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Dietary phytoestrogens and breast cancer risk[1–3]

Lital Keinan-Boker, Yvonne T van Der Schouw, Diederick E Grobbee, and Petra HM Peeters

ABSTRACT

Background: A high intake of phytoestrogens, particularly isoflavones, has been suggested to decrease breast cancer risk. Results from human studies are inconclusive.

Objective: We investigated the association between phytoestrogen intake and breast cancer risk in a large prospective study in a Dutch population with a habitually low phytoestrogen intake.

Design: The study population consisted of 15 555 women aged 49–70 y who constituted a Dutch cohort of the European Prospective Investigation into Cancer and Nutriton (EPIC; 1993–1997). Data concerning habitual dietary intake in the preceding year were obtained by using a validated food-frequency questionnaire. The content of isoflavones and lignans in relevant food items was estimated through a literature search, the use of food-composition tables, and contact with experts. Newly diagnosed breast cancer cases up to 1 January 2001 were identified through linkage with the Comprehensive Cancer Center Middle Netherlands. Hazard ratios for the disease were estimated by Cox proportional hazard analysis for quartiles of isoflavone and lignan intake. Associations were adjusted for known breast cancer risk factors and daily energy intake.

Results: A total of 280 women were newly diagnosed with breast cancer during follow-up. The median daily intakes of isoflavones and lignans were 0.4 (interquartile range: 0.3–0.5) and 0.7 (0.5–0.8) mg/d, respectively. Relative to the respective lowest intake quartiles, the hazard ratios for the highest intake quartiles for isoflavones and lignans were 1.0 (95% CI: 0.7, 1.5) and 0.7 (0.5, 1.1), respectively. Tests for trend were nonsignificant.

Conclusion: In Western populations, a high intake of isoflavones or mammalian lignans is not significantly related to breast cancer risk. *Am J Clin Nutr* 2004;79:282–8.

KEY WORDS Breast cancer, phytoestrogens, isoflavones, lignans, Prospect–European Prospective Investigation into Cancer and Nutrition (EPIC), Netherlands

INTRODUCTION

Phytoestrogens, natural estrogen-like substances in plant food, are subdivided into 3 main classes: isoflavones, lignans, and coumestans. Two of the major plant isoflavones, genistein and daidzein, may also be metabolized from other isoflavonoid precursors, eg, biochanin A and formononetin, respectively. Enterolactone and enterodiol, the main mammalian lignans, are formed from the plant lignans matairesinol and secoisolariciresinol, respectively, as well as others, by gut microflora. The main compound in the coumestan subgroup is coumesterol (1–4).

Isoflavones resemble estrogen structurally, are able to bind to the estrogen receptor (ER), and have ER-mediated estrogenic properties (transcriptional activity) (1, 2). They also act as antiestrogens by competing with the more potent endogenous estrogen for the ERs (5). Additionally, phytoestrogens have antioxidative, antiproliferative, and antiangiogenic activities, which are hormonally independant (1–8). The plant lignans show hardly any binding affinity to ERs (6). However, in animal and in vitro studies, lignans were reported to have antioxidative activity (9) and to reduce tumor progression and metastasis (10, 11).

Soy foods are rich in isoflavones, and Asian populations habitually consume large amounts of soy foods. Ecologic observations suggest that the intake of soy foods plays a role in the prevention of breast cancer (12–14). Few prospective studies have been conducted in this field (15). Several studies, but not all, showed protective effects of soy in Asian populations (16–18). No such associations were suggested for Western subjects (19, 20). In Western populations, soy consumption is infrequent, but isoflavones are consumed in small amounts from other sources. Lignans are more widespread and occur more frequently in Western diets than do isoflavones.

A method of estimating daily phytoestrogen intake on the basis of daily intakes of certain food items for which values of isoflavone and lignan content are available has been developed (21–26) and used in Western populations (27–29). To prospectively study the effect of phytoestrogens on breast cancer risk in a Western population, we conducted a cohort study on habitual intake of isoflavones and lignans in 15 555 Dutch women.

SUBJECTS AND METHODS

Subjects

The study population consisted of a Dutch cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC); this Dutch portion of the EPIC was conducted in Utrecht, Netherlands, and is referred to here as "Prospect-

[1] From the Julius Center for Health Sciences and Primary Care, University Medical Center, Utrecht, Netherlands.

