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Research Article

Dietary Phytoestrogens and the Risk of Ovarian Cancer in the Women's Lifestyle and Health Cohort Study

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Abstract

Background: Dietary intake of phytoestrogens has been inversely associated to hormone-dependent cancers, such as prostate and breast cancers. Few studies have investigated the association between ovarian cancer and intake of phytoestrogens. We evaluated the associations between intake of phytoestrogens (isoflavonoids/lignans/coumestrol) and fiber (vegetable/cereal) and risk of ovarian cancer.

Methods: In 1991–1992 a prospective population-based cohort study among Swedish women was conducted, including 47,140 women with complete dietary questionnaire data. During follow-up until December 2007, 163 women developed invasive ($n = 117$) and borderline ($n = 46$) ovarian cancers. The median follow-up time was 16 years and total person year was 747,178. Cox proportional hazards models were conducted to estimate multivariate risk ratios, 95% CI for associations with risk of ovarian cancer.

Results: We found no association between intake of phytoestrogens or fiber and overall ovarian cancer risk. In addition, we found no statistically significant association between intake of specific food items rich in phytoestrogens (berries, nuts, beans/soy, and crisp or whole-grain bread) and ovarian cancer risk overall. Fiber and coumestrol was inversely associated with borderline ovarian cancer, but not with invasive ovarian cancer.

Conclusions: We found no association between intake of phytoestrogens or fiber and overall ovarian cancer risk.

Impact: Phytoestrogens do not play a major etiologic role in ovarian cancer, at least among women in this Swedish cohort with low bean/soy intake. However, our results of a difference in the effect of fiber or coumestrol between invasive and borderline ovarian cancer need to be evaluated in larger studies. *Cancer Epidemiol Biomarkers Prev; 20(2); 1–10. ©2011 AACR.*

Introduction

Phytoestrogens, compounds naturally found in plant foods and structurally related to endogenous estrogens, have been inversely associated to hormone-dependent cancers, such as prostate and breast cancers (1, 2). Few have investigated the relationship between ovarian cancer and intake of phytoestrogens (3, 4).

Isoflavonoids, a class of phytoestrogens found in soy-based foods, have estrogenic, antiestrogenic, and antiproliferative effects and inhibit the growth and proliferation of ovarian cancer (5–8). Results from a meta-analysis showed that high intake of isoflavonoids or soy-based food were associated with a decreased risk of ovarian cancer (4). Half of these studies were conducted in an Asian population were the intake of soy products are much higher than in a Western population. Dietary sources of isoflavonoids in Western populations are based on intake of other beans, pea, and various other vegetables and fruits. However, there is no clear etiologic role of vegetable intake in ovarian cancer (9). Many studies showed no association between vegetable intake and ovarian cancer risk (10–12), some found a protective effect (3, 13, 14), whereas some found an increased risk with high vegetable intake (15).

Lignans, another class of phytoestrogens, have a lower estrogenic activity than isoflavonoids (16), but have also antiestrogenic and anticarcinogenic effects (17). Compared with isoflavonoids, the intake of lignans is more in Western populations; some of the human dietary sources of lignans are flaxseed, grain (especially rye), seeds, and berries (18). In one small American case–control study, high intake of the lignan compound, matairesinol

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(MAT) and secoisolariciresinol (SECO), were found to be inversely related to ovarian cancer risk (3).

To our knowledge, only 1 cohort study (11) and 2 case–control (3, 19) studies have examine the intake of phytoestrogens in relation to ovarian cancer in a Western setting and no one have investigated the phytoestrogen compound, coumestrol. Furthermore, no study has yet included the newly identified lignan precursors lariciresinol, pinoresinol, syringaresinol, and medioresinol when calculating total intake of lignans. Lignans are especially found in the fiber-rich outer layer of cereal grains (20), and a diet high in fiber interferes with the enterohepatic metabolism of estrogens reducing their levels in the body (21). Use of antibiotics could affect the serum levels of bioactive phytoestrogens, because the colonic microflora is involved in the biotransformation of phytoestrogens (22, 23).

In this prospective, population-based study of Swedish women we examined the association between risk of ovarian cancer and dietary intake of total isoflavonoids, lignans, and coumestrol. We also evaluated the association between dietary intake of total fiber, cereal, and vegetable fiber and the risk of ovarian cancer.

