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Vegetable and fruit consumption and the risk of hormone receptor–defined breast cancer in the EPIC cohort[1,2]

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ABSTRACT

Background: The recent literature indicates that a high vegetable intake and not a high fruit intake could be associated with decreased steroid hormone receptor–negative breast cancer risk.

Objective: This study aimed to investigate the association between vegetable and fruit intake and steroid hormone receptor–defined breast cancer risk.

Design: A total of 335,054 female participants in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort were included in this study (mean ± SD age: 50.8 ± 9.8 y). Vegetable and fruit intake was measured by country-specific questionnaires filled out at recruitment between 1992 and 2000 with the use of standardized procedures. Cox proportional hazards models were stratified by age at recruitment and study center and were adjusted for breast cancer risk factors.

Results: After a median follow-up of 11.5 y (IQR: 10.1–12.3 y), 10,197 incident invasive breast cancers were diagnosed [3479 estrogen and progesterone receptor positive (ER+PR+); 1021 ER and PR negative (ER−PR−)]. Compared with the lowest quintile, the highest quintile of vegetable intake was associated with a lower risk of overall breast cancer ($HR_{quintile\ 5–quintile\ 1}$: 0.87; 95% CI: 0.80, 0.94). Although the inverse association was most apparent for ER−PR− breast cancer (ER−PR−: $HR_{quintile\ 5–quintile\ 1}$: 0.74; 95% CI: 0.57, 0.96; *P*-trend = 0.03; ER+PR+: $HR_{quintile\ 5–quintile\ 1}$: 0.91; 95% CI: 0.79, 1.05; *P*-trend = 0.14), the test for heterogeneity by hormone receptor status was not significant (*P*-heterogeneity = 0.09). Fruit intake was not significantly associated with total and hormone receptor–defined breast cancer risk.

Conclusion: This study supports evidence that a high vegetable intake is associated with lower (mainly hormone receptor–negative) breast cancer risk. *Am J Clin Nutr* 2016;103:168–77.

Keywords: breast cancer, estrogen receptor, fruit, progesterone receptor, vegetables

INTRODUCTION

During the past decades, several prospective cohort studies have investigated the association between vegetable and fruit intake and breast cancer risk (1–8). Vegetables and fruit are hypothesized to prevent breast cancer occurrence because of their anticarcinogenic substances (i.e., vitamins C and E, minerals, fiber, carotenoids, and many other bioactive compounds) (9). For instance, cruciferous vegetables and berries have great potential due to the high amounts of glucosinolates in cruciferous vegetables (10) and antioxidants and polyphenols in berries (11).

Despite the extensive research on this association and the biological plausibility of the ability of vegetables and fruit to reduce breast cancer risk, the World Cancer Research Fund reported in 2007 that the existent evidence is "too limited or inconclusive for a conclusion to be made" (12). A meta-analysis of the Continuous Update Project, which included 15 prospective studies published through April 2011, showed that high fruit intake was associated with a borderline significant lower breast cancer risk ($RR_{200\text{-g increment}}$: 0.94; 95% CI: 0.89, 1.00), whereas no association was found for vegetable intake ($RR_{200\text{-g increment}}$: 1.00; 95% CI: 0.95, 1.06) (13, 14). It was suggested that the association might depend on the characteristics of the tumor (14). Breast cancer is a heterogeneous disease that represents different molecular entities with different therapy sensitivity and prognosis, and likely also a different etiology. The major subtypes are based on steroid hormone receptor status. Unfortunately, at that time, the number of studies with steroid hormone receptor status data available was too low to take this into account (14). However, recently, the Pooling Project was able to perform steroid hormone receptor–defined analyses (15). Published and unpublished results of 20 prospective cohort studies [of which 6 (4, 5, 16–19) were also included in the analyses of the Continuous Update Project (13)] were pooled. Here, a protective effect of high vegetable consumption on estrogen receptor (ER)[42] negative (ER−) breast cancer risk was observed with an RR of 0.82 (95% CI: 0.74, 0.90) comparing the highest intake quintile with the lowest (15). Furthermore, several subgroups of vegetables and fruit were associated with lower ER− breast cancer risk (15). No association was found for total and ER-positive (ER+) breast cancer risk. A limitation of a pooled analysis as such is the between-studies variation in the assessment of exposure, confounders, and outcomes (15).

We aimed to investigate the association between intakes of vegetables and fruit and their subgroups and steroid hormone receptor–defined breast cancer risk within the EPIC (European Prospective Investigation into Cancer and Nutrition) (20). EPIC is a European-wide cohort, which resulted in much variability in vegetable and fruit consumption (21). Dietary habits were assessed with country-specific questionnaires, which were developed according to a common format (20). In 2005, we reported on the relation between vegetable and fruit consumption and total breast cancer risk in EPIC when 3659 breast cancer cases were documented after 5 y of follow-up in a study population containing 285,526 women. No significant associations were found for total breast cancer (8). For the current investigation, numbers of breast cancers have increased to >10,000 cases, and we collected data to perform hormone receptor–defined analyses.

METHODS

Subjects

EPIC is a prospective multicenter cohort study initiated to investigate nutrition, metabolic factors, and hormones in relation to cancer and other chronic diseases (20, 22). Between 1992 and 2000, 521,448 participants (age range: 25–70 y) were recruited from 23 centers in 10 European countries. At recruitment, anthropometric measurements were obtained, and participants completed dietary, lifestyle, and health questionnaires. The study has been described in detail elsewhere (20, 22).

Our study population comprised female EPIC participants without a prevalent cancer diagnosis (excluding nonmelanoma skin cancer) (n = 345,158). Participants who did not complete the dietary or nondietary questionnaires were excluded (n = 3345). To reduce the influence of implausible extreme values on the analysis, in addition we excluded participants in the top or bottom 1% of the ratio of reported energy intake compared with calculated energy requirement (n = 6753). Furthermore, participants with incomplete follow-up information were excluded (n = 6), which left 335,054 women for the current analyses.