[2] Supported by grant no. 21000027 from Zorg Onderzoek Nederland, grant no. 2000/30 from the World Cancer Research Fund, and the Europe Against Cancer Program of the European Commission (SANCO).

[3] Address reprint requests to PHM Peeters, Julius Center for Health Sciences and Primary Care, D01.335, University Medical Center Utrecht, PO Box 85500, 3508 GA Utrecht, Netherlands. E-mail: p.h.m.peeters@jc.azu.nl.

Received November 1, 2002.

Accepted for publication August 6, 2003.

EPIC" (30). The cohort includes 17 357 women aged 50–69 y who reside in Utrecht or its vicinity and were recruited between 1993 and 1997 through a regional program for breast cancer screening (34.5% response) (30). At recruitment, each participant filled out a general questionnaire and a food-frequency questionnaire (FFQ). In addition, pulse rate, blood pressure, and anthropometric measurements (height, weight, and waist and hip circumference) were taken, and a blood sample (30 mL) was donated by participants and stored at −196 °C under liquid nitrogen (30).

For the present study, eligible participants were all Prospect-EPIC participants with complete questionnaires who signed an informed consent form (n = 17 235). Exclusion criteria included a daily intake of <500 or >6000 kcal/d (n = 95), prevalent cancer at any site at the time of enrollment (n = 1136), and residency in areas not fully covered by the Comprehensive Cancer Center Middle Netherlands (n = 449). Consequently, there were 15 555 eligible subjects.

The cohort data were linked to the databases of the municipal registries to ascertain the vital status of all participants and to obtain information about subjects who moved from the region or emigrated abroad (≈100% completeness). Linkage of the cohort to the Comprehensive Cancer Center Middle Netherlands (complete since 1989) provided us with information regarding incident cancer cases, which were coded according to the 9th revision of the *International Classification of Diseases*. Additional causes of death were obtained through general practitioners and were coded according to the 10th revision of the *International Classification of Diseases*.

The endpoint for the study was the diagnosis of primary breast cancer. For cases of breast cancer, follow-up ended at the date of diagnosis or at the date of death due to breast cancer, whichever occurred first. For women diagnosed with other primary cancers, follow-up was censored at the date of diagnosis. For women who left the region or country, the censoring date was the date of emigration. For those who died, follow-up was censored at the date of death. For all others, the censoring date was 1 January 2001. The Institutional Review Board of the University Medical Center Utrecht approved the study.

Prospect-EPIC general questionnaire, FFQ, and anthropometric measurements

The general questionnaire included questions regarding demographic factors, lifestyle habits, obstetric and gynecologic history, and past and current morbidity. Menopause was defined as the complete cessation of menstrual bleeding during the 12 mo preceding enrollment due to natural, chemical, or surgical causes (according to self-report). Marital status and education level were determined categorically. Parity was categorically defined as "yes" or "no." Use of oral contraceptives or hormone replacement therapy was categorically defined as "ever" or "never." Smoking was described by a categorical variable (past, current, or never smoker) and by the continuous variable of total pack-years smoked (number of cigarettes smoked per day multiplied by the number of smoking years and divided by 20). The Voorrips score is a summary measure that combines household, occupational, and recreational physical activity (31). Body mass index was computed as weight (in kg) divided by the square of height (in m) (30).

The self-administered FFQ included questions on the subjects' habitual intake of 178 food items during the preceding year (32, 33). The questionnaire contained color photographs of 2–4 different-sized portions of 21 food items, which helped in assessing serving sizes. The frequency of consumption of each food item could be indicated on a daily, weekly, monthly, or yearly scale or as "never." The FFQ also included open-ended questions, in which the names of the brands used (eg, for margarine) could be filled out.

