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EPIDEMIOLOGY

Dietary flavonoid and lignan intake and breast cancer risk according to menopause and hormone receptor status in the European Prospective Investigation into Cancer and Nutrition (EPIC) Study

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Abstract Evidence on the association between dietary flavonoids and lignans and breast cancer (BC) risk is inconclusive, with the possible exception of isoflavones in Asian countries. Therefore, we investigated prospectively dietary total and subclasses of flavonoid and lignan intake and BC risk according to menopause and hormonal receptor status in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. The study included 334,850 women, mostly aged between 35 and 70 years from ten European countries. At baseline, country-specific validated dietary questionnaires were used. A flavonoid and lignan food composition database was developed from the US

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Department of Agriculture, the Phenol-Explorer and the UK Food Standards Agency databases. Cox regression models were used to analyse the association between dietary flavonoid/lignan intake and the risk of developing BC. During an average 11.5-year follow-up, 11,576 incident BC cases were identified. No association was observed between the intake of total flavonoids [hazard ratio comparing fifth to first quintile ($HR_{Q5–Q1}$) 0.97, 95 % confidence interval (CI): 0.90–1.04; P trend = 0.591], isoflavones ($HR_{Q5–Q1}$ 1.00, 95 % CI: 0.91–1.10; P trend = 0.734), or total lignans ($HR_{Q5–Q1}$ 1.02, 95 % CI: 0.93–1.11; P trend = 0.469) and overall BC risk. The stratification of the results by menopausal status at recruitment or the differentiation of BC cases according to oestrogen and progesterone receptors did not affect the results. This study shows no associations between flavonoid and lignan intake and BC risk, overall or after taking into account menopausal status and BC hormone receptors.

Keywords Flavonoids · Lignans · Breast cancer · Hormone receptors · EPIC

List of Abbreviations

BC	Breast cancer
EPIC	European prospective investigation into cancer and nutrition
ER	Oestrogen receptor
PR	Progesterone receptor

Introduction

Breast cancer (BC) is a complex and heterogeneous disease, with oestrogen receptor (ER) and progesterone receptor (PR) status being one of the markers for breast tumour classification [1]. Differences have been observed in the aetiology, treatment and prognosis of hormone receptor status-positive and -negative BC [2, 3]. Because of the importance of menopause as an effect modifier, studies should stratify for menopause status [1].

Polyphenols are secondary plant metabolites widely spread throughout the plant kingdom [4]. They are usually divided into five classes: flavonoids (anthocyanidins, flavonols, flavanones, flavones, flavanols and isoflavones), phenolic acids, stilbenes, lignans and other polyphenols. Flavonoids have many biological effects that may play a role in BC prevention, including a reduction of reactive oxygen species production, antimutagenic and antiproliferative properties, regulation of cell signalling and cell cycle, and inhibition of angiogenesis [5, 6]. In addition, phyto-oestrogens, such as isoflavones and lignans, have a weak oestrogen-like activity; therefore, phyto-oestrogens could interact with ERs in the development of BC [7, 8].

Previous case–control studies have shown that the intake of some subclasses of flavonoids, especially flavones and flavonols, was associated with a reduced risk of BC [9]. However, evidence from prospective cohort studies remains controversial [10–15]. A recent meta-analysis [16] on the role of isoflavones on BC risk suggested a

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significantly inverse association in certain Asian countries, particularly in post-menopausal women, in whom soy intake is notably high [17]. To date, no association has been observed in Western countries [16]. With respect to lignans, the evidence is abundant but inconclusive [18–20]. The French postmenopausal European Prospective Investigation into Cancer and Nutrition (EPIC) cohort showed a significant protective association of dietary lignan intake which was limited to ER- and PR-positive tumours [21]. Indeed, in one of the Swedish EPIC cohorts, the plasma enterolactone concentration, a lignan intake biomarker, was inversely associated with BC risk in ERα positive, particularly when ERβ is negative [22]. However, in the Danish EPIC cohort, a significant inverse association was only observed between plasma enterolactone concentrations and ER-negative tumours [23], whereas no significant associations were reported between dietary, urinary and serum levels of both lignans and isoflavones in the Norfolk EPIC Study [24]. This inconsistency might be due to the limited number of cases by BC subtypes, or low levels and/or low variability of dietary intake. Therefore, larger epidemiological studies are needed to investigate the potential protective association of flavonoid and lignan intake as well as a possible modification of this effect by menopausal or hormone receptor status.

The aim of the current study was to evaluate the association of dietary intake of flavonoids and lignans on the risk of BC, by menopause and hormone receptor status, within the EPIC Study [25], a large prospective cohort with considerable variability in flavonoid and lignan intake amongst participants [26, 27].

Materials and methods

Subjects and study design

EPIC is a multicentre prospective cohort study primarily designed to investigate the relation between diet, lifestyle and environmental factors and cancer. All participants were enroled between the years 1992 and 2000 from 23 centres in ten European countries: Denmark, France, Germany, Greece, Italy, The Netherlands, Norway, Spain, Sweden and the United Kingdom. Participants were mainly recruited from the general population with some exceptions: Turin and Ragusa (Italy) and Spain recruited mostly blood donors, France recruited mostly teachers, Oxford (United Kingdom) recruited a high proportion of health-conscious individuals and Utrecht (The Netherlands) and Florence (Italy) recruited women attending mammographic screening programmes. The rationale and study design of the EPIC Study have been published elsewhere [25, 28]. Approval for this study was obtained from the ethical review boards of the International Agency for Research on Cancer (IARC) and from the local ethics committees in participating countries. All cohort members provided written informed consent.