Subjects and Methods

Study population

The study design and exposure assessment have been previously described in detail (24, 25). The source population of this study was women ages 30 to 49 years, residing in the Uppsala Health Care Region in Sweden between 1991 and 1992. From this source population, 96,000 women were randomly selected from 4 age strata (30–34, 35–39, 40–44, and 45–49 years) and were invited to participate in the Swedish component of the Scandinavian Women's Lifestyle and Health Cohort (WLH study). The women were asked to fill in a questionnaire, and of those invited, 49,259 returned the questionnaires and were enrolled in the study. The Swedish Data Inspection Board and the regional Ethical Committee approved the study.

Among the 49,248 Swedish women with dietary data included in the study, 171 epithelial invasive ($n = 122$) or borderline ($n = 49$) ovarian cancer cases were diagnosed by the end of follow-up in December 2007. The following were excluded, in order: subject who have undergone bilateral oophorectomy (information from the baseline questionnaire) and therefore not at risk for ovarian cancer ($n = 433$), those with energy intakes outside the first (1,852 kJ/day) and 99th (12,465 kJ/day) percentiles ($n = 977$), and those with missing values in any of the adjustment covariates ($n = 696$). A total of 47,140 women (117 invasive and 46 borderline ovarian cancer cases) were included in the final analysis. The median follow-up time was 16 years and total person year was 747,178.

Classification of phytoestrogens

Phytoestrogens are naturally occurring hormone-like compounds found in plant food and can be subdivided into coumestans, isoflavonoids, and lignans. Coumestrol (a coumestan) and the isoflavonoids genistein, daidzein, and their plant precursors biochanin A and formononetin, are mainly found in soybeans and clover. Plant lignans, such as MAT and SECO, are converted by the colonic microflora to mammalian lignans, now enterolignans (23), enterolactone, and enterodiol (20). Until recently, only 2 plant lignan precursors for mammalian lignans were known: SECO and MAT. Lariciresinol, pinoresinol, syringaresinol, and medioresinol are newly identified enterolactone precursors, found mainly in cereals and seeds.

Exposure assessment

Known and potential risk factors for ovarian cancer were assessed with a self-administered questionnaire, including average intake of foods and beverages (24). Dietary habits during the 6 months before the enrolment in the study were ascertained through a validated semi-quantitative food-frequency questionnaire (FFQ) that covered the frequency of consumption and quantity of approximately 80 food items and beverages, including 50 items containing phytoestrogens (26). For example, participants were asked how often, on average, they ate bean, soy, or lentil; green pea; pea soup; broccoli; cauliflower; white or red cabbage; spinach; onion or leek; carrot; swede or beetroot; porridge or gruel; wheat or oat bran; cereals or müsli, nut or almond; apple or pear: never/seldom, 1–3 times/month, 1 time/week, 2 times/week, 3–4 times/week, 5–6 times/week, 1 time/day, 2 times/day, or 3 times/day. The participants were also asked how many slices of bread they ate per day or week; wheat bread, whole meal bread (bread baked of coarse and whole meal flour), or crisp bread (mostly baked of rye meal). To estimate individual intake of energy and nutrients, we linked the dietary information from the questionnaire to the nutrient database created by the Swedish National Food Administration (food table of 1989; ref. 27). Furthermore, to estimate the intake of specific phytoestrogens, we created a database (25, 28) with information from published analytical data for the content of isoflavonoids (genistein, daidzein, biochanin A, formononetin, equol), coumestans (coumestrol), and lignans (MAT, SECO) in food products. The content of the lignans lariciresinol, pinoresinol, syringaresinol, and medioresinol in different grain flours was used to estimate lignan content of bread and cereal products, and added to the database. Information from recently published analytical data for the content of lariciresinol and pinoresinol in vegetables and fruits were added to the database (17, 29–31). Content of syringaresinol and medioresinol in vegetables and fruits were available only for tomato, orange, citrus, strawberry, and lingonberry (17). On the basis of the levels of genistein, daidzein, equol, MAT, enterodiol, and enterolactone in raw cow milk, we estimated the content of these compounds in different kinds of milk products, and the information was added to the database (28). Most of the analyses of phytoestrogen compounds in

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food products were carried out by isotope dilution gas chromatography–mass spectrometry performed in a laboratory in Finland (20, 31). Analyses of the content of lariciresinol and pinoresinol in vegetables and fruits were carried out by alkaline hydrolysis (29) followed by gas chromatography–mass spectrometry (20, 31) or by liquid chromatography–tandem mass spectrometry (29). In addition to the FFQ part of the questionnaire, participants were asked how often, on average, they ate berries: times/week, times/month, or seldom/never. The reported frequency together with an estimated portion size (115 g berries/portion for all women) was used to calculate intake of berries (g/day).

