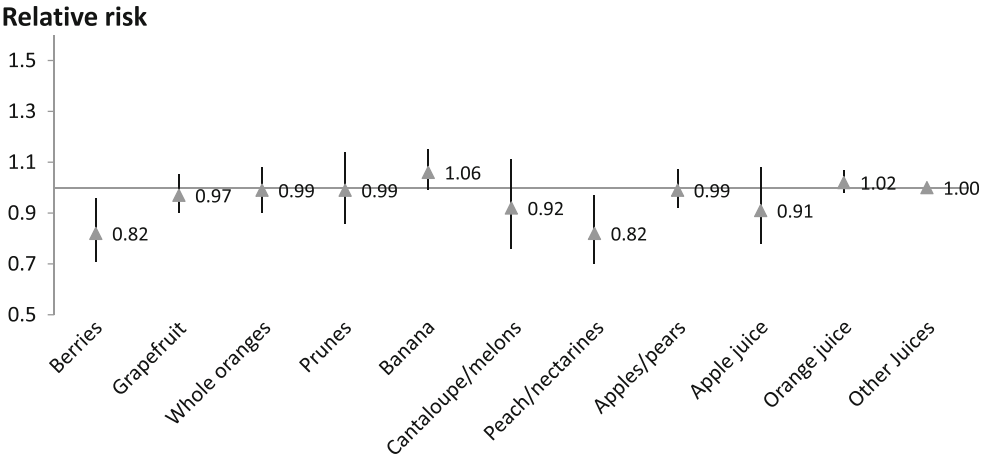

Fig. 1 Multivariate (adjusted for age, energy intake, smoking, alcohol, weight change since age 18, height, postmenopausal hormone use, physical activity, BMI at age 18, family history, history of benign breast disease, modified Alternate Mediterranean Diet score) RRs for ER− breast cancer of specific fruits for every 2 servings/week intake. *Error bars* represent 95 % CI

Both strawberry and blueberry extracts have shown to reduce growth in breast cancer cell lines [14–16]. In mice, strawberries extract has reduced tumor progression by enhancing apoptosis [17]. In addition, one study showed quercetin and chlorogenic acid extracted from peaches reduced proliferation in estrogen-independent breast cancer cell lines [18]. Fruits and vegetables may also reduce breast cancer risk as sources of antioxidants [19, 20].

The large number of postmenopausal breast cancer during 24 years of followup allowed us to examine specifically ER− tumors, which have been inversely associated with intake of fruits and vegetables. We have extensive

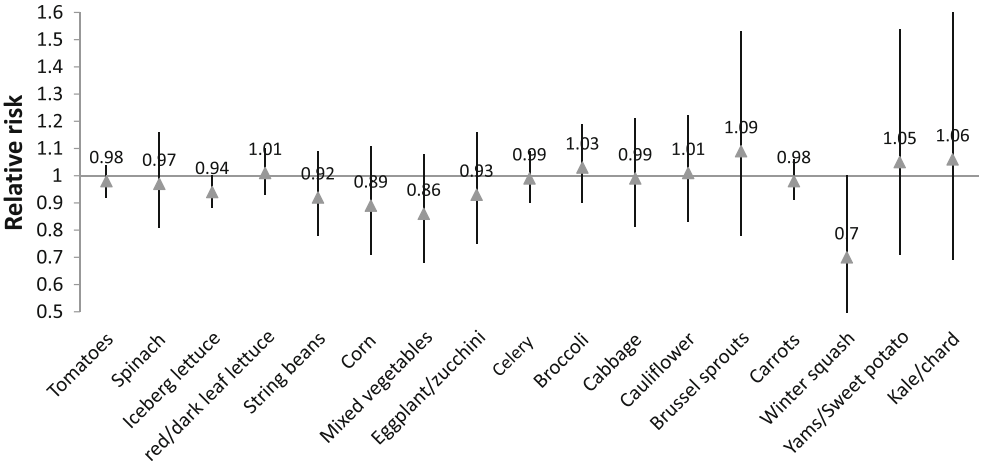

Fig. 2 Multivariate (adjusted for age, energy intake, smoking, alcohol, weight change since age 18, height, postmenopausal hormone use, physical activity, BMI at age 18, family history, history of benign breast disease, modified Alternate Mediterranean Diet score) RRs for ER− breast cancer of specific vegetables for every 2 servings/week intake. *Error bars* represent 95 % CI

Table 3 Multivariate RRs (95 % CI) for selected fruits intake

	Never	Up to 2/month	>2/month to <1/week	>1+/week	1/week to <2/week	2+/week	p_{trend}
Total berries							
Number of cases	59	232	184		283	34	
Person years	100,500	330,065	234,597		484,552	71,322	
Multivariate RR[a]	1	1.04 (0.78, 1.40)	1.11 (0.81, 1.50)		0.87 (0.65, 1.17)	0.75 (0.49, 1.16)	0.01
Strawberries							
Number of cases	63	353	213		142	21	
Person years	114,010	496,968	288,904		283,880	37,276	
Multivariate RR[a]	1	1.14 (0.86, 1.50)	1.12 (0.83, 1.50)		0.81 (0.59, 1.10)	1.02 (0.62, 1.69)	0.04
Blueberries							
Number of cases	326	352	75	39			
Person years	268,245	282,908	81,907	44,866			
Multivariate RR[a]	1	1.00 (0.85, 1.17)	0.79 (0.60, 1.03)	0.69 (0.50, 0.95)			0.02
Peaches/nectarines							
Number of cases	74	306	165		220	27	
Person years	118,385	445,096	233,514		353,017	71,025	
Multivariate RR[a]	1	1.00 (0.77, 1.29)	0.98 (0.73, 1.30)		0.87 (0.66, 1.15)	0.59 (0.37, 0.93)	0.02

[a] Adjusted for age, energy intake, smoking, alcohol, BMI, weight change since age 18, height, postmenopausal hormone use, physical activity, BMI at age 18, family history, history of benign breast disease

information on potential confounders which we carefully controlled for in our analysis. Although error in assessment of long-term diet was reduced by many repeated measure, some degree of error is unavoidable as diet and lifestyle information was obtained through self-report.

In conclusion, we observed a lower risk for ER− tumors with higher intake of berries and peaches. Our finding of lower risks with higher intake of blueberries needs to be confirmed in other populations.

Acknowledgments We would like to thank the participants and staff of the Nurses' Health Study, for their valuable contributions as well as the following state cancer registries for their help: AL, AZ, AR, CA, CO, CT, DE, FL, GA, ID, IL, IN, IA, KY, LA, ME, MD, MA, MI, NE, NH, NJ, NY, NC, ND, OH, OK, OR, PA, RI, SC, TN, TX, VA, WA, WY. NIH Grants CA87969, HL60712, CA95589, and 1U54CA155626-01.

Conflict of interest The authors declare that they have no conflict of interest.

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