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EPIC recruited 367,903 women, mostly aged between 35 and 70 years. Women with prevalent cancer diagnosis at baseline ($n = 19{,}853$), missing diagnosis or censoring date ($n = 2{,}892$), missing dietary or lifestyle information ($n = 3{,}339$), or in the top and bottom 1 % of the ratio of reported total energy intake to estimated energy requirement ($n = 6{,}752$) were excluded. In addition, 217 non-first BC cases were censored, leaving 334,850 women with complete exposure information for the current analysis.

Dietary assessment and data collection

Habitual diet over the previous 12 months was measured by country-specific validated questionnaires [28]. Most centres used self-administered questionnaires, whereas in Greece, Spain and Ragusa (Italy), a face to face interview was performed. Questionnaires in most of the centres were quantitative, estimating portion sizes systematically. In Denmark, Norway, Umeå (Sweden) and Naples (Italy), semiquantitative food-frequency questionnaires were administered. In Malmö (Sweden), a modified diet history method was used, combining a 7-day diet record, a semi-quantitative questionnaire and 1-h dietary interview. Daily food intake was calculated in g/day. Ethanol (g/day), total dietary fibre (g/day) and total energy (kcal/day) intake was computed using the EPIC Nutrient Database [29]. A separate lifestyle questionnaire gathered information on socio-demographic characteristics, lifetime smoking and alcohol consumption, physical activity, education and medical history [25]. In addition, anthropometric measures were obtained at recruitment [30]. Body mass index was calculated as weight (kg) per height (m) squared.

Identification and follow-up of BC cases

In most countries (Denmark, Italy, The Netherlands, Norway, Spain, Sweden and United Kingdom), incident BC cases were identified through a linkage with population-based cancer registries. In Greece, Germany, Naples (Italy) and France, active follow-up of cancer was using health insurance records, cancer and pathology registries, and direct contact with participants or their next of kin. In all EPIC centres, cancer diagnosis was confirmed by review of pathology reports. Vital status was collected from regional or national mortality registries. Subjects were followed up from study entry and until cancer diagnosis (except for nonmelanoma skin cancer), death, emigration or until the end of the follow-up period, whichever occurred first. The follow-up periods ended at the following times: December 2004 Asturias (Spain), December 2006 [Florence, Varese and Ragusa (Italy); and Granada and San Sebastian (Spain)], December 2007 [Murcia and Navarra (Spain), Oxford (United Kingdom), Bilthoven and Utrecht (The Netherlands), and Denmark], June 2008 Cambridge (United Kingdom), December 2008 [Turin (Italy), Malmö and Umea (Sweden), and Norway]. For study centres with active follow-up, the end of follow-up was considered to be the last known contact with study participants: December 2006 for France and Naples (Italy), December 2008 for Potsdam (Germany), December 2009 for Greece and June 2010 for Heidelberg (Germany). We used the Tenth Revision International Classification of Diseases, Injury and Causes of Death (ICD-10), and invasive BC was defined as C50.0–50.9. Information on ER and PR status was provided by each centre on the basis of pathology reports. To standardize the quantification of receptor status, the following criteria for a positive receptor status were applied: $\geq$% cells stained, any 'plus-system' description, $\geq$20 fmol/mg, an Allred score of $\geq$3, an immunoreactive score (IRS) $\geq$2, or an H-score $\geq$10 [31].

Flavonoid and lignan intake

Dietary flavonoid and lignan intake was estimated by matching food items on the country-specific dietary questionnaires with a comprehensive food composition database (FCDB) on flavonoids and lignans based on the US Department Agriculture FCDBs [32–34], Phenol-Explorer [35] and the UK Food Standards Agency FCDB [24]. Furthermore, our FCDB was expanded using retention factors, calculating flavonoid content of recipes, estimating missing values based on similar foods (by species and plant part), obtaining consumption data for food group items and employing botanical data for logical zeros. Data on flavonoids and lignans are expressed as aglycones' equivalents, after conversion of the flavonoid glycosides into aglycone contents using their respective molecular weights. Our FCDB contains composition data on lignans (secoisolariciresinol, matairesinol, lariciresinol, pinoresinol, enterolactone and enterodiol) and the six flavonoid subclasses: anthocyanidins, flavanols (including flavan-3-ols monomers, proanthocyanidins and theaflavins), flavonols, flavones, flavanones and isoflavones [26, 36–38]. The final FCDB contains 1,877 food items, including both raw and cooked foods, and recipes.

Statistical analysis

Flavonoid and lignan intake was assessed by the mean and its standard deviation (SD) as well as the median and the tenth and ninetieth centiles (P10th, P90th) since the data were skewed to the right. The association between dietary intake of flavonoids and lignans and the risk of developing BC was assessed by means of the hazards ratio (HR) and its 95 % confidence interval (CI) using Cox regression models. Tests and graphs based on Schoenfeld [39] residuals