In 2002–2003, a second questionnaire was sent to those women who were alive and living in Sweden and of those 34,415 answered the follow-up questionnaire. The participants were asked about their use of antimicrobial medication, "How many times in your life have you been treated with penicillin or other antibiotics?" (never, 1–2 times, 3–10 times, more than 10 times).

Follow-up

Follow-up of the cohort was achieved through linkages with existing nationwide health registers in Sweden. Because each resident in Sweden has a unique national registration number, one can link the data from the cohort with these registers for virtually complete follow-up with respect to death and emigration. From the total population registers, we received information on the dates of death for women who died during the follow-up period and dates of emigration until December 31, 2007. The national cancer registry provided data on prevalent cancer cases at cohort enrolment and on epithelial invasive or borderline ovarian ((ICD7 = 175) cancer diagnosed in the cohort during follow-up and SNOMED (Systematized Nomenclature of Medicine) codes (Borderline: 84423, 84513, 84623, 84723; invasive: 80203, 81403, 82603, 83103, 83803, 84403, 84413, 84603, 84703, 826031, 838031, 838032, 838033, 844131, 844133, 846032, 846033, 847031, 847032). The start of follow-up was defined as the date of return of the questionnaire. Observation time was calculated from date of entry into the cohort until the occurrence of epithelial invasive or borderline ovarian cancer, emigration, death, or end of the observation period (December 31, 2007).

Statistical methods

The association between phytoestrogens and risk of ovarian cancer was evaluated by Cox proportional hazards models utilizing attained age as time scale (32). We interpreted relative hazards as estimates of relative risks (RR), given with 95% confidence intervals (CI). This corresponds to a 2-sided 5% level of significance.

Nutrient density was obtained through dividing the estimated intake of phytoestrogens (μg/day) and other nutrients or specific food items by the total energy intake (MJ/day; Multivariate Nutrient Density Model; ref. 33).

Food items with the highest lignan, isoflavonoid, or coumestrol content per edible portion within the FFQ were analyzed separately, and these food items were berries, nuts, beans (beans, soy, and lentil), or crisp and whole-grain bread. All exposures except coumestrol, bean, and nuts were categorized into quartiles and for each comparison of dietary intake; the lowest quartile was used as the reference category. Coumestrol, beans, and nuts were split into 3 categories because 50%–60% of the women had zero intakes, and they form the reference group. The remaining women were split into 2 equally large groups (cutoff at median: 0.014 μg/day·MJ for coumestrol, 0.73 g/day·MJ for beans, and 0.063 g/day·MJ for nuts). However, the correlation between coumestrol and bean intake was 1.0 and the number of women were equally divided into the categories of the coumestrol and the bean variable, indicating that the dietary source of coumestrol was exclusive beans, soy and lentils. Therefore, only results for coumestrol are presented.

Age- and energy-adjusted models were fitted, and models adjusted for additional potential confounders including body mass index (BMI), level of education, country of birth, smoking, use of oral contraceptives, age at menarche, parity, use of postmenopausal hormone replacement therapy, and selected food groups and nutrient densities categorized into quartiles. Variables in the models were continues or categorized as defined in Table 1. We initially tested all covariates, and selection of the covariates to be included in the final models was based on statistical significance and previous subject matter knowledge. Those included in the final models were known risk factors or considered to be important confounding factors for the relation between the main exposure and ovarian cancer, and are listed in the table footnotes (Tables 2 and 3). In analysis of the different phytoestrogen compounds we adjusted mutually for other classes of phytoestrogen (isoflavonoids/lignans/coumestrol). Also, in analysis of different types of fiber we mutually adjusted cereal fiber or vegetable fiber; however, the risk estimates did not change and therefore the variables were not included in the final model.