All participants gave written or oral informed consent. The study was approved by the International Agency for Research on Cancer Ethical Review Committee and by local ethical committees at the participating centers.

Exposure assessment

At recruitment, validated, country-specific dietary questionnaires were used to measure the habitual dietary intake over the

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[2]Supplemental Figures 1–5 and Supplemental Tables 1 and 2 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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[42] Abbreviations used: EPIC, European Prospective Investigation into Cancer and Nutrition; ER, estrogen receptor; PR, progesterone receptor; +, positive; −, negative.

12 mo before study enrollment. These questionnaires were designed to capture local dietary habits and to provide high compliance (details are provided in the **Supplemental Materials**) (22). To improve the comparability of dietary data across the centers and to adjust for potential systematic over- and underestimation in dietary intake measurements, standardized, computer-based, 24-h dietary recall measurements were collected from a stratified random sample covering all centers (i.e., 36,000 participants) (23).

This study evaluated the effects of vegetables, fruit, and vegetables and fruit combined. In addition, we examined subgroups of vegetables (leafy, fruiting, root, cabbages, mushrooms, grain/pod, onions/garlic, stalk/sprouts, and mixed salads) and fruit (citrus fruit, apples/pears, grapes, stone fruit, berries, bananas, and kiwis) (21). Legumes, potatoes, and other tubers were not included as vegetables, because they differ in energy and carbohydrate contents (21). Although, for example, fresh orange juice is rich in vitamin C, fruit and vegetable juices were not included in our analyses, because they are nutritionally different from whole fruit and vegetables (e.g., added water, sugars, and vitamins) (21).

Outcome assessment

The outcome was incident primary invasive breast cancer. Cases were identified either through linkage with population cancer registries or by active follow-up (details are provided in the Supplemental Materials). The International Classification of Diseases, 10th Revision, was used to classify cancer data, with breast cancer defined as C50. Steroid hormone receptor status data and information on the available laboratory methods and quantification descriptions used to determine receptor status were collected across 20 centers (details are provided in the Supplemental Materials). Mortality data were collected from registries at the regional or national level.

Statistical analysis

Cox proportional hazard models were used to calculate HRs and their 95% CIs. Attained age was used as the underlying time variable, with entry time defined as age at recruitment and exit time defined as age at breast cancer diagnosis or age at censoring (i.e., age at death, loss to follow-up, end of follow-up, or diagnosis of other cancers or ductal carcinoma in situ, whichever came first). An aggregate 1-stage analysis was performed.

Vegetable and fruit intakes were expressed in grams per day and were analyzed both as categorical variables by using EPIC-wide quintiles (reference = quintile 1) and continuous variables (increment = 100 g/d for separate vegetable and fruit consumption; 200 g/d for total consumption). Subgroups of vegetables and fruit were categorized into EPIC-wide quartiles of intake. Tests for linear trend were performed by using the medians of categories modeled continuously. To examine the shape of the association under study, cubic spline analysis was performed with 3, 4, and 5 knots placed by using the continuous variables of vegetable and fruit consumption. The cubic spline model with the lowest Akaike's information criterion was assumed to fit the data best.

Associations for total breast cancer and breast cancer subtypes were assessed. Breast cancer subtypes were classified by their joint estrogen and progesterone status. We examined associations for ER+/progesterone receptor–positive (ER+PR+) and ER−PR− breast cancer cases separately, because these subtypes have different risk factors (24). Furthermore, we examined associations for ER+PR− breast cancer cases. The number of ER−PR+ cases was too low to be considered as a separate outcome category ($n = 210$).

Models were stratified by age at recruitment and study center to reduce violations of the proportional hazards assumption (confirmed by log-minus-log plots). Analyses were adjusted for potential confounding factors collected at recruitment. These were selected a priori on the basis of current knowledge of breast cancer risk factors. Models were adjusted for estimated total energy intake to control for potential confounding and to remove extraneous variation in vegetable and fruit intake due to differences in energy requirements (25). To improve the measurement error correction, estimated energy intake was divided into energy from fat and nonfat sources, because the nonfat components contributed mostly to vegetable and fruit consumption (26). A third reason to adjust for total energy intake was for the purpose of making isoenergetic comparisons (25), which implied in this study, substitution models where possible substitutions were restricted to foods providing the same amount of energy from fat and nonfat as vegetables and fruit. The models were further adjusted for saturated fat intake, age at menarche, oral contraceptive use, age at first full-term pregnancy, menopausal status, hormone replacement therapy use, BMI, physical activity, smoking status and intensity, alcohol user or nonuser, alcohol consumption, and educational level (units and categories are described in the Supplemental Materials). An interaction term BMI × menopausal status was added to the model, because it is known that the association between BMI and breast cancer risk varies by menopausal status (12). In the separate vegetable and fruit analyses, the final models were mutually adjusted (i.e., adjustment for fruit intake in the vegetable analysis; adjustment for vegetable intake in the fruit analysis).

Heterogeneity of the association according to hormone receptor–defined breast cancer subtypes (ER+PR+ compared with ER−PR−) was assessed by using a data-augmentation method described by Lunn and McNeil (27). A log-likelihood ratio test was used to compare the models with and without interaction terms between the continuous vegetable and fruit variable and breast cancer subtype. Women who developed the competing breast cancer subtype (e.g., ER+PR+, ER−PR+, or ER+PR− breast cancer in the ER−PR− analysis) or women with missing receptor status information were censored at the time of occurrence.

Country-specific results were computed and presented for the continuous variables of vegetable and fruit consumption. To assess possible heterogeneity by country, models with and without the cross-product terms (i.e., continuous vegetable and fruit variable multiplied by country) were compared by using a log-likelihood ratio test. To assess possible effect modification by menopausal status on the association under study, models with and without the cross-product terms (i.e., continuous vegetable and fruit variable multiplied by menopausal status at recruitment) were compared by using a log-likelihood ratio test.