were used to assess the proportional hazards' assumption. Age was the primary time variable and entry time was defined as age at enrolment and exit time as age at diagnosis (for cases) or censoring (for at-risk subjects). The Breslow method was adopted for handling ties [40]. All models were stratified by centre to control for differences in questionnaire design and follow-up procedures amongst centres and by age at baseline (1 year intervals). All models were also adjusted for menopausal status at recruitment [post-menopausal (including surgical) vs peri- or pre-menopausal, as defined in [41]], smoking status (never, former, current and unknown), educational level (none, primary school, technical/professional school, secondary school, university or higher and unknown), physical activity (inactive, moderately inactive, moderately active, active and unknown), age at menarche (<12, 12–14, >14 year, unknown), age at first full-term birth (nulliparous, <21, 21–30, >30 year), ever use of contraceptive pills (ever, never, unknown), ever use of hormones (ever, never, unknown) and age at menopause (≤50, >50 year). All models were also adjusted for the following continuous variables: height (cm), weight (kg), total energy (kcal/day), alcohol (g/day) and fibre (g/day) intake at baseline. The primary exposure of interest, that is, total flavonoids, total lignans and flavonoid subclasses (mg/day), were assessed as cohort-wide quintiles. In addition, tests for linear trend were performed by assigning the median of each quintile as scores. The continuous flavonoid variables (mg/day) were $\log_2$ transformed since they were not normally distributed. The natural logarithm is the most common transformation used to normalize right-skewed data; we used a $\log_2$ transformation because it produces the same normalizing effect, but the HR is more easily interpretable because it corresponds to the reduction of BC risk for doubling the intake. Flavonoid and lignan intake was also energy-adjusted using the residual method [42], but the results did not change substantially. The interactions between BMI status (<25; 25–30; >30 kg/m^2) or alcohol consumption (as tertiles) and total flavonoid intake were tested using likelihood ratio tests based on the models with and without the interaction terms. In addition, separate models were defined to assess the risk of BC by menopausal status (pre- and post-menopausal status) at the recruitment after the exclusion of women with a history of ovariectomy and unknown menopausal status. The associations were also evaluated according to ER and PR status, as well as for combinations of them. Sensitivity analyses were performed by excluding women who developed BC during the first 2 years of follow-up from the analysis. All p-values presented are two-tailed and were considered to be statistically significant when $P < 0.05$. All statistical analyses were conducted using SAS version 9.3 software (SAS Institute, Inc., NC).

Results

During a median follow-up time of 11.5 years (3,670,436 person-years), 11,576 incident BC cases were identified. The Table 1 shows the distribution of incident BC cases by country, menopausal and hormone receptor status. ER and PR status was available in only 63 and 52 % of cancer cases, respectively, and was distributed as follows: 80 % ER-positive (ER^+) and 20 % ER^- tumours, and 64 % PR^+ and 36 % PR^- tumours.

Women with the highest intake of total flavonoids were more likely to be older, taller and with a lower weight and BMI (Table 2). Moreover, these women used more oral contraceptives, had the highest educational level, the lowest tobacco consumption, tended to be more physically active and had a higher consumption of energy, alcohol and fibre than those in the bottom quintile of the total flavonoid intake. Table 3 shows the mean, median and percentiles 10 and 90 of the total and subclasses of flavonoid and lignan intake and their main food sources.

Total flavonoid intake was not associated with BC overall (hazard ratio comparing fifth to first quintile ($HR_{Q5–Q1}$) 0.97, 95 % CI: 0.90–1.04; P trend = 0.591) in pre-menopausal women ($HR_{Q5–Q1}$ 0.98, 95 % CI: 0.84–1.15; P trend = 0.656) or in post-menopausal women ($HR_{Q5–Q1}$ 0.96, 95 % CI: 0.86–1.06; P trend = 0.622) (Table 4). The results obtained for total lignan or flavonoid subclasses (including isoflavones) did not show any association either. For total flavonoid intake, no interaction was observed with BMI status (P for interaction 0.864) or alcohol consumption (P for interaction 0.674).

BC cases were classified according to oestrogen and PRs. Baseline characteristics and the intake of flavonoids and lignans of BC cases with and without hormone receptor status information were assessed. No major differences in demographic characteristics and nutritional intake were found between cases without and with available information on ER status, except that BC cases with missing information on PR status were more likely to be post-menopausal.

When cases were stratified by hormone receptor status, no significant association was found between any flavonoid and lignan intake and ER^-/PR^-, ER^+/PR^-, ER^-/PR^+, and ER^+/PR^+ BC incidence (Table 5), although an inverse trend, though not significant, was observed between doubling in the intake of total lignan (HR for $\log_2$ 0.88, 95 % CI: 0.76–1.01) and ER^-/PR^- tumours. In a sensitivity analysis, where 136 ER^-/PR^- BC cases diagnosed within the first 2 years of follow-up were removed, the inverse associations with lignan intake (HR for $\log_2$ 0.85, 95 % CI: 0.73–0.99) were slightly strengthened in comparison with the results based on the whole cohort. In the rest of sensitivity analysis excluding BC cases diagnosed within the

Table 1 Distribution of participants and breast cancer cases according to menopausal status or breast cancer phenotype in ten countries participating in the European Prospective Investigation into Cancer and Nutrition (EPIC) study

Country	All	PY	Breast cancer cases						
			All	Pre-menopausal[a]	Post-menopausal[a]	ER^-/PR^{-b}	ER^-/PR^{+b}	ER^+/PR^{-b}	ER^+/PR^{+b}
France	67,356	699,216	3,187	755	1,417	377	102	487	1,359
Italy	30,498	341,417	1,047	382	462	123	41	164	496
Spain	24,846	299,575	495	256	164	38	6	39	129
United Kingdom	52,513	586,165	1,480	440	787	53	4	36	174
The Netherlands	26,839	315,551	916	184	523	63	5	74	275
Greece	15,224	148,594	198	65	107	9	1	13	45
Germany	27,390	272,011	834	269	407	89	11	46	317
Sweden	26,339	349,110	1,095	122	655	84	25	57	128
Denmark	28,693	316,601	1,340	88	997	108	10	94	296
Norway	35,152	342,195	984	266	353	106	12	123	434
Total	334,850	3,670,436	11,576	2,827	5,872	1,050	217	1,133	3,653

PY person-years, *ER* oestrogen receptor, *PR* progesterone receptor

[a] Excluding peri-menopausal women 63,340 (18.9 %) and women with a bilateral ovariectomy 9,634 (2.9 %)

[b] Missing data for ER: 4,308 (37.2 %); for PR: 5,508 (47.6 %)

first 2 years of follow-up, the results were almost identical to the whole cohort.