As we have only incomplete information about menopausal status after the start of follow-up, we considered the average age at menopause in Sweden, 50 years (34), as applicable for all cohort members. Menopausal status was evaluated by fitting separate models for ovarian cancer occurring at less or more than 50 years of age. Of the total 163 ovarian cancer cases, 58 occurred before 50 years of age. When analyzing the time course starting at 50 years of age 36,634 women were available for analysis (when censoring women with ovarian cancer before 50 years of age, women who died or emigrated or who were too young to reach 50 years at the end of follow-up). Among these 36,634 women, 105 ovarian cancer cases occurred.

In addition, we tested for interactions between phytoestrogens and use of antibiotics (≤2 times respective >2 times in life) to evaluate whether the effect of phytoestrogens was modified by antibiotic treatment.

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Table 1. Selected baseline characteristics of participants with questionnaire data in 1991–1992 in the Swedish Women's Lifestyle and Health Cohort Study

Characteristics	Cohort without ovarian cancer (n = 46,977)	Cohort with ovarian cancer (n = 163)
Age at entry, years, mean (SD)	40 (5.7)	43 (4.8)
BMI, kg/m^2, mean (SD)	23.5 (3.7)	24 (3.4)
BMI, kg/m^2, *n* (%)		
<25 (normal weight)	33,919 (72)	101 (62)
25–29.9 (overweight)	8,875 (19)	45 (28)
>=30 (obese)	2,475 (5)	10 (6)
Missing	1,708 (4)	7 (4)
Years of education, *n* (%)		
0–10	13,633 (29)	59 (36)
11–13	18,105 (38)	58 (36)
>13	14,351 (31)	44 (27)
Missing	888 (2)	2 (1)
Smoking status, *n* (%)		
Never	19,004 (41)	62 (38)
Ever	27,830 (59)	101 (62)
Missing	143 (0.3)	0 (0)
Use of oral contraceptives, *n* (%)		
Never	7,611 (16)	39 (24)
Ever	39,366 (84)	124 (76)
Parity, *n* (%)		
Nulliparous	6,516 (14)	25 (15)
1 child	7,258 (15)	22 (14)
2 children	20,304 (43)	64 (39)
3 children	9,752 (21)	41 (25)
≥4 children	3,147 (7)	11 (7)
Use of antibiotics, lifetime, *n* (%)		
Never	716 (2)	3 (2)
1–2 times	6,199 (13)	19 (12)
3–10 times	16,840 (36)	56 (34)
>10 times	9,078 (19)	20 (12)
Missing	14,144 (30)	65 (40)
Age at menarche, years, mean (SD)	13 (1.4)	13 (1.3)
Use of hormone replacement therapy		
Never	45,499 (97)	154 (95)
Ever	1,478 (3)	9 (5)
Ovarian cancer subtypes[a]		
Invasive	-	117
Borderline	-	46
Total energy intake, kJ, mean (SD)	6,520 (1,890)	6,140 (1,920)
Proportion of total energy intake,%		
Fat	31	31
Protein	17	17
Carbohydrate	52	52
Alcohol	1	1
Dietary intake, g/day, mean (5th–95th percentiles)		
Meat	80 (21–148)	74 (22–134)
Fish	19 (0–42)	17 (3–38)

(Continued on the following page)

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Table 1. Selected baseline characteristics of participants with questionnaire data in 1991–1992 in the Swedish Women's Lifestyle and Health Cohort Study (Cont'd)

Characteristics	Cohort without ovarian cancer (n = 46,977)	Cohort with ovarian cancer (n = 163)
Vegetables	87 (20–181)	86 (13–185)
Fruit	124 (11–310)	121 (11–334)
Beans	3 (0–10)	3 (0–10)
Total fiber	14 (7–23)	14 (6–24)
Vegetable fiber	5 (2–11)	5 (2–11)
Cereal fiber	8 (3–14)	8 (3–15)
Dietary phytoestrogen intake, μg/day, mean (5th–95th percentiles)		
Total isoflavonoids[b]	74 (2–247)	69 (2–247)
Total lignans[c]	2,336 (1,019–4,007)	2,317 (1,054–4,356)
Coumestrol	0.05 (0–0.2)	0.05 (0–0.2)

[a]See Subjects and Methods for definitions.
[b]Including genistein, daidzein, formononetin, biochanin A, and equol.
[c]Secoisolariciresinol (sum of anhydrosecoisolariciresinol and secoisolariciresinol), matairesinol, lariciresinol, isolariciresinol, pinoresinol, syringaresinol, and medioresinol.