Scoring of phytoestrogen intake

The method used and the guidelines applied for assigning an individual phytoestrogen intake score for each participant were described in detail previously (25, 26). Briefly, published laboratory analysis data on the phytoestrogen contents of relevant food items were located by conducting a search of the medical (MEDLINE; National Library of Medicine, Bethesda, MD) and agricultural (AGRICOLA; National Agricultural Library, Beltsville, MD) scientific literature and by contacting experts in the field of phytoestrogens and several Dutch food manufacturers. Search terms included isoflavones, coumestans, plant lignans, and mammalian lignans. Note that mammalian lignans are not naturally present in plant foods, and their food content values are based on in vitro production of enterolactone and enterodiol from certain food items, according to published databases. Literature data for the contents of the isoflavones daidzein, genistein, formononetin, and biochanin A; the coumestan coumestrol; the plant lignans matairesinol and secoisolariciresinol; and the mammalian lignans enterolactone and enterodiol were grouped in 7 categories according to their exact values. This was done to avoid implying a degree of accuracy for which the current available data are too limited and too preliminary (25, 26). Subsequently, the phytoestrogen score of each food item was multiplied by the quantity consumed per day per participant. The resulting phytoestrogen score was then summed up across food items to obtain daily intake scores of daidzein, genistein, formononetin, biochanin A, coumsterol, matairesinol, secoisolariciresinol, enterolactone, and enterodiol for each participant. We then summarized the individual intake scores of daidzein, genistein, formononetin, and biochanin A into a total isoflavone score; the individual intake scores of matairesinol and secoisolariciresinol into a total plant lignan score; and the individual intake scores of enterolactone and enterodiol into a total mammalian lignan score.

Statistical analysis

Our analyses refer to the subgroup of mammalian lignans (enterolactone and enterodiol) rather than to the plant lignans (matairesinol and secoisolariciresinol). The mammalian lignans—end products of various plant precursors metabolized by the gut microflora—are the bioactive forms, which might be more relevant in analytic studies on cancer associations. Additionally, besides matairesinol and secoisolariciresinol, enterolactone and enterodiol are produced from other plant lignans (3, 4), for most of which no food-composition data are available. Thus, the use of mammalian lignan scores might account for the lack of data on some of their plant precursors. Isoflavones are found both in plants and in body fluids, so no distinction was made between their plant and bioactive forms. Coumesterol was not used in our analyses because of its very low intake in this study population.

Quartiles of total isoflavone (daidzein, genistein, biochanin

A, and formononetin) and total mammalian lignan (enterolactone and enterodiol) intake were computed according to the distribution of the total cohort. General characteristics of the study population are presented by quartiles of total phytoestrogen intake (ie, summed intakes of daidzein, genistein, biochanin A, formononetin, enterolactone, and enterodiol). Daily intake is expressed in medians because the distribution curves appeared to be skewed.

Hazard ratios for breast cancer were estimated by Cox proportional hazard analysis and are presented, together with 95% CIs, for quartiles of isoflavone and lignan intake, with the lowest quartile as the reference. We first present the results of a model adjusted only for age at enrollment. Subsequently, we present the results of models adjusted for established risk factors for breast cancer (age at enrollment, age at first full-term delivery, height, weight, parity, physical activity score, use of oral contraceptives, use of hormone replacement therapy, menopausal status, marital status, and academic education). Finally, we further adjust the fully adjusted models for daily energy intake (as a continuous variable). Trends were assessed by testing the significance of the models' linearity (Wald test) by using phytoestrogen intake as a categorical variable (quartiles) in polynomial contrast. We repeated the analyses by excluding all cases that were diagnosed <1 y after enrollment.

Previous studies suggested that the effects of phytoestrogens on breast cancer risk differ according to menopausal status. Therefore, we also conducted a subanalysis of all participants who were postmenopausal at enrollment (and thus also postmenopausal at breast cancer diagnosis). In addition to the adjustment variables used for the total population, the models for postmenopausal participants were also adjusted for age at menopause. The SPSS statistical package for WINDOWS, version 9.0, was used for all statistical analyses (34), and all tests were two-sided.

RESULTS

Among the 15 555 eligible participants in the study, 280 (1.8%) were diagnosed with their first case of incident breast cancer during follow-up. A total of 549 (3.5%) subjects were diagnosed with other types of cancer, and 581 (3.7%) subjects left the region. One hundred forty-two (0.9%) participants died, and 14 003 (90.0%) subjects were alive and free from cancer at the end of follow-up (1 January 2001). The women were followed up for a total of 80 215 person-years. The median follow-up for the total cohort was 5.2 y. Most of the cohort participants (n = 11 655, 74.9%) were postmenopausal at enrollment.