Discussion

In this large prospective study including women from ten Western European countries with a large variation in flavonoid and lignan intake, we found no association between total flavonoid, total lignan and flavonoid subclass intake and overall, pre- and post-menopausal BC risk. The analyses differentiating BC cases according to oestrogen and PRs did not show any difference. To our knowledge, this is the largest study with information on hormone receptor status to date to explore this association.

Our results are in agreement with previous prospective studies [10–14], showing no association between the intake of total flavonoids and flavonoid subclasses (not considering isoflavones) and overall, pre- and post-menopausal BC risk. In a nested case–control study, plasma tea polyphenols, basically flavan-3-ol monomers, were not related to overall BC risk [43]. However, several case–control studies, which are susceptible to recall bias, showed inverse associations with flavones and flavonols and inconsistent results with flavan-3-ol monomers [9]. In a case–control study, stratification by hormone receptor status showed a reduced risk of BC for increasing flavonol and flavone intake in ER^+/PR^+ post-menopausal women; however, BC cases in other subtypes were too low for a meaningful conclusion [44]. No significant associations between BC risk by hormone receptor status and any flavonoid subclasses were observed in our study. A recent prospective study suggested that flavonoids were inversely associated with overall BC risk in non-to-low alcohol drinkers (<6.5 g alcohol/day) and were positively associated in moderate-to-heavy alcohol drinkers [45]. In our study, no significant interaction was observed with alcohol consumption.

For isoflavones, our findings suggest no association with BC risk (overall, by menopausal or hormone receptor status). Studies on BC risk and soy or isoflavones, measured using dietary questionnaires or plasma/urine biomarkers, have found no associations in Western countries [16] as in the previous data on the Dutch EPIC cohort [46] or even amongst the vegetarian participants in the EPIC Oxford (UK) Study [47]. However, in Asian countries, isoflavones were related to a lower BC incidence and recurrence, particularly in post-menopausal women [16, 48]. Menopausal status might be an important modifier of the effect of phyto-oestrogens on the risk for BC because mechanisms that mediate the effect could involve the ovarian synthesis of sex hormones or the alteration of other menstrual cycle characteristics [49]. However, in our study, we did not observe any association with BC risk in post-menopausal women, even in the double-positive receptor status tumours. The large difference in isoflavone intake between countries (<1 and >30 mg/day in Western and Asian countries, respectively) is the most likely explanation for these inconsistent results [17, 26]. In addition, the early exposure to phyto-oestrogens (during the childhood

Table 2 Baseline characteristics according to quintiles of total flavonoid intake in the European Prospective Investigation into Cancer and Nutrition (EPIC) study

	Quintiles of total flavonoids (mg/day)				
	Q1: <176	Q2: 176–275	Q3: 276–403	Q4: 404–654	Q5: >654
No of participants	66,970	66,970	66,970	66,970	66,970
Age (year)[a]	50.2 (8.7)	50.8 (9.4)	50.8 (9.4)	51.1 (9.9)	51.1 (11.4)
Height (cm)[a]	160.9 (7.0)	161.0 (6.9)	161.3 (6.9)	162.1 (6.7)	163.3 (6.4)
Weight (kg)[a]	67.5 (12.5)	67.1 (12.1)	66.6 (11.8)	66.1 (11.6)	65.4 (11.4)
BMI (kg/cm^2)[a]	26.2 (4.9)	26.0 84.8)	25.6 (4.6)	25.2 (4.4)	24.5 (4.2)
Educational level (%)					
None	6.0	6.6	5.7	3.4	1.0
Primary school	31.4	28.0	25.1	21.1	13.7
Technical school	27.5	21.1	17.6	19.1	22.3
Secondary school	20.5	23.0	26.0	26.2	22.1
University or higher	13.3	19.2	23.2	26.0	31.5
Unknown	1.3	2.0	2.5	4.3	9.4
Smoking status (%)					
Never	44.4	55.8	59.5	59.9	58.7
Former	21.2	20.7	20.7	23.4	26.7
Smoker	31.7	21.2	17.6	14.5	12.3
Unknown	2.7	2.3	2.2	2.2	2.3
Physical activity (%)					
Inactive	19.6	24.1	23.9	21.0	18.7
Moderately inactive	22.0	31.3	34.7	35.5	34.6
Moderately active	13.4	19.7	22.9	24.7	26.0
Active	8.3	11.8	13.8	16.7	19.1
Missing	36.7	13.1	4.7	2.1	1.5
Use of contraceptive pill (%)					
Never	41.3	43.6	42.4	39.4	34.6
Ever	55.7	54.1	54.9	58.8	62.7
Unknown	3.0	2.3	2.7	1.7	2.7
Use of hormones (%)					
Never	67.8	69.3	70.1	69.9	68.9
Ever	24.1	22.4	22.9	24.9	26.9
Unknown	8.1	8.3	6.9	5.2	4.1
Menopausal status (%)					
Pre-menopausal	34.2	34.8	35.5	34.4	35.3
Post-menopausal	40.8	42.7	42.9	44.9	45.6
Peri-menopausal	23.0	19.8	18.5	17.5	15.8
Bilateral ovariectomy	2.1	2.8	3.1	3.2	3.2
Energy (kcal/day)[a]	1,633 (435)	1,860 (475)	2,006 (522)	2,074 (562)	2,085 (559)
Alcohol (g/day)[a]	4.5 (7.4)	6.8 (9.9)	8.8 (11.9)	10.3 (13.7)	10.4 (13.8)
Total fibre (g/day)[a]	17.5 (5.4)	20.4 (5.7)	22.5 (6.2)	24.2 (7.1)	26.1 (8.6)

[a] Mean (SD)

and adolescence as observed in Asian countries) may play an important role in their cancer-preventive effects [50]. Further research is needed to evaluate the effect of early phyto-oestrogen intake on hormonal-related cancers, such as BC.