Separate models were fitted for borderline respective invasive ovarian cancer. All analyses were done using the STATA version 10.1.

Results

Characteristics of study participants

Baseline characteristics and intake of nutrients among the study participants are presented in Table 1. As expected, women who developed ovarian cancer tended to be older, use oral contraceptives less frequently and use hormone replacement therapy more often than women who did not develop ovarian cancer. Ninety-seven percent of the women were born in one of the Northern countries (92% were born in Sweden; data not shown). Intake of main groups of macronutrients and dietary mean intake of fiber, meat, fish, vegetables, fruit, or beans were very similar between the 2 groups. The intake of phytoestrogens among women who developed ovarian cancer did not differ substantially from the intake among women who did not develop ovarian cancer (Table 1). In both groups, the mean daily intake of lignans was more than that of isoflavonoids and coumestrol and the highest median intakes of lignans were seen for syringaresinol and medioresinol (data not shown). Rye bread, wheat bread, cereals, and berries contributed the most to the intake of lignans, whereas beans (bean, soy, and lentil) were the most important dietary source of isoflavonoids and exclusive source for coumestrol (data not shown).

Dietary phytoestrogens, fiber, and epithelial invasive or borderline ovarian cancer risk

We found no statistically significant association between risk of ovarian cancer and dietary intakes of total lignan, total isoflavonoid compounds, or coumestrol (Table 2). There was no statistically significant association with risk of ovarian cancer for specific food items rich in phytoestrogens (berries, nuts, beans/soy, crisp and whole-grain bread). For example, the RRs comparing the highest to the lowest quartile of berry or crisp and whole-grain bread was 1.5 (95% CI: 0.95–2.32) and 1.48 (95% CI: 0.95–2.31), respectively. The RRs comparing the highest to the lowest category of nut intake was 0.88 (95% CI: 0.56–1.38; data not shown).

We found no statistically significant association between intake of total dietary fiber, vegetable fiber, or cereal fiber and ovarian cancer, although there was marginally significant decreased risk among women with an intermediate intake of total fiber. For example, the RR comparing the third to the lowest quartile of fiber intake was 0.67 (95% CI: 0.42–1.08; ref. Table 2). In additional analysis we found no association between risk of overall ovarian cancer and intake of total carbohydrates, total cereals products, or total vegetables (data not shown).

When borderline and invasive ovarian cancer cases were examined separately, the RRs comparing the highest to the lowest quartile of isoflavonoids, coumestrol, total fiber, cereal fiber, or vegetable fiber intake were different between the 2 subtypes of ovarian cancer, with a tendency of a protective effect for borderline ovarian cancer but not for invasive cancer (Table 3). For example, for intake of coumestrol, there was a decreased risk for borderline ovarian cancer among women with the highest intake of coumestrol (RR = 0.29; 95% CI: 0.09–0.94) compared with women with no intake; however, after multivariate adjustment the inverse relationship was only marginally significant (RR = 0.32; 95% CI: 0.10–1.07). The corresponding RR for invasive ovarian cancer was 1.47 (95% CI: 0.94–2.29),

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Table 2. Risk of epithelial ovarian cancer in relation to estimated dietary intake of total lignans, total isoflavonoids, coumestrol, and fiber, estimated as RR with 95% CI