The participants who had the highest (4th quartile) intake scores for phytoestrogens were significantly younger at enrollment and significantly older at delivery of their first child than were the participants who had lower intake scores. The subjects with the highest intake scores also smoked significantly less, were significantly taller, and had significantly lower BMI values and significantly higher physical activity scores. They also had significantly higher daily intakes of energy, fiber, fruit, vegetables, and soy products (**Table 1**).

The median daily total intake of phytoestrogens was ≈1 mg/d. The daily intake of mammalian lignans (0.67 mg/d) was ≈80% higher than that of isoflavones (**Table 2**).

Risk estimates for breast cancer by quartiles of isoflavone intake indicated no associations. For mammalian lignans, the estimates indicated a possible protective trend: with the lowest quartile as the reference, the hazard ratio for the highest quartile was 0.7 (95% CI: 0.5, 1.1) (P for trend = 0.06). However, none of the results were significant (**Table 3**).

When only breast cancer cases that were diagnosed ≥12 mo after enrollment were included in the analyses (n = 203), the results were very similar (data not shown). The same was true when only postmenopausal participants (at enrollment and at diagnosis) were included (for mammalian lignans in the fully adjusted model, P for trend = 0.06; data not shown). An interaction term for menopausal status in the model that included all women was not significant.

DISCUSSION

The results of the present study, which focused on Western women whose habitual diet is low in phytoestrogens, showed no protective effects of isoflavones or lignans against breast cancer. The main advantages of this study are its prospective nature, the large sample size, the completeness of the risk factor data and of the follow-up, and the detailed dietary assessment. The FFQs, which included questions on habitual dietary intake during the year preceding enrollment, were filled out by the participants long before the diagnosis of breast cancer and thus could not have been influenced by awareness of the disease. The fact that participants were recruited through an existing population-based breast cancer–screening program ensured the disease-free state of the cases at enrollment; they were all mammographically free of the disease. The completeness of the Comprehensive Cancer Center Middle Netherlands ensured a complete follow-up. The incidence of invasive breast tumors in our study population was ≈300/100 000 women [≈86% of total breast tumors ($0.86 \times 280 \times 100\,000/80\,215$)], and the incidence of invasive breast cancer that was expected on the basis of Dutch cancer rates published in 1997 (weighted by the relevant age categories) was similar (ie, 285/100 000 women) (35). Additionally, the updated data from the municipal registries provided detailed follow-up information about vital status and lowered the proportion of subjects who were lost to follow-up.

The semiquantitative FFQ was not specifically designed to estimate phytoestrogen intake and did not include questions about specific food items that contain high amounts of phytoestrogens (eg, soy foods for isoflavones and flaxseed or linseed for lignans). However, these foods are infrequently consumed in the Netherlands. On the other hand, the FFQ was specifically designed to estimate the habitual intake of foods from food groups that contribute significantly to phytoestrogen intake in Western societies, such as grains, fruit, nuts and seeds, and alcoholic beverages. For these food groups, the correlation between the consumption estimated from the FFQ and that estimated from twelve 24-h dietary recalls during a period of 1 y was high (Spearman correlation coefficients ≥ 0.7) (26, 32, 33).

Published epidemiologic studies regarding dietary intake of soy foods—a proxy for isoflavone intake—and breast cancer risk were largely concerned with Asian populations having a high traditional intake of soy (16–18). Only a few studies referred to dietary intake in a Western population (27, 28, 36).

TABLE 1
General characteristics of the study population by quartiles of total phytoestrogen intake[1]