In our prospective study, no association was observed between total lignan intake and overall BC risk and by menopausal status. Our results are in concordance with four of the six prospective studies conducted to date [19, 20, 24], except the EPIC French and Swedish

Table 3 Total and subclasses of flavonoid and lignan intake (mg/day) and their main food sources in the European Prospective Investigation into Cancer and Nutrition (EPIC) study

	Mean	SD	Median	P10th	P90th	Four main food sources (%)
Total flavonoids	434.4	330.7	332.2	123.3	922.1	Tea (21.3 %), apples and pears (19.6 %), wine (8.9 %), stone fruits (6.7 %)
Flavanols	350.8	304.1	246.6	82.2	808.3	Tea (49.3 %), apples and pears (16.7 %), wine (6.3 %), stone fruits (5.2 %)
Flavan-3-ols monomers	177.5	254.1	43.8	12.4	531.6	Tea (86.3 %), apples and pears (2.9 %), wine (2.4 %), chocolates (1.8 %)
Proanthocyanidins	167.5	109.6	148.5	58.8	294.7	Apples and pears (33.2 %), wine (11.0 %), stone fruits (10.0 %), chocolates (6.3 %)
Teaflavins	5.9	9.8	0.4	0.0	19.3	Tea (100 %)
Anthocyanidins	29.5	22.8	23.6	8.2	58.2	Wine (15.6 %), grapes (15 %), berries (13.3 %), apple and pears (12.6 %)
Flavonols	27.2	17.6	22.2	9.8	52.4	Tea (30.3 %), bouillons (9.8 %), leafy vegetables (8.2 %), apple and pears (8.1 %)
Flavanones	21.8	21.7	16.1	3.4	45.6	Citrus fruit (49.6 %), fruit juices (42.2 %), wine (3.6 %), jams (0.5 %)
Flavones	3.5	3.9	2.5	0.7	7.0	Herbal tea (36.0), wine (13.6 %), leafy vegetables (8.4 %), citrus fruit (8.4 %)
Total isoflavones	1.5	4.8	0.5	0.1	2.6	Soya products (44.3 %), chocolates (7.6 %), coffee (7.3 %), breads (7.1 %)
Total lignans	1.4	0.8	1.2	0.7	2.4	Breads (12.4 %), cabbages (12.4 %), Tea (12.1 %), COFFEE (8.0 %)

post-menopausal cohorts [21, 51]. Likewise, most of the case–control studies showed protective associations on BC [18, 19]. Of these, one study investigated the role of dietary intake during adolescence, reporting a protective effect in adulthood for high plant lignan intake early in life [52]. Using nutritional biomarkers in serum or plasma, to evaluate lignan intake, the results were also inconsistent [18, 22, 24, 53]. In the Danish EPIC cohort, a significant inverse association was observed between plasma lignan levels and ER-negative tumours [23]. Our results show an inverse trend, though not significant, between dietary intake of total lignans and ER-/PR- breast tumours. This borderline association may be observed by chance, although, similarly, a case–control study found an inverse association between dietary total lignan and ER- tumours in pre-menopausal women [54]. This suggests a potential protective non-hormonal-related effect of lignans on BC. A plausible mechanism of action for this effect could be through downregulation of insulin-like growth factor 1(IGF-1), decreased epidermal growth factor receptor (EGFR) expression and tumour vascular endothelial growth factor (VEGF) expression [55]. These growth factors play important roles in tumour growth and progression through stimulation of cell proliferation, such as angiogenesis, synthesis of DNA, RNA and cellular proteins, and inhibition of apoptosis [56, 57]. Further epidemiological evidence on the potential association between lignan intake and ER-/PR- breast tumours is warranted.

One of the limitations of the present study is the use of a single baseline assessment of diet and other lifestyle variables. Therefore, changes in lifestyle could not be taken into account in these analyses. Another limitation may be the measurement error in collecting dietary intake since country-specific validated questionnaires were used [20, 25, 26]. It is particularly relevant in the case of soya products (the main source of isoflavones) because some countries did not include soy-based foods in their dietary questionnaires as they were rarely consumed in the 1990s in most of the European countries. In addition, flavonoid and lignan intake is likely to be underestimated since substantial data were lacking in the flavonoid database (although an extensive common database was used) [26, 27] and herb/plant supplement intake was not taken into account in these analyses (up to 5 % in Denmark, the highest consumer country) [58]. This misclassification is likely to be random and therefore any association between intake and disease risk is likely underestimated. Another limitation is the potential modification of diet during the early prediagnostic period of the disease; however, sensitivity analyses excluding incident cases diagnosed in the first 2 years of follow-up did not alter the associations. Finally, we realize that our study is prone to the well-known drawback of multiple comparisons. The strengths of our study include its prospective and population-based design, detailed information on diet and a large sample of BC cases with data on hormone receptor status of breast tumours, which allows greater power for subgroup analyses.

In conclusion, this large prospective analysis of flavonoid and lignan intake and BC risk suggests no associations between dietary intake of total flavonoids, total lignans and any flavonoid subclasses and BC risk in Western European women overall or after taking into account menopausal status and oestrogen and PRs of BC tumours.