The multiple imputation technique as described by Rubin (28) was used to impute missing covariate values ($m = 5$; details are provided in the Supplemental Materials). The missing indicator technique was used in the heterogeneity, interaction, and calibration analyses.

To judge whether the receptor status was available in a selective population, we repeated the analyses including only cases with receptor status data available. To exclude reverse causation due to dietary changes during the preclinical period, we conducted a sensitivity analysis restricted to women with a follow-up of ≥2 y. High vegetable and fruit consumption was accompanied by a low percentage of current smokers. Even though results were adjusted by smoking status, there may still be residual confounding. Therefore, we conducted a sensitivity analysis restricted to never smokers.

Calibration

The 24-h dietary recall measurements were regressed on dietary questionnaire values of vegetables, fruit, and total vegetables and fruit combined in country-specific models (23, 29–34). The models were adjusted for age at recruitment, study center, and the covariates included in the breast cancer risk models. Results were weighted for day of the week and season of the year of the 24-h dietary recall measurement. Nonconsumers were kept in the models and negative predictive values were set to 0. To take into account the uncertainty related to measurement error correction, 95% CIs were calculated by using the bootstrap sampling technique (n = 10 repetitions). The continuous models were based on both observed and calibrated measurements, whereas categorical analyses were based on the observed measurements.

Two-tailed P values <0.05 were considered significant. Analyses were performed by using SAS version 9.2 and R version 14.2.

RESULTS

In total, 335,054 women were included in this study. The median vegetable intake was 137 g/d (IQR: 60–238 g/d) and the median fruit intake was 170 g/d (IQR: 53–317 g/d) (**Table 1**). Median vegetable and fruit consumption in the EPIC cohort by country showed a north-to-south gradient, with the highest total intakes in Spain, Italy, France, and Greece.

The mean (±SD) age of our study population was 50.8 ± 9.8 y (**Table 2**). Approximately half of the study population (46.3%) was postmenopausal at recruitment. During a median follow-up of 11.5 y (IQR: 10.1−12.3 y), 10,197 primary invasive breast cancer cases were diagnosed, and data on receptor status were available in 57% of the cases among whom 3479 were ER+PR+ cases and 1021 were ER−PR− cases. We next examined the study population characteristics according to vegetable and fruit intake quintiles. Women with the highest vegetable and fruit intakes had higher intakes of energy from fat and nonfat sources than did women with the lowest intakes. In addition, these women were more often highly educated. Oral contraceptive use and tobacco use were lower in women with the highest vegetable and fruit intakes. Women with the highest fruit intake were more frequently postmenopausal and nonconsumers of alcohol compared with women with the lowest intake.

Compared with the lowest quintile, the highest vegetable intake quintile was associated with a significantly lower risk of overall breast cancer (adjusted $HR_{\text{quintile 5 vs. quintile 1}}$: 0.87; 95% CI: 0.80, 0.94; P-trend < 0.01; mutually adjusted $HR_{\text{quintile 5 vs. quintile 1}}$: 0.86; 95% CI: 0.80, 0.94; P-trend < 0.01) (**Table 3**). In the continuous analysis, a borderline significant inverse association was found (100-g/d increased intake of vegetables: observed HR: 0.97; 95% CI: 0.96, 0.99; calibrated HR: 0.96; 95% CI: 0.91, 1.00). Fruit consumption was not associated with overall breast cancer risk (adjusted $HR_{\text{quintile 5 vs. quintile 1}}$: 0.99; 95% CI: 0.93, 1.07; P-trend = 0.86; mutually adjusted $HR_{\text{quintile 5 vs. quintile 1}}$: 1.01; 95% CI: 0.94, 1.09; P-trend = 0.70).

In analyses by hormone receptor status, high vegetable consumption was only significantly associated with ER−PR− breast cancer risk (adjusted $HR_{\text{quintile 5 vs. quintile 1}}$: 0.74; 95% CI: 0.57, 0.96; P-trend = 0.03) (**Table 4**). In this adjusted analysis, the most important confounders were energy intake (fat and nonfat sources) and saturated fat intake. Additional adjustment for fruit intake did not materially change the effect estimate (mutually adjusted $HR_{\text{quintile 5 vs. quintile 1}}$: 0.76; 95% CI: 0.58, 0.98). Although the association was stronger for receptor-negative breast cancer than for receptor-positive breast cancer risk, the test for heterogeneity by hormone receptor status did not reach significance (P-heterogeneity = 0.09). No significant associations were found for fruit intake and hormone receptor–defined breast cancer risk.

TABLE 1
Description of the EPIC cohort by country[1]

Country	Participants, n (%)	Median age at recruitment, y (IQR)	Total follow-up time, person-years	Median follow-up time, person-years (IQR)	No. of incident breast cancer cases	Median consumption,[2] g/d (IQR)		
						Vegetables	Fruit	Vegetables and fruit
France	67,385 (20.1)	51.5 (47.0–57.4)	699,360	11.8 (9.8–12.0)	2676	185 (94–304)	195 (100–328)	411 (252–591)
Italy	30,512 (9.1)	50.9 (44.4–56.7)	341,489	11.7 (10.6–12.6)	945	150 (65–268)	279 (136–442)	467 (282–671)
Spain	24,854 (7.4)	47.7 (41.3–54.9)	299,617	12.6 (11.2–13.4)	439	158 (62–287)	290 (135–500)	488 (284–739)
United Kingdom	52,543 (15.7)	47.9 (36.4–57.9)	586,301	11.5 (10.4–12.6)	1299	155 (81–255)	143 (34–267)	317 (185–485)
The Netherlands	26,866 (8.0)	52.7 (46.0–58.9)	315,683	12.2 (11.0–13.3)	804	114 (52–186)	150 (9–278)	277 (142–439)
Greece	15,225 (4.5)	53.6 (43.1–63.9)	148,604	10.7 (8.0–12.0)	188	172 (88–289)	150 (20–298)	369 (208–555)
Germany	27,411 (8.2)	48.4 (41.1–57.0)	272,105	10.9 (8.1–11.8)	748	144 (64–243)	160 (13–315)	332 (189–526)
Sweden	26,368 (7.9)	50.6 (46.7–60.1)	349,229	13.9 (12.8–15.3)	981	106 (47–174)	129 (42–239)	251 (144–389)
Denmark	28,722 (8.6)	56.3 (52.8–60.4)	316,745	11.6 (10.9–12.3)	1264	120 (43–212)	146 (0–296)	282 (151–469)
Norway	35,168 (10.5)	48.0 (44.4–51.8)	342,279	10.1 (10.0–10.1)	853	101 (42–181)	124 (0–228)	238 (125–389)
Overall	335,054 (100)	51.0 (44.9–57.5)	3,671,411	11.5 (10.1–12.3)	10,197	137 (60–238)	170 (53–317)	336 (185–522)