	Quartile 1: 0.10–0.84 mg/d[2] (n = 3891)	Quartile 2: 0.85–1.06 mg/d (n = 3888)	Quartile 3: 1.07–1.35 mg/d (n = 3893)	Quartile 4: 1.36–79.08 mg/d (n = 3883)	P for trend[3]
Age at enrollment (y)	57.3 ± 0.10[4]	57.2 ± 0.10	57.2 ± 0.09	56.6 ± 0.09	<0.001
Age at menarche (y)	13.5 ± 0.03	13.4 ± 0.03	13.4 ± 0.03	13.3 ± 0.03	0.005
Age at delivery of first child (y)	24.9 ± 0.07	25.2 ± 0.07	25.1 ± 0.07	25.3 ± 0.07	0.001
Age at menopause (y)	47.3 ± 0.07	47.6 ± 0.10	47.7 ± 0.11	47.5 ± 0.11	0.333
Weight (kg)	70.0 ± 0.19	70.4 ± 0.18	70.8 ± 0.18	69.6 ± 0.18	0.230
Height (cm)	163.5 ± 0.10	164.2 ± 0.10	164.4 ± 0.10	164.9 ± 0.10	<0.001
Pack-years smoked until enrollment or cessation of smoking (y)	8.1 ± 0.18	6.7 ± 0.16	6.1 ± 0.15	5.9 ± 0.15	<0.001
BMI (kg/m^2)	26.2 ± 0.07	26.1 ± 0.06	26.2 ± 0.07	25.6 ± 0.06	<0.001
Waist-to-hip ratio	0.8 ± 0.001	0.8 ± 0.001	0.8 ± 0.001	0.8 ± 0.001	<0.001
Physical activity score[5]	5.7 ± 0.07	6.5 ± 0.08	7.1 ± 0.09	7.8 ± 0.09	<0.001
Intake					
Energy (kcal/d)	1593.9 ± 6.16	1755.3 ± 6.08	1858.0 ± 6.40	1972.1 ± 7.69	<0.001
Fat (g/d)	65.5 ± 0.33	70.6 ± 0.33	73.8 ± 0.36	76.4 ± 0.42	<0.001
Fiber (g/d)	17.6 ± 0.06	21.6 ± 0.06	24.3 ± 0.06	26.8 ± 0.09	<0.001
Fruit (g/d)	155.8 ± 1.54	218.3 ± 1.83	260.2 ± 2.09	295.7 ± 2.68	<0.001
Vegetables (g/d)	103.9 ± 0.58	133.9 ± 0.64	157.7 ± 0.78	178.7 ± 0.94	<0.001
Soy products (g/d)	0.01 ± 0.03	0.1 ± 0.01	0.2 ± 0.02	4.9 ± 0.16	<0.001
Alcohol (g/d)	9.3 ± 0.22	9.1 ± 0.20	8.8 ± 0.09	9.0 ± 0.19	0.124
Marital status [n (%)]					
Married or living together	3007 (77.3)	3079 (79.2)	3080 (79.1)	2807 (72.3)	
Widowed	422 (10.9)	350 (9.0)	341 (8.8)	337 (8.7)	
Divorced	290 (7.5)	264 (6.8)	249 (6.4)	432 (11.1)	
Not married	169 (4.3)	195 (5.0)	221 (5.7)	307 (7.9)	<0.001
Academic education [n (%)]	456 (11.9)	517 (13.3)	554 (14.2)	991 (25.5)	<0.001
Ever taken OC [n (%)]	2425 (62.4)	2481 (63.8)	2547 (65.4)	2598 (66.9)	<0.001
Ever received HRT [n (%)]	956 (24.6)	956 (24.6)	1040 (26.7)	999 (25.7)	0.070
Ever had children [n (%)]	3517 (90.5)	3466 (89.1)	3439 (88.5)	3339 (85.9)	<0.001
Menopausal status [n (%)]					
Premenopausal	399 (10.3)	385 (9.9)	397 (10.2)	440 (11.3)	
Postmenopausal	2891 (74.4)	2986 (76.8)	2927 (75.3)	2848 (73.3)	
Perimenopausal or unknown	598 (15.4)	517 (13.3)	564 (14.5)	600 (15.4)	0.015
Familial history of breast cancer in mother or sister [n (%)]	465 (12.0)	453 (11.7)	494 (12.7)	467 (12.0)	0.597

[1] Total phytoestrogen intake = intakes of daidzein, genistein, formononetin, biochanin A, enterolactone, and enterodiol. OC, oral contraceptives; HRT, hormone replacement therapy.
[2] Range.
[3] For continuous variables, computed by univariate linear regression models with quartile of phytoestrogen intake as a continuous variable; for all categorical variables except marital status and menopausal status, computed by "linear-by-linear" chi-square test. For marital status and menopausal status, the overall chi-square distribution was tested.
[4] $\bar{x}$ ± SE.
[5] The Voorrips score, a summary measure that combines household, occupational, and recreational physical activity (31); range of possible scores: 0.1–37.