Table 4 Multivariable HRs (95 % CI) for breast cancer by quintile of flavonoid or lignan intake overall and by menopausal status in the European Prospective Investigation into Cancer and Nutrition (EPIC) study

		Overall			Pre-menopausal			Post-menopausal		
		BC			BC			BC		
		PY	Cases	HR (95 % CI)[a]	PY	Cases	HR (95 % CI)[b]	PY	Cases	HR (95 % CI)[b]
Total flavonoids										
Quintile 1	<176.0	719,894	2,110	1.00 (ref.)	247,232	495	1.00 (ref.)	294,306	1,009	1.00 (ref.)
Quintile 2	176.0–276.2	734,702	2,226	0.98 (0.92–1.04)	256,615	593	1.07 (0.94–1.21)	312,900	1,050	0.94 (0.86–1.03)
Quintile 3	276.3–403.6	739,302	2,328	0.97 (0.91–1.04)	266,083	571	0.95 (0.83–1.09)	314,406	1,185	0.99 (0.90–1.09)
Quintile 4	403.7–654.0	737,369	2,482	0.99 (0.93–1.07)	257,394	630	1.06 (0.92–1.22)	327,820	1,286	0.98 (0.89–1.08)
Quintile 5	>654.0	739,172	2,430	0.97 (0.90–1.04)	264,713	538	0.98 (0.84–1.15)	333,797	1,342	0.96 (0.86–1.06)
P trend				0.591			0.656			0.622
Continuous (log_2)				0.99 (0.97–1.01)			1.00 (0.95–1.04)			0.99 (0.96–1.02)
Flavanols										
Quintile 1	<121.2	719,289	2,086	1.00 (ref.)	247,785	495	1.00 (ref.)	292,008	991	1.00 (ref.)
Quintile 2	121.2–198.7	734,056	2,254	0.99 (0.93–1.05)	255,431	583	1.05 (0.92–1.19)	312,505	1,072	0.95 (0.87–1.04)
Quintile 3	198.8–308.2	738,083	2,348	0.98 (0.92–1.05)	262,448	583	0.98 (0.86–1.12)	316,619	1,187	0.98 (0.90–1.08)
Quintile 4	308.3–550.5	738,602	2,481	1.00 (0.94–1.08)	259,007	648	1.09 (0.95–1.25)	327,129	1,278	0.98 (0.89–1.08)
Quintile 5	>550.5	740,410	2,407	0.97 (0.90–1.04)	267,367	518	0.94 (0.81—1.10)	334,968	1,344	0.95 (0.86–1.06)
P trend				0.444			0.271			0.524
Continuous (log_2)				0.99 (0.98–1.01)			1.00 (0.96–1.04)			0.99 (0.96–1.02)
Flavan-3-ol monomers										
Quintile 1	<18.2	717,614	1,867	1.00 (ref.)	254,980	503	1.00 (ref.)	298,216	874	1.00 (ref.)
Quintile 2	18.2–31.7	733,193	2,285	1.01 (0.94–1.07)	248,946	565	1.00 (0.88–1.13)	308,839	1,090	0.98 (0.89–1.08)
Quintile 3	31.8–81.0	734,331	2,505	1.02 (0.96–1.10)	257,285	620	0.97 (0.85–1.11)	314,824	1,239	1.01 (0.91–1.11)
Quintile 4	81.1–379.8	741,226	2,500	1.00 (0.93–1.08)	263,690	619	0.99 (0.86–1.14)	318,057	1,281	1.00 (0.90–1.10)
Quintile 5	>379.8	744,076	2,419	1.01 (0.93–1.09)	267,137	520	0.96 (0.82–1.13)	343,293	1,388	1.00 (0.90–1.11)
P trend				0.856			0.700			0.932
Continuous (log_2)				1.00 (0.99–1.01)			1.00 (0.98–1.02)			1.00 (0.99–1.02)
Proanthocyanidins										
Quintile 1	<84.2	730,001	2,175	1.00 (ref.)	253,757	514	1.00 (ref.)	300,710	1,069	1.00 (ref.)
Quintile 2	84.2–126.8	740,141	2,253	0.96 (0.90–1.02)	259,008	525	0.93 (0.82–1.05)	318,404	1,147	0.95 (0.87–1.04)
Quintile 3	126.9–170.7	735,051	2,432	1.00 (0.93–1.06)	254,487	596	1.00 (0.88–1.14)	323,897	1,249	0.98 (0.90–1.07)
Quintile 4	170.8–232.5	732,243	2,379	0.95 (0.89–1.01)	256,883	572	0.92 (0.80–1.05)	321,573	1,231	0.94 (0.85–1.03)
Quintile 5	>232.5	733,003	2,337	0.96 (0.89–1.04)	267,903	620	1.01 (0.86–1.17)	318,645	1,176	0.91 (0.82–1.01)
P trend				0.354			0.724			0.079
Continuous(log_2)				0.99 (0.97–1.02)			1.01 (0.96–1.06)			0.98 (0.95–1.02)
Theaflavins										
Quintile 1	0	1,465,200	4,435	1.00 (ref.)	472,410	1,061	1.00 (ref.)	622,820	2,115	1.00 (ref.)
Quintile 2							–			
Quintile 3	0.01–1.98	730,975	2,243	1.03 (0.97–1.10)	294,469	641	1.06 (0.94–1.20)	302,026	1,094	1.01 (0.92–1.10)
Quintile 4	1.99–13.88	737,650	2,502	1.02 (0.96–1.08)	261,076	609	1.05 (0.93–1.18)	317,676	1,285	1.01 (0.93–1.10)