			Age and energy adjusted		Multivariate[c]	
Dietary intake[a]	Mean (interquartile range)	Cases/person years	RR[b]	95% CI[b]	RR[b]	95% CI[b]
Total lignans, μg/d·MJ[d]	225 (13–282)	32/185,592	1.00	Reference	1.00	Reference
	317 (283–351)	48/187,176	1.35	0.86–2.11	1.35	0.85–2.13
	389 (352–431)	37/187,507	0.97	0.60–1.56	0.97	0.59–1.59
	528 (432–1,713)	46/186,904	1.09	0.69–1.72	1.04	0.63–1.72
Total isoflavonoids, μg/d·MJ[e]	0.5 (0–0.73)	34/186,437	1.00	Reference	1.00	Reference
	0.9 (0.74–1.1)	38/187,165	1.07	0.67–1.70	1.09	0.67–1.77
	6.5 (1.2–15)	48/186,852	1.37	0.88–2.13	1.47	0.90–2.38
	38 (16–750)	43/187,725	1.15	0.74–1.81	1.26	0.79–2.01
Coumestrol, μg/d·MJ[f]	None	99/469,949	1.00	Reference	1.00	Reference
	<0.014	32/139,436	1.20	0.79–1.81	1.25	0.82–1.90
	≥0.014	32/137,794	1.03	0.69–1.54	1.10	0.73–1.65
Total fiber, g/d·MJ	1.6 (0–1.82)	43/185,414	1.00	Reference	1.00	Reference
	2.0 (1.83–2.1)	40/187,271	0.86	0.56–1.33	0.87	0.56–1.35
	2.2 (2.2–2.4)	34/187,568	0.68	0.43–1.07	0.67	0.42–1.08
	3.0 (2.5–10)	46/186,926	0.84	0.55–1.27	0.82	0.50–1.35
Cereal fiber, g/d·MJ	0.8 (0–0.9)	35/186,112	1.00	Reference	1.00	Reference
	1.1(1.0–1.2)	49/186,810	1.41	0.91–2.18	1.42	0.92–2.21
	1.3 (1.3–1.4)	33/187,351	0.92	0.57–1.48	0.93	0.57–1.51
	1.8 (1.5–5.7)	46/186,905	1.19	0.77–1.85	1.17	0.74–1.87
Vegetable fiber, g/d·MJ	0.4 (0–0.57)	38/186,324	1.00	Reference	1.00	Reference
	0.7 (0.58–0.78)	39/186,987	0.96	0.62–1.51	1.01	0.64–1.59
	0.9 (0.79–1.0)	41/187,278	0.94	0.61–1.47	1.00	0.63–1.58
	1.4 (1.1–9.5)	45/186,589	0.94	0.60–1.45	1.02	0.63–1.64

[a]Exposure is categorized into quartiles and the lowest quartile was used as the reference category.
[b]RR (95% CI) was obtained by Cox proportional hazards models.
[c]Adjusted for age, oral contraceptives, age at menarche, parity, hormone replacement therapy, and intake of total energy intake, alcohol, saturated fat, meat, and fish.
[d]Secoisolariciresinol, matairesinol, lariciresinol, isolariciresinol, pinoresinol, syringaresinol, and medioresinol.
[e]Including genistein, daidzein, formononetin, biochanin A, and equol.
[f]Intake of coumestrol is categorized into 3 groups, where the reference group contains those with zero intakes.

and the point estimate for borderline ovarian cancer was outside this CI, indicating a difference of the coumestrol effect for the 2 subtypes of ovarian cancer. For lignans the estimates were similar across sub-types of ovarian cancer (Table 3) and also for nuts, berries and crisp and whole-grain breads (data not shown).

For a subset of the women with information on use of antibiotic we carried out stratified analysis and examined heterogeneity of the association with phytoestrogens and ovarian cancer, between those with no or low intake of antibiotics (≤2 times in life) and those with high intake of antibiotics (>2 times in life). Although the point estimates were different for isoflavonoids, coumestrol, and specific food items rich in phytoestrogens, between the groups of antibiotic intake there was no significant heterogeneity for any association with phytoestrogen intake between the 2 groups of antibiotic intake. For example, the RR comparing the highest to the lowest quartile of crisp and whole-grain bread intake was 0.4 (95% CI: 0.16–1.20) and 1.3 (95% CI: 0.75–2.36) for those with low and high intake of antibiotics, respectively ($P = 0.12$; data not shown).

We repeated all analyses separately for women ages less or more than 50 years, and the estimates were similar across age groups (data not shown). However, for all stratified analyses the sample sizes were small, leading to unstable estimates.