[1] n = 335,054. EPIC, European Prospective Investigation into Cancer and Nutrition.
[2] Estimated from 24-h recall data.

TABLE 2
Characteristics of female EPIC participants according to EPIC-wide quintiles of vegetable and fruit intake[1]

		Total vegetable intake		Total fruit intake	
	Total	Q1	Q5	Q1	Q5
n	335,054	67,010	67,010	67,011	67,011
Vegetable intake,[2] g/d	185 (118–286)[3]	77 (57–92)	402 (352–489)	127 (83–195)	262 (170–384)
Fruit intake,[2] g/d	209 (120–323)	130 (72–230)	303 (200–429)	63 (36–86)	460 (399–565)
Follow-up, y	11.5 (10.1–12.3)	11.3 (10.0–12.7)	11.6 (10.0–12.0)	11.1 (10.0–12.2)	11.7 (10.2–12.5)
Continuous covariates					
Age at recruitment, y	50.8 ± 9.8[4]	50.3 ± 9.5	51.2 ± 10.5	49.0 ± 9.6	51.7 ± 9.9
Age at menarche,[5] y	13.1 ± 1.5	13.2 ± 1.5	12.9 ± 1.6	13.2 ± 1.5	13.0 ± 1.6
Age at first full-term pregnancy,[6] y	24.8 ± 4.3	24.4 ± 4.4	24.9 ± 4.3	24.4 ± 4.4	25.0 ± 4.3
BMI, kg/m^2	25.0 ± 4.4	25.0 ± 4.4	25.3 ± 4.7	24.7 ± 4.4	25.5 ± 4.6
Alcohol use, g/d	3.3 (0.5–10.7)	2.2 (0.4–8.2)	3.3 (0.5–10.6)	3.8 (0.8–11.9)	2.2 (0.3–9.2)
Daily energy from fat, kcal/d	684 ± 242	586 ± 206	797 ± 271	635 ± 227	737 ± 267
Daily energy from nonfat, kcal/d	1247 ± 351	1114 ± 313	1333 ± 379	1102 ± 317	1402 ± 373
Saturated fat, g/d	29.4 ± 11.9	26.9 ± 10.8	31.0 ± 13.0	28.5 ± 11.6	29.6 ± 12.5
Categorical covariates[7]					
Parity, %					
Nulliparous	15.1	14.8	15.3	16.2	15.3
Parous	84.9	85.2	84.7	83.8	84.7
OAC use, %					
Never	42.2	39.4	50.4	32.8	52.8
Past	52.6	54.3	46.2	60.2	43.6
Current	5.2	6.3	3.4	7.0	3.6
Menopausal status, %					
Pre	34.8	35.2	35.7	40.2	33.2
Peri	18.9	19.3	17.4	20.2	16.2
Post	46.3	45.6	47.0	39.6	50.6
HRT use, %					
Never	74.7	74.3	77.3	73.3	77.5
Past	7.7	7.1	7.4	6.8	8.3
Current	17.6	18.6	15.3	19.9	14.2
Physical activity, %					
Inactive	24.3	24.2	27.2	23.0	28.6
Moderately inactive	35.8	36.4	34.5	36.9	34.1
Moderately active	24.2	22.6	25.0	24.3	22.2
Active	15.8	16.8	13.4	15.8	15.1
Educational level, %					
None	4.7	3.1	8.0	2.6	8.3
Primary school	24.8	31.7	21.7	25.1	28.2
Technical school	22.4	29.5	12.6	29.5	15.4
Secondary school	24.5	19.7	28.3	22.0	24.9
Longer education	23.6	16.0	29.5	20.7	23.2
Alcohol user, %					
Yes	83.6	82.1	81.9	86.0	78.0
No	16.4	17.9	18.1	14.0	22.0
Smoking status, %					
Never	49.8	46.2	53.7	40.8	56.2
Past	22.4	21.6	20.9	22.0	20.9
Current	19.3	27.4	12.9	31.5	13.5
Pipe or occasional[8]	8.5	4.8	12.5	5.7	9.4
Incident breast cancer cases					
Total *n*	10,197	2091	1856	2003	1960
ER+PR+, %	34.1	34.2	34.8	36.3	34.5
ER+PR−, %	10.5	10.0	10.1	10.4	10.6
ER−PR+, %	2.1	2.2	2.4	1.6	2.1
ER−PR−, %	10.0	11.7	9.1	10.3	9.5
Missing receptor status, %	43.3	1.9	43.6	41.4	43.4

[1]EPIC, European Prospective Investigation into Cancer and Nutrition; ER, estrogen receptor; HRT, hormone replacement therapy; OAC, oral contraceptive; PR, progesterone receptor; Q, quintile; +, positive; −, negative.
[2]Median intakes were estimated from food-frequency questionnaire data.
[3]Median; IQR in parentheses (all such values).
[4]Mean ± SD (all such values).
[5]Including only menstruating women.
[6]Including only parous women.
[7]Missing values were not included in the distribution (range of missing values: 0–11.6%).
[8]Including pipe or cigar smokers and occasional smokers.