Table 4 continued

		Overall			Pre-menopausal			Post-menopausal		
		BC			BC			BC		
		PY	Cases	HR (95 % CI)[a]	PY	Cases	HR (95 % CI)[b]	PY	Cases	HR (95 % CI)[b]
Quintile 5	>13.88	736,615	2,396	1.02 (0.95—1.10)	264,085	516	1.04 (0.90–1.20)	340,707	1,378	1.02 (0.92–1.12)
P trend				0.857			0.988			0.756
Continuous (log_2)				1.00 (1.00–1.00)			1.00 (1.00–1.01)			1.00 (1.00–1.01)
Anthocyanidins										
Quintile 1	<12.1	743,639	2,170	1.00 (ref.)	249,795	467	1.00 (ref.)	339,940	1,179	1.00 (ref.)
Quintile 2	12.1–19.4	744,989	2,141	0.99 (0.93–1.05)	265,622	520	1.05 (0.92–1.19)	326,072	1,128	0.98 (0.90–1.07)
Quintile 3	19.5–28.4	736,734	2,178	1.00 (0.94–1.07)	277,063	557	1.05 (0.92–1.20)	308,553	1,109	1.00 (0.91–1.09)
Quintile 4	28.5–43.6	729,323	2,313	0.99 (0.93–1.06)	275,386	642	1.09 (0.95–1.26)	296,365	1,075	0.94 (0.86–1.04)
Quintile 5	>43.6	715,754	2,774	1.02 (0.94–1.10)	224,173	641	1.09 (0.93—1.28)	312,299	1,381	1.01 (0.90–1.13)
P trend				0.560			0.323			0.829
Continuous (log_2)				1.01 (0.99–1.03)			1.02 (0.97–1.06)			1.00 (0.97–1.03)
Flavonols										
Quintile 1	<12.8	730,767	2,093	1.00 (ref.)	261,133	544	1.00 (ref.)	303,965	996	1.00 (ref.)
Quintile 2	12.9–18.7	737,662	2,139	0.98 (0.92–1.05)	271,730	556	0.95 (0.84–1.07)	308,094	1,065	1.02 (0.93–1.11)
Quintile 3	18.8–26.7	738,205	2,260	0.98 (0.92–1.05)	267,990	592	0.94 (0.83–1.07)	313,212	1,116	1.01 (0.91–1.11)
Quintile 4	26.8–39.8	733,606	2,485	0.98 (0.91–1.05)	253,619	571	0.90 (0.78–1.03)	323,182	1,275	1.00 (0.90–1.10)
Quintile 5	>39.8	730,199	2,599	0.96 (0.88–1.03)	237,567	564	0.91 (0.78–1.06)	334,776	1,420	1.00 (0.90–1.12)
P trend				0.259			0.316			0.893
Continuous (log_2)				0.97 (0.95–1.00)			0.95 (0.90–1.01)			0.98 (0.94–1.02)
Flavanones										
Quintile 1	<6.2	726,556	2,207	1.00 (ref.)	265,038	540	1.00 (ref.)	303,621	1,076	1.00 (ref.)
Quintile 2	6.2–12.6	722,347	2,418	0.97 (0.92–1.03)	258,848	596	0.94 (0.83–1.06)	291,066	1,168	1.03 (0.95–1.12)
Quintile 3	12.7–20.2	727,114	2,418	0.95 (0.90–1.01)	248,676	583	0.95 (0.84–1.08)	314,023	1,222	1.00 (0.91–1.09)
Quintile 4	20.3–33.0	745,491	2,454	1.02 (0.96–1.09)	254,202	585	1.05 (0.92–1.19)	335,356	1,267	1.04 (0.95–1.13)
Quintile 5	>33.0	748,931	2,079	0.99 (0.93–1.06)	265,276	523	1.02 (0.89–1.18)	339,163	1,139	1.04 (0.95–1.15)
P trend				0.562			0.293			0.401
Continuous (log_2)							1.00 (0.97–1.02)			1.01 (0.99–1.03)
Flavones										
Quintile 1	<1.12	733,989	2,095	1.00 (ref.)	263,027	527	1.00 (ref.)	296,684	986	1.00 (ref.)
Quintile 2	1.12–2.01	738,165	2,250	1.00 (0.94–1.07)	267,049	546	0.92 (0.81–1.06)	319,146	1,173	1.06 (0.97–1.16)
Quintile 3	2.02–3.04	739,074	2,337	0.99 (0.92–1.06)	266,429	573	0.90 (0.78–1.05)	325,942	1,200	1.02 (0.92–1.12)
Quintile 4	3.05–4.88	731,616	2,570	1.03 (0.96–1.11)	252,599	636	0.94 (0.81–1.10)	319,408	1,312	1.11 (1.00–1.23)
Quintile 5	>4.88	727,596	2,324	0.99 (0.91–1.07)	242,934	545	0.86 (0.73–1.02)	322,049	1,201	1.10 (0.98–1.23)
P trend				0.729			0.162			0.120
Continuous (log_2)				1.00 (0.98–1.02)			0.95 (0.91–1.00)			1.04 (1.01–1.06)
Isoflavones										
Quintile 1	<0.22	690,923	1,903	1.00 (ref.)	227,330	466	1.00 (ref.)	292,501	907	1.00 (ref.)