Although the first studies showed clear protective effects of a high soy intake, later studies were less clear. The results of the few prospective studies were mostly negative (17, 18, 28). The results of the present study showed no protective effects of isoflavones and are therefore in accordance with previously published data for Western women.

The results of several studies suggest that isoflavone consumption should be high at certain ages (eg, prepuberty) to yield protective effects (37–39). This may explain why we observed no beneficial effects of isoflavone intake in our study: overall consumption was very low, and we obtained data regarding recent consumption only, but not consumption at a young age.

Another hypothesis was recently introduced in an attempt to explain the inconsistent results for isoflavones. Equol is exclusively the product of intestinal bacterial metabolism of dietary daidzein. Only 50–70% of the healthy population produce equol in response to a dietary challenge by daidzein. Because equol has greater antioxidant activity than do all other isoflavones, it was suggested that only subjects who are equol producers benefit from consuming isoflavones (40). We have no data concerning the equol production ability of the study participants; therefore, we cannot examine this hypothesis.

Although less studied, lignans are more common in Western diets (25, 26, 41, 42); thus, the role of lignans might be more relevant than that of isoflavones. Only 3 previous studies

TABLE 2
Phytoestrogen intakes in the total study population[1]

	Median	Interquartile range
	mg/d	
Daidzein	0.13	0.09–0.19
Genistein	0.14	0.09–0.22
Formononetin	0.08	0.06–0.11
Biochanin A	0.00	0.00–0.00
Enterolactone[2]	0.40	0.31–0.50
Enterodiol[2]	0.27	0.21–0.33
Isoflavones[3]	0.37	0.26–0.54
Lignans[4]	0.67	0.53–0.83
Total[5]	1.07	0.85–1.36

[1] $n = 15\,555$.
[2] Refers to in vitro production of mammalian lignans from known food items, according to food-composition tables.
[3] Sum of daidzein, genistein, formononetin, and biochanin A.
[4] Sum of enterolactone and enterodiol.
[5] Sum of daidzein, genistein, formononetin, biochanin A, enterolactone, and enterodiol.

assessed the relation between lignan intake and breast cancer risk in Western populations (27–29). Two of them (27, 28) focused on plant lignan intake, and their results did not indicate a protective effect of high intakes. The third study (29) indicated that high intakes of mammalian lignans are associated with a low risk of breast cancer, mostly in premenopausal women and in carriers of a certain genotype of the *CYP17* gene (29).

The methodology in the prospective study by Horn-Ross et al (28) was similar to ours. However, Horn-Ross et al used estimates of plant lignan (matairesinol and secoisolariciresinol) intake, whereas we used estimates of mammalian lignan (enterolactone and enterodiol, the bioactive forms) intake. The mammalian lignan content of foods is determined by in vitro fermentation with human fecal microbiota (thus simulating colon fermentation) and subsequent measurement of the quantity of enterolactone and enterodiol produced (43). It should be kept in mind, though, that in vitro values of mammalian lignans are only an indicator of in vivo values because the in vitro values do not incorporate individual metabolic capacity. Additionally, the published values for the mammalian lignan content of foods may have been overestimated if the high background of bacterial mammalian lignans was not accounted for.

When we used estimates of plant lignan intake instead of mammalian lignan intake in our analyses (data not shown), we observed a slightly higher risk of breast cancer with high intakes, although the results were not significant. The discrepancy noted between our results for plant lignans and those for mammalian lignans, as well as similar discrepancies found in previous studies (28, 29), warrants clarification. One explanation is the incompleteness of food-composition tables with regard to both plant and mammalian lignans. For example, coffee and tea and, to a lesser extent, alcoholic beverages are frequently consumed by Dutch women. These drinks contain plant lignans but lack values for mammalian lignans. This causes differences in classification. In fact, of 15 555 participants, only one-third were classified into identical intake quartiles for plant and mammalian lignans. Therefore, the different risk estimates for plant and mammalian lignans may simply be caused by a lack of accurate food-composition data.

Alternatively, the discrepancy could partly reflect real differences between lignan precursors and bioactive compounds. Besides being produced from matairesinol and secoisolariciresinol, the bioactive compounds enterolactone and enterodiol are produced from other plant precursors, such as hydroxymatairesinol, secoisolariciresinol diglucoside, lariciresinol, pinoresinol, syringaresinol, and others, which to date are not included in any of the food-composition tables (3, 4). The effects noted for mammalian lignans may actually be produced by some other plant lignans, not necessarily matairesinol and secoisolariciresinol, for which composition data (and thus intake data) are unavailable.