TABLE 3
HRs (95% CIs) for total breast cancer in relation to vegetable and fruit intake among female EPIC participants[1]

	EPIC-wide quintiles						Continuous[2]	
	1	2	3	4	5	*P*-trend	Observed	Calibrated
Total vegetables								
Intake, g/d	77 (57–92)[3]	130 (118–143)	185 (170–201)	261 (239–286)	402 (352–489)			
Person-years	739,446	737,453	737,109	735,160	722,242			
Cases, *n* (*n* per 1000 person-years)	2091 (2.83)	1994 (2.70)	2162 (2.93)	2094 (2.85)	1856 (2.57)			
HR (95% CI)[4]	1.00 (reference)	0.93 (0.87, 0.99)	0.98 (0.92, 1.04)	0.94 (0.88, 1.01)	0.90 (0.83, 0.97)	0.01	0.98 (0.96, 1.00)	0.94 (0.89, 1.00)
HR (95% CI)[5]	1.00 (reference)	0.91 (0.86, 0.97)	0.96 (0.90, 1.02)	0.92 (0.85, 0.98)	0.87 (0.80, 0.94)	<0.01	0.97 (0.96, 0.99)	0.96 (0.91, 1.00)
HR (95% CI)[6]	1.00 (reference)	0.91 (0.86, 0.97)	0.95 (0.89, 1.02)	0.91 (0.85, 0.98)	0.86 (0.80, 0.94)	<0.01	0.97 (0.95, 0.99)	0.95 (0.91, 1.00)
Total fruit								
Intake, g/d	63 (36–86)	136 (120–153)	209 (189–230)	296 (269–323)	460 (399–565)			
Person-years	722,137	732,187	735,987	739,779	741,321			
Cases, *n* (*n* per 1000 person-years)	2003 (2.77)	2104 (2.87)	2036 (2.77)	2094 (2.83)	1960 (2.64)			
HR (95% CI)[4]	1.00 (reference)	1.00 (0.94, 1.06)	0.94 (0.88, 1.00)	0.98 (0.92, 1.04)	0.97 (0.91, 1.03)	0.31	1.00 (0.98, 1.01)	0.99 (0.96, 1.02)
HR (95% CI)[5]	1.00 (reference)	1.01 (0.95, 1.08)	0.95 (0.89, 1.02)	0.99 (0.93, 1.06)	0.99 (0.93, 1.07)	0.86	1.00 (0.99, 1.02)	1.00 (0.97, 1.03)
HR (95% CI)[7]	1.00 (reference)	1.01 (0.95, 1.08)	0.96 (0.90, 1.02)	1.00 (0.94, 1.07)	1.01 (0.94, 1.09)	0.70	1.01 (0.99, 1.02)	1.01 (0.98, 1.04)
Total vegetables and fruit								
Intake, g/d	182 (139–215)	301 (274–330)	414 (386–445)	549 (511–593)	798 (710–946)			
Person-years	728,288	735,104	738,787	737,100	732,132			
Cases, *n* (*n* per 1000 person-years)	2064 (2.83)	2062 (2.81)	2111 (2.86)	2086 (2.83)	1874 (2.56)			
HR (95% CI)[4]	1.00 (reference)	0.94 (0.88, 1.00)	0.93 (0.87, 0.99)	0.92 (0.86, 0.98)	0.90 (0.84, 0.97)	0.01	0.99 (0.97, 1.00)	0.97 (0.93, 1.01)
HR (95% CI)[5]	1.00 (reference)	0.93 (0.87, 0.99)	0.93 (0.87, 0.99)	0.91 (0.85, 0.98)	0.90 (0.83, 0.97)	0.02	0.99 (0.97, 1.01)	0.98 (0.94, 1.02)

[1]Values were estimated by using Cox proportional hazards models; $n = 335{,}054$. Total breast cancer, $n = 10{,}197$. EPIC, European Prospective Investigation into Cancer and Nutrition.

[2]The increment is 100 g/d for the separate vegetable and fruit analyses and 200 g/d for vegetables and fruit combined.

[3]Median; IQR in parentheses (all such values). Median intakes were estimated from food-frequency questionnaire data.

[4]Stratified by age and center.

[5]Stratified by age and center and adjusted for energy intake (kcal/d, continuous) divided into energy from fat and energy from nonfat sources, saturated fat intake (g/d, continuous), age at menarche (never; <12, 12–14, or >14 y), oral contraceptive use (never, past, or current), age at first full-term pregnancy (nulliparous; ≤20, >20 and ≤25, >25 and ≤30, or >30 y), menopausal status (premenopausal, perimenopausal/unknown, or postmenopausal), hormone replacement therapy use (never, past, or current), BMI (kg/m^2, continuous), BMI × menopausal status, physical activity (inactive, moderately inactive, moderately active, or active), smoking status and intensity (never; former: quit >20 y ago, quit 11–20 y ago, or quit ≤10 y ago; current: pipe/cigar smoking, 1–15 cigarettes/d, 16–25 cigarettes/d, or ≥26 cigarettes/d), alcohol user (yes or no), alcohol consumption (g/d, continuous), educational level (none, primary school, technical/professional school, secondary school, or university degree).

[6]Adjusted as in footnote 5 and also adjusted for fruit intake (g/d, continuous).

[7]Adjusted as in footnote 5 and also adjusted for vegetable intake (g/d, continuous).