Table 4 continued

		Overall			Pre-menopausal			Post-menopausal		
		BC			BC			BC		
		PY	Cases	HR (95 % CI)[a]	PY	Cases	HR (95 % CI)[b]	PY	Cases	HR (95 % CI)[b]
Quintile 2	0.22–0.39	744,644	2,378	0.98 (0.91–1.06)	236,820	562	1.05 (0.90–1.22)	354,245	1,269	0.95 (0.86–1.05)
Quintile 3	0.40–0.65	748,866	2,583	0.99 (0.92–1.08)	224,423	571	1.00 (0.85–1.19)	351,304	1,376	1.00 (0.90–1.12)
Quintile 4	0.66–1.36	748,014	2,584	0.99 (0.91–1.08)	234,073	570	0.97 (0.80–1.17)	341,279	1,343	0.99 (0.88–1.11)
Quintile 5	>1.36	737,992	2,128	1.00 (0.91–1.10)	369,392	658	0.94 (0.77–1.16)	243,899	977	1.00 (0.87–1.14)
P trend				0.734			0.351			0.702
Continuous($\log_2$)				1.00 (0.98–1.02)			1.00 (0.96–1.04)			0.99 (0.96–1.02)
Lignans										
Quintile 1	<0.82	740,984	2,153	1.00 (ref.)	258,427	559	1.00 (ref.)	315,689	1,038	1.00 (ref.)
Quintile 2	0.82–1.09	738,044	2,247	0.98 (0.92–1.04)	260,129	572	0.95 (0.84–1.08)	309,167	1,079	0.96 (0.88–1.05)
Quintile 3	1.10–1.40	732,578	2,327	0.97 (0.91–1.03)	255,233	578	0.94 (0.83–1.08)	311,536	1,135	0.93 (0.84–1.02)
Quintile 4	1.41–1.89	728,584	2,422	0.97 (0.90–1.04)	250,910	573	0.97 (0.84–1.12)	321,476	1,270	0.93 (0.84–1.03)
Quintile 5	>1.89	730,249	2,427	1.02 (0.93–1.11)	267,339	545	1.04 (0.87–1.24)	325,361	1,350	0.95 (0.84–1.07)
P trend				0.469			0.459			0.589
Continuous ($\log_2$)				0.98 (0.94–1.03)			1.02 (0.93–1.11)			0.94 (0.89–1.00)

PY person-years, *BC* breast cancer

[a] Multivariable model: stratified by centre and age (1 year) and adjusted for baseline menopausal status (pre-menopausal plus unknown, post-menopausal plus women who underwent an ovariectomy), weight (kg), height (cm), smoking status (never, former, current, unknown), educational level (none, primary, technical, secondary, university or higher, unknown), physical activity (inactive, moderately inactive, moderately active, active, unknown), age at menarche (<12, 12–14, >14 year, unknown), age at first full-term birth (nulliparous; <21, 21–30, >30 year), ever use of contraceptive pills (never, ever, unknown), ever use of hormones (never, ever, unknown), age at menopause (<=50, >50 year), energy intake (kcal/day), alcohol intake (g/day), and fibre intake (g/day)

[b] The model was adjusted as in footnote 1 but without adjustment for menopausal status and with the exclusion of women with a history of ovariectomy or unknown menopausal status

Table 5 Multivariable HRs (95 % CI) for breast cancer by doubling in flavonoid or lignan intake (mg/day) according to breast cancer phenotype in the EPIC study

	ER^-/PR^- HR (95 % CI)[a]	ER^-/PR^+ HR (95 % CI)[a]	ER^+/PR^- HR (95 % CI)[a]	ER^+/PR^+ HR (95 % CI)[a]
Total flavonoids	0.99 (0.92–1.07)	1.00 (0.85–1.19)	0.99 (0.92–1.07)	1.02 (0.98–1.06)
Flavanols	0.99 (0.93–1.06)	1.01 (0.87–1.17)	0.99 (0.93–1.05)	1.02 (0.99–1.06)
Flavan-3-ol monomers	0.99 (0.95–1.03)	1.02 (0.94–1.11)	0.99 (0.95–1.03)	1.02 (1.00–1.04)
Proanthocyanidins	1.01 (0.92–1.10)	0.99 (0.81–1.21)	0.98 (0.90–1.06)	1.02 (0.97–1.06)
Theaflavins	1.00 (0.99–1.01)	1.01 (0.99–1.03)	1.00 (0.99–1.01)	1.00 (1.00–1.01)
Anthocyanidins	1.02 (0.95–1.10)	1.12 (0.94–1.35)	0.99 (0.92–1.06)	1.00 (0.96–1.04)
Flavonols	0.96 (0.87–1.05)	0.94 (0.76–1.17)	0.98 (0.90–1.08)	1.01 (0.96–1.06)
Flavanones	0.99 (0.95–1.03)	1.00 (0.90–1.11)	0.99 (0.95–1.03)	1.00 (0.98–1.02)
Flavones	0.99 (0.92–1.06)	1.07 (0.91–1.27)	0.97 (0.90–1.04)	1.00 (0.96–1.03)
Isoflavones	0.98 (0.92–1.06)	0.94 (0.80–1.10)	1.03 (0.96–1.11)	0.99 (0.96–1.03)
Lignans	0.88 (0.76–1.01)	1.17 (0.82–1.68)	0.89 (0.75–1.05)	1.04 (0.96–1.13)

Number of breast cancer cases by hormone receptor status: ER^-/PR^- ($n = 1{,}050$), ER^-/PR^+ ($n = 217$), ER^+/PR^- ($n = 1{,}133$), ER^+/PR^+ ($n = 3{,}653$)

EPIC European Prospective Investigation into Cancer and Nutrition, *ER* oestrogen receptor, *PR* progesterone receptor

[a] Multivariable model: stratified by centre and age (1 year) and adjusted for baseline menopausal status (pre-menopausal plus unknown, post-menopausal plus women who underwent an ovariectomy), weight (kg), height (cm), smoking status (never, former, current, unknown), educational level (none, primary, technical, secondary, university or higher, unknown), physical activity (inactive, moderately inactive, moderately active, active, unknown), age at menarche (<12, 12–14, >14 year, unknown), age at first full-term birth (nulliparous, <21, 21–30, >30 year), ever use of contraceptive pills (never, ever, unknown), ever use of hormones (never, ever, unknown), age at menopause (<=50, >50 year), energy intake (kcal/day), alcohol intake (g/day), and fibre intake (g/day)

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