Table 3. Risk of epithelial invasive or borderline ovarian cancer in relation to estimated dietary intake of total lignans, total isoflavonoids, coumestrol, and fiber, estimated as RR with 95% CI*. Exposures are categorized into quartiles and the lowest quartile was used as the reference category[a]

Dietary intake (μg/day·MJ)	Invasive ovarian cancer					Borderline ovarian cancer				
	Cases/ person years	Age and energy adjusted		Multivariate[c]		Cases/ person years	Age and energy adjusted		Multivariate[c]	
		RR[b]	95% CI[b]	RR[b]	95% CI[b]		RR[b]	95% CI[b]	RR[b]	95% CI[b]
Total lignans[d]	23/185,509	1.00	Reference	1.00	Reference	9/185,387	1.00	Reference	1.00	Reference
	34/187,073	1.31	0.77–2.23	1.29	0.75–2.22	14/186,866	1.46	0.63–3.38	1.49	0.63–3.51
	24/187,383	0.87	0.49–1.55	0.84	0.46–1.52	13/187,278	1.24	0.53–2.90	1.33	0.54–3.27
	36/186,813	1.22	0.72–2.07	1.06	0.59–1.90	10/186,536	0.78	0.31–1.93	0.95	0.35–2.58
Total isoflavonoids[e]	22/186,329	1.00	Reference	1.00	Reference	12/186,257	1.00	Reference	1.00	Reference
	24/187,064	1.02	0.57–1.82	1.03	0.57–1.87	14/186,902	1.17	0.54–2.54	1.26	0.57–2.82
	34/186,732	1.42	0.83–2.45	1.44	0.80–2.59	14/186,541	1.25	0.58–2.72	1.61	0.68–3.80
	37/186,654	1.51	0.89–2.56	1.58	0.91–2.74	6/186,366	0.49	0.18–1.29	0.59	0.22–1.62
Coumestrol	66/469,675	1.00	Reference	1.00	Reference	33/469,317	1.00	Reference	1.00	Reference
	22/139,351	1.11	0.68–1.84	1.16	0.67–1.85	10/139,229	1.50	0.71–3.16	1.56	0.74–3.33
	29/137,752	1.41	0.91–2.18	1.47	0.94–2.29	3/137,521	0.29	0.09–0.94	0.32	0.10–1.07
Total fiber	27/185,299	1.00	Reference	1.00	Reference	16/185,167	1.00	Reference	1.00	Reference
	28/187,158	0.95	0.55–1.61	0.94	0.55–1.62	12/187,027	0.72	0.34–1.53	0.75	0.35–1.62
	24/187,476	0.76	0.44–1.32	0.72	0.40–1.30	10/187,315	0.55	0.25–1.22	0.59	0.25–1.37
	38/186,845	1.13	0.68–1.85	1.06	0.59–1.89	8/186,558	0.37	0.16–0.87	0.41	0.16–1.09
Cereal fiber	23/186,018	1.00	Reference	1.00	Reference	12/185,904	1.00	Reference	1.00	Reference
	35/186,701	1.49	0.88–2.53	1.49	0.87–2.53	14/186,488	1.27	0.59–2.76	1.32	0.61–2.89
	22/187,233	0.90	0.50–1.62	0.88	0.49–1.61	11/187,136	0.98	0.43–2.23	1.05	0.45–2.43
	37/186,827	1.44	0.85–2.42	1.37	0.79–2.39	9/186,539	0.70	0.30–1.67	0.74	0.30–1.85
Vegetable fiber	23/186,206	1.00	Reference	1.00	Reference	15/186,108	1.00	Reference	1.00	Reference
	27/186,889	1.08	0.62–1.89	1.12	0.64–1.97	12/186,729	0.80	0.37–1.71	0.86	0.39–1.88
	30/187,189	1.14	0.66–1.96	1.17	0.66–2.06	11/187,011	0.66	0.30–1.44	0.75	0.33–1.69
	37/186,495	1.31	0.77–2.22	1.35	0.76–2.39	8/186,219	0.40	0.17–0.94	0.50	0.20–1.27

[a]Intake of Coumestrol is categorized into 3 groups, where the reference group contains those with zero intakes.
[b]RR (95% CI) was obtained by Cox proportional hazards models.
[c]Adjusted for age, oral contraceptives, age at menarche, parity, hormone replacement therapy, and intake of total energy intake, alcohol, saturated fat, meat, and fish.
[d]Secoisolariciresinol, matairesinol, lariciresinol, isolariciresinol, pinoresinol, syringaresinol, and medioresinol.
[e]Including genistein, daidzein, formononetin, biochanin A, and equol.

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Discussion

In this large population-based prospective cohort study, we found no evidence that specific phytoestrogens or fiber intake are associated with overall ovarian cancer risk or ages less or more than 50 years. However, we detected a difference in the effects of phytoestrogens or fiber between invasive and borderline ovarian cancer risk.