TABLE 4
HRs (95% CIs) for hormone receptor–defined breast cancer in relation to vegetable and fruit intake among female EPIC participants[1]

	EPIC-wide quintiles						Continuous[2]	
	1	2	3	4	5	*P*-trend	Observed	Calibrated
ER+PR+ (*n* = 3479)								
Total vegetables								
Cases, *n* (*n* per 1000 person-years)	715 (0.97)	701 (0.95)	726 (0.98)	691 (0.94)	646 (0.89)			
HR (95% CI)[3]	1.00 (reference)	0.98 (0.88, 1.09)	1.01 (0.90, 1.12)	0.93 (0.83, 1.05)	0.94 (0.83, 1.06)	0.22	0.99 (0.96, 1.02)	0.96 (0.87, 1.06)
HR (95% CI)[4]	1.00 (reference)	0.97 (0.87, 1.08)	0.99 (0.89, 1.11)	0.92 (0.81, 1.04)	0.91 (0.79, 1.05)	0.14	0.98 (0.95, 1.02)	1.01 (0.92, 1.12)
HR (95% CI)[5]	1.00 (reference)	0.97 (0.87, 1.08)	0.99 (0.88, 1.11)	0.92 (0.81, 1.04)	0.90 (0.79, 1.04)	0.13	0.98 (0.95, 1.02)	1.02 (0.92, 1.13)
Total fruit								
Cases, *n* (*n* per 1000 person-years)	726 (1.01)	703 (0.96)	671 (0.91)	703 (0.95)	676 (0.91)			
HR (95% CI)[3]	1.00 (reference)	0.97 (0.87, 1.08)	0.90 (0.80, 1.00)	0.91 (0.81, 1.01)	0.92 (0.82, 1.03)	0.15	0.99 (0.97, 1.01)	0.97 (0.92, 1.02)
HR (95% CI)[4]	1.00 (reference)	0.99 (0.89, 1.10)	0.91 (0.82, 1.02)	0.94 (0.84, 1.05)	0.97 (0.86, 1.09)	0.57	1.00 (0.98, 1.03)	0.98 (0.94, 1.03)
HR (95% CI)[6]	1.00 (reference)	0.99 (0.89, 1.10)	0.92 (0.82, 1.03)	0.94 (0.84, 1.06)	0.98 (0.86, 1.10)	0.70	1.00 (0.98, 1.03)	0.99 (0.93, 1.04)
Total vegetables and fruit								
Cases, *n* (*n* per 1000 person-years)	733 (1.01)	715 (0.97)	674 (0.91)	714 (0.97)	643 (0.88)			
HR (95% CI)[3]	1.00 (reference)	0.95 (0.86, 1.06)	0.85 (0.76, 0.95)	0.87 (0.78, 0.98)	0.86 (0.76, 0.97)	0.01	0.99 (0.96, 1.02)	0.95 (0.88, 1.02)
HR (95% CI)[4]	1.00 (reference)	0.95 (0.86, 1.06)	0.86 (0.76, 0.96)	0.88 (0.78, 0.99)	0.86 (0.76, 0.99)	0.04	0.99 (0.96, 1.03)	0.98 (0.91, 1.06)
ER+PR− (*n* = 1075)								
Total vegetables								
Cases, *n* (*n* per 1000 person-years)	210 (0.28)	198 (0.27)	248 (0.34)	232 (0.32)	187 (0.26)			
HR (95% CI)[3]	1.00 (reference)	0.96 (0.79, 1.17)	1.13 (0.93, 1.38)	0.97 (0.79, 1.20)	0.82 (0.65, 1.02)	0.04	0.94 (0.89, 0.99)	0.83 (0.70, 1.00)
HR (95% CI)[4]	1.00 (reference)	0.94 (0.77, 1.15)	1.10 (0.90, 1.35)	0.95 (0.76, 1.18)	0.80 (0.62, 1.03)	0.05	0.93 (0.87, 0.99)	0.89 (0.75, 1.05)
HR (95% CI)[5]	1.00 (reference)	0.95 (0.77, 1.15)	1.11 (0.91, 1.36)	0.96 (0.77, 1.19)	0.81 (0.63, 1.05)	0.07	0.93 (0.87, 0.99)	0.89 (0.75, 1.06)
Total fruit								
Cases, *n* (*n* per 1000 person-years)	209 (0.29)	213 (0.29)	210 (0.29)	236 (0.32)	207 (0.28)			
HR (95% CI)[3]	1.00 (reference)	0.96 (0.79, 1.16)	0.88 (0.72, 1.07)	0.91 (0.75, 1.11)	0.86 (0.70, 1.06)	0.17	0.97 (0.94, 1.01)	0.96 (0.88, 1.04)
HR (95% CI)[4]	1.00 (reference)	0.95 (0.78, 1.16)	0.88 (0.72, 1.07)	0.92 (0.75, 1.12)	0.87 (0.70, 1.09)	0.26	0.98 (0.94, 1.02)	0.99 (0.91, 1.08)
HR (95% CI)[6]	1.00 (reference)	0.96 (0.79, 1.17)	0.89 (0.73, 1.09)	0.94 (0.77, 1.16)	0.91 (0.73, 1.14)	0.50	0.99 (0.95, 1.03)	1.01 (0.92, 1.11)
Total vegetables and fruit								
Cases, *n* (*n* per 1000 person-years)	195 (0.27)	223 (0.30)	224 (0.30)	246 (0.33)	187 (0.26)			
HR (95% CI)[3]	1.00 (reference)	1.04 (0.86, 1.27)	0.94 (0.77, 1.15)	0.97 (0.79, 1.20)	0.80 (0.64, 1.01)	0.03	0.94 (0.89, 0.99)	0.89 (0.78, 1.02)
HR (95% CI)[4]	1.00 (reference)	1.02 (0.84, 1.24)	0.92 (0.75, 1.13)	0.96 (0.77, 1.19)	0.80 (0.62, 1.02)	0.04	0.94 (0.88, 1.00)	0.94 (0.82, 1.08)
ER−PR− (*n* = 1021)								
Total vegetables								
Cases (*n* per 1000 person-years)	245 (0.33)	201 (0.27)	205 (0.28)	201 (0.27)	169 (0.23)			
HR (95% CI)[3]	1.00 (reference)	0.91 (0.75, 1.10)	0.93 (0.76, 1.13)	0.89 (0.72, 1.09)	0.79 (0.63, 0.99)	0.05	0.94 (0.88, 0.99)	0.84 (0.70, 1.00)
HR (95% CI)[4]	1.00 (reference)	0.88 (0.73, 1.07)	0.88 (0.72, 1.08)	0.84 (0.67, 1.04)	0.74 (0.57, 0.96)	0.03	0.92 (0.86, 0.98)	0.82 (0.68, 0.98)
HR (95% CI)[5]	1.00 (reference)	0.88 (0.73, 1.07)	0.89 (0.73, 1.09)	0.85 (0.68, 1.06)	0.76 (0.58, 0.98)	0.05	0.92 (0.86, 0.99)	0.83 (0.69, 0.99)
Total fruit								
Cases, *n* (*n* per 1000 person-years)	206 (0.29)	221 (0.30)	192 (0.26)	216 (0.29)	186 (0.25)			
HR (95% CI)[3]	1.00 (reference)	1.10 (0.90, 1.33)	0.93 (0.76, 1.14)	1.03 (0.84, 1.26)	0.94 (0.76, 1.16)	0.39	0.99 (0.95, 1.03)	0.97 (0.89, 1.06)
HR (95% CI)[4]	1.00 (reference)	1.08 (0.89, 1.31)	0.90 (0.73, 1.11)	0.98 (0.80, 1.21)	0.87 (0.70, 1.10)	0.16	0.97 (0.93, 1.02)	0.94 (0.85, 1.02)
HR (95% CI)[6]	1.00 (reference)	1.09 (0.90, 1.32)	0.92 (0.75, 1.13)	1.02 (0.83, 1.26)	0.92 (0.73, 1.16)	0.35	0.98 (0.94, 1.03)	0.95 (0.86, 1.04)
Total vegetables and fruit								
Cases, *n* (*n* per 1000 person-years)	227 (0.31)	217 (0.30)	202 (0.27)	206 (0.28)	169 (0.23)			