Our findings for mammalian lignans were not significant but seem to show a protective trend. This is supported by the results of the case-control study of McCann et al (29), in which they reported that, relative to postmenopausal women in the

TABLE 3
Hazard rates (HRs) for breast cancer risk by quartiles of isoflavone and lignan intake[1]

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* for trend
Isoflavones					
Cases/noncases (*n*)	67/3822	71/3817	75/3813	67/3821	
Median	0.19	0.31	0.44	0.77	
HR[2]	1.00	1.01 (0.99, 1.03)	1.08 (0.78, 1.51)	1.15 (0.83, 1.61)	0.70
HR[3]	1.00	0.98 (0.67, 1.44)	1.11 (0.76, 1.62)	0.99 (0.67, 1.49)	0.86
HR[4]	1.00	0.98 (0.66, 1.44)	1.10 (0.75, 1.61)	0.98 (0.65, 1.48)	0.92
Lignans					
Cases/noncases (*n*)	68/3821	87/3802	68/3821	57/3831	
Median	0.59	0.64	0.69	0.77	
HR[2]	1.00	1.29 (0.94, 1.77)	1.01 (0.72, 1.41)	0.85 (0.60, 1.21)	0.20
HR[3]	1.00	1.16 (0.80, 1.66)	0.91 (0.62, 1.34)	0.75 (0.49, 1.13)	0.09
HR[4]	1.00	1.13 (0.78, 1.83)	0.87 (0.59, 1.30)	0.70 (0.46, 1.09)	0.06

[1] $n = 15\,555$ (280 breast cancer cases). 95% CIs in parentheses.
[2] Adjusted for age.
[3] Adjusted for age at enrollment, age at first full-term delivery, height, weight, parity, physical activity score, use of oral contraceptives or hormone replacement therapy, marital status, and academic education.
[4] Adjusted for age at enrollment, age at first full-term delivery, height, weight, parity, physical activity score, use of oral contraceptives or hormone replacement therapy, marital status, academic education, and daily energy intake.

lowest tertile of mammalian lignan intake, those in the highest tertile had a breast cancer hazard ratio of 0.59 (95% CI: 0.28, 1.27).

Apart from attempts to estimate dietary intakes of lignans, several studies focused on measurements of enterolactone in body fluids. We were unable to show protective effects of high urinary excretion of enterolactone in a comparable Dutch cohort for which we had no dietary data (20). Urinary excretion of enterolactone reflects not only the individual dietary intake but also the results of the metabolic process involving the bacterial colonic flora, which might be influenced by a recent use of antibiotics or gastrointestinal diseases. Urinary excretion reflects short-term intake (24–72 h) (41), whereas our FFQ reflected the habitual diet during the preceding year. We believe that when studying associations with cancer, habitual exposure to phytoestrogens may be more relevant.

Two other studies assessed serum or plasma enterolactone concentrations in relation to breast cancer risk (44, 45). Both of them showed slightly protective, nonsignificant results for high concentrations in older women, which is in accordance with the results of the present study.

Lignan intake in our study population was rather low (median: 0.7 mg/d). Similar intakes were reported in American women (29). True consumption is probably higher given the incompleteness of available food-composition tables. Consumption of vegetables, fruit, grain products, and nuts accounted for most of the lignan intake (26). Similar sources have been reported for Finnish (44) and American (24, 25) women. Because these food groups are frequently consumed in Western diets, it is reasonable to assume that the intake of lignans is stable over the course of a lifetime and occurs at younger ages also, which is important if lignan intake is relevant only at younger ages, as has been suggested for isoflavones. In conclusion, the results of this prospective study in a large cohort of Dutch women do not show protective effects of high dietary intakes of isoflavones or lignans against breast cancer risk.

LK-B, YTvdS, and PHMP provided the conceptual basis for the study and performed the data and statistical analyses. DEG supervised the study design and the statistical analysis. LK-B wrote the final manuscript, which was critically reviewed by all the other coauthors. No conflicts of financial or personal interest in any company or organization are reported.

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