(Continued)

TABLE 4 (*Continued*)

	EPIC-wide quintiles						Continuous[2]	
	1	2	3	4	5	*P*-trend	Observed	Calibrated
HR (95% CI)[3]	1.00 (reference)	0.97 (0.80, 1.17)	0.88 (0.72, 1.07)	0.87 (0.71, 1.07)	0.78 (0.62, 0.97)	0.02	0.95 (0.90, 1.01)	0.90 (0.79, 1.03)
HR (95% CI)[4]	1.00 (reference)	0.93 (0.77, 1.13)	0.82 (0.67, 1.01)	0.80 (0.65, 1.00)	0.70 (0.54, 0.89)	<0.01	0.93 (0.87, 0.99)	0.85 (0.73, 0.98)
P-heterogeneity by hormone receptor status (ER+PR+ vs. ER−PR−)	0.09							

[1] n = 335,054. Values were estimated by using Cox proportional hazards models. EPIC, European Prospective Investigation into Cancer and Nutrition; ER, estrogen receptor; PR, progesterone receptor; +, positive; −, negative.

[2] The increment is 100 g/d for the separate vegetable and fruit analyses and 200 g/d for vegetables and fruit combined.

[3] Stratified by age and center.

[4] Stratified by age and center and adjusted for energy intake (kcal/d, continuous) divided into energy from fat and energy from nonfat sources, saturated fat intake (g/d, continuous), age at menarche (never; <12, 12–14, or >14 y), oral contraceptive use (never, past, or current), age at first full-term pregnancy (nulliparous; ≤20, >20 and ≤25, >25 and ≤30, or >30 y), menopausal status (premenopausal, perimenopausal/unknown, or postmenopausal), hormone replacement therapy use (never, past, or current), BMI (kg/m^2, continuous), BMI × menopausal status, physical activity (inactive, moderately inactive, moderately active, or active), smoking status and intensity (never; former: quit >20 y ago, quit 11–20 y ago, or quit ≤10 y ago; current: pipe/cigar smoking, 1–15 cigarettes/d, 16–25 cigarettes/d, or ≥26 cigarettes/d), alcohol user (yes or no), alcohol consumption (g/d, continuous), educational level (none, primary school, technical/professional school, secondary school, or university degree).

[5] Adjusted as in footnote 4 and additionally adjusted for fruit intake (g/d, continuous).

[6] Adjusted as in footnote 4 and additionally adjusted for vegetable intake (g/d, continuous).

Restricted cubic spline analyses were performed for vegetable and fruit intake and ER−PR− breast cancer risk. The lowest Akaike's information criterions were observed for the cubic spline models with 3 knots (**Supplemental Figures 1** and **2**).

The analysis that examined vegetable and fruit subgroups showed that fruiting, leafy, and root vegetables made the largest contribution to total vegetable consumption, whereas apples and pears, citrus fruit, and stone fruit made the largest contribution to the total fruit consumption (**Supplemental Figures 3** and **4**). The inverse association between high vegetable intake and ER−PR− breast cancer risk cannot be attributed to a particular vegetable subgroup (**Supplemental Table 1**). Similar to total fruit intake, subgroups of fruit were not associated with ER−PR− breast cancer risk (**Supplemental Table 2**). The country-specific results indicated no heterogeneity in the association between vegetable intake and ER−PR− breast cancer risk by country (*P*-heterogeneity = 0.52; **Supplemental Figure 5**).

We found no evidence that menopausal status at recruitment modified the associations between vegetables, fruit, and vegetables and fruit combined and breast cancer risk (*P*-interaction ≥ 0.43). The results of the sensitivity analyses did not alter our conclusions (data not shown).

DISCUSSION

Within this European-wide prospective cohort study with >10,000 incident breast cancer cases observed, we investigated the association between vegetable and fruit consumption and breast cancer risk. Both exposure and outcome were assessed according to standardized procedures. Our study results support evidence that high vegetable intake is associated with lower (mainly hormone receptor–negative) breast cancer risk.

We analyzed the data using isoenergetic models, which meant that an increase in vegetable and/or fruit intake had to be accompanied by a reduction in intake of other foods to obtain energy balance (i.e., isocaloric comparisons). We did not specify these food groups, but they had to provide the same amount of energy from fat and nonfat as vegetables and fruit.

Almost 10 y ago we reported that there was no association between vegetable ($HR_{quintile\ 5–quintile\ 1}$: 0.98; 95% CI: 0.84, 1.14) or fruit ($HR_{quintile\ 5–quintile\ 1}$: 1.09; 95% CI: 0.94, 1.25) consumption and total breast cancer risk (8). At that time, only 3659 incident cases were included and receptor status data were not available. We conducted the current analysis with Greece and Norway included and longer follow-up, and therefore larger power, to discover potential small effects.

Country-specific results showed that the inverse association between high vegetable consumption and ER−PR− breast cancer risk was observed in 8 of 10 countries (with the exceptions of Germany and The Netherlands), although this was not significant in most countries due to the small sample size within each subgroup (i.e., country). The test for heterogeneity by country was not significant (*P*-heterogeneity = 0.52). On the basis of these results, together with the fact that all of the models were stratified by study center, it seems unlikely that the association between vegetable consumption and ER−PR− breast cancer risk is caused by regional differences in breast cancer incidence.

Our study is in agreement with the findings of the Pooling Project (15). For ER− breast cancer, the pooled RR comparing

the highest with the lowest quintile of vegetable consumption was 0.82 (95% CI: 0.74, 0.90), whereas a nonsignificant inverse association was found for fruit consumption ($RR_{quintile\ 5–quintile\ 1}$: 0.94; 95% CI: 0.85, 1.04) (15). A large number of women (n = 993,466) and a large number of ER− breast cancer cases (n = 4821) were analyzed in this pooled project, with the disadvantage that there is a lack of standardized measurements of exposure and outcome in the design phase of included studies (8, 15). However, to minimize this influence, the between-study variation in exposure and outcome measurements was taken into account in the analysis (15).

In our study as well as in the Pooling Project (15) a protective association between vegetable intake only (not fruit) and hormone receptor–negative (not hormone receptor–positive) breast cancer was observed. An explanation could be that the protective effect of vegetables is easier to detect in relation to hormone receptor–negative breast cancer than in relation to hormone receptor–positive breast cancer, in which this effect may be negligible compared with that of hormonal risk factors (35). Therefore, a weak inverse association between high vegetable intake and hormone receptor–positive breast cancer risk cannot be excluded. In a Japanese cohort study, a possible inverse association between the intake of cruciferous vegetables and the development of ER+PR+ breast cancer was observed (36).

We observed that vegetables and fruit were both inversely associated with ER−PR− breast cancer risk, but the association for vegetables was stronger. Therefore, it might be that although vegetables and fruit are both rich in various bioactive compounds, antioxidants and fiber from vegetables and from fruit are not equally effective (15, 37). We observed in the EPIC cohort that only fiber from vegetables, and not fruit, were associated with a lower ER−PR− breast cancer risk ($HR_{quintile\ 5–quintile\ 1}$: 0.74; 95% CI: 0.59, 0.93) (37). However, note that in a Swedish prospective cohort study the strongest protective effects were observed for fiber from fruit (38).

Because vegetables and fruit are heterogeneous groups of foods each with their own micronutrients, subgroups were also investigated. Most of the subgroups showed a nonsignificant protective effect among women with the highest intakes, indicating that the protective effect cannot be attributed to a specific subgroup. The Pooling Project had more detailed subgroup information available (i.e., at the food item level) that included information on intakes of cruciferous vegetables—for example, broccoli. They found (non)significant protective associations for most of the subgroups, including broccoli, and concluded that there was a beneficial effect of overall vegetable intake rather than an effect of certain subgroups (15).

Observational studies that investigate risk factors that are highly correlated to lifestyle behavior are prone to residual confounding because a high vegetable and fruit intake is often accompanied by a health-conscious lifestyle (39). In our study we observed, for example, that never smokers consumed more vegetables and fruit. Although we cannot rule out residual confounding, we expect it to be limited, because we were able to adjust for many (lifestyle) risk factors. In addition, we performed a sensitivity analysis restricted to never smokers and the effect estimates did not change.

A limitation of our study is the single exposure measurement (i.e., at recruitment). This may have caused misclassification, which is expected to be nondifferential (i.e., not related to breast cancer occurrence), because it is unlikely that women changed their dietary pattern due to preclinical disease, which was confirmed in a sensitivity analysis excluding women with a follow-up of <2 y. In addition, risk factor information was only available at recruitment. It might be that these risk factors influence breast cancer risk mainly in adult life or have cumulative effects that lead to residual confounding. Another limitation is the lack of data on molecular breast cancer subtypes (40, 41). Molecular subtypes may further refine the association between vegetable and fruit intake and subtype-defined breast cancer risk.

A major strength of our study is that it includes countries ranging from the north to the south of Europe, resulting in a large variety in vegetable and fruit consumption. Furthermore, we assessed exposure, outcome, and confounders using standardized procedures. In addition, calibration analyses were performed to enhance the validity of the measurements.

In conclusion, our study was conducted within the single largest prospective European cohort and adds substantial evidence to the findings of the large Pooling Project, which shows that high vegetable intake is associated with a 20–25% lower risk of hormone receptor–negative breast cancer.

The authors' responsibilities were as follows—MJE, PHMP, MFB, and CHvG: designed the research, analyzed the data, performed the statistical analysis, and wrote the manuscript; PHMP, KO, A Tjønneland, IR, RK, HB, A Trichopoulou, S Panico, RT, EL, JRQ, M-JS, MD, HBB-d-M, K-TK, TJK, and ER: collected the data; and MJE, PHMP, MFB, KO, A Tjønneland, AO, IR, PF, LD, MCB-R, LB, RTF, RK, HB, A Trichopoulou, PL, DT, GM, VP, S Panico, RT, S Polidoro, GS, EL, EW, JRQ, NT, M-JS, M-DC, EA, MD, AW, MW, HBB-d-M, K-TK, RCT, TJK, DA, MG, ER, and CHvG: revised the manuscript. All of the authors read and approved the final manuscript. None of the authors had a conflict of interest.

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