We found a marginally inverse association between high intake of fiber or coumestrol (mainly reflecting bean intake) and borderline ovarian cancer risk, but not for invasive ovarian cancer risk. To our knowledge, such a finding has not been reported before. However, the sample sizes were small, leading to unstable estimates. Also, there are few studies on diet (non- on phytoestrogens) and ovarian cancer, which has separated borderline and invasive ovarian cancer, to compare our results with and it is therefore difficult to rule out whether these are chance findings. In a case–control study, an association was found between egg cholesterol and invasive ovarian cancer risk, but not for borderline ovarian cancer risk (35). Furthermore, in the same study, the observed inverse relationship for vegetable fiber did not differ between borderline or invasive cancer risks. In another case–control study, galactose consumption was associated with an increased risk of borderline, but not invasive ovarian cancer (36). However, in case–control studies with retrospective collected dietary data, it is difficult to rule out whether dietary differences between invasive and borderline cancer cases are due to etiologic differences or due to disease related changes as a result of a more symptomatic stage of disease among invasive cancer cases. However, results from the prospective US Nurses Health study indicate that associations with several ovarian cancer risk factors vary by histologic subtype, and these differences were consistent with known similarities between each major histologic subtype and its normal tissue counterpart (37). If tumors with low malignant potential (borderline ovarian tumors) are not considered as an intermediate precursor in the progression to high-grade ovarian tumors, it is conceivable that invasive and borderline tumors may have different risk factors as well. There is growing evidence that low- and high-grade serous carcinomas are distinctly different neoplasms with different pathogenesis, behavior, and response to treatment (38, 39). Also, it is possible that the progression to high-grade tumors differs between the histologic subtypes of ovarian cancer (40–42). However, prospective studies with sufficient power are needed to rule out if exogenous factors influence low-grade tumors differently than high-grade tumors.

The lack of association between total lignans and ovarian cancer risk in this study does not confirm earlier findings from the only study that has investigated lignan intake in relation to ovarian cancer risk (3). In this study, McCann and colleagues found a reduced risk of ovarian cancer among women with high intake of the lignan compounds SECO and MAT. Discrepancy between the study by McCann and ours are as follows: first, the dietary sources of lignan differs—in the McCann study the largest contributors were coffee, carrots, cucumber, and strawberry, whereas in our study the lignan sources were rye bread, wheat bread, cereals, and berries. This difference is probably a result of a larger intake of bread and cereals in our study population, but also due to that we included other lignan compounds than SECO and MAT in the total lignan intake. This is strengthening by the lack of association for grain products in the McCann study. Second, the study by McCann was a case–control study, a study design that is more vulnerable to certain bias than cohort studies, for example, recall bias.

We found no association between intake of isoflavonoids and ovarian cancer and this result is not in agreement with earlier findings of a protective effect of soy and isoflavonoid intake on ovarian cancer risk (4). Most of the earlier studies were conducted in populations with a higher intake of soy products, for example, in our cohort the median value of the upper tertile of intake for bean, soy, and lentil was 4.9 g/day, which is 10 times less than the lowest quartile of intake among women in a Chinese case–control study (43). However, an inverse relationship between isoflavonoids and risk of ovarian cancer was found among Italian women (19), who have a lower intake of isofalvonoids than Asian women. In the Italian study, the inverse relationship was only seen for women in the highest quintile of isofalvonoids intake, which was in fact less than the highest intake among women in our cohort. The largest dietary contributors to the isoflavonoid intake in the Italian study was soy and soy milk (19), food items that is very uncommon in the Swedish diet, and the contributors to the isoflavonoid intake in our study was most likely other types of beans or vegetables. These differences in dietary habits could maybe be one of the reasons for lack of an association between isoflavonoids and risk of ovarian cancer in our study, or again, differences in study designs.

In this study, we found no association between ovarian cancer risk and phytoestrogen-rich food items such as berries, nuts, beans, crisp bread, or whole-grain bread. For comparison, some other studies, investigating dietary pattern, have reported an increased risk for a starch-rich (44), "bread and pasta" (14), or high carbohydrate dietary pattern (15), whereas others found no relationship with ovarian cancer risk and intake of grain products (3). Furthermore, several studies have examined the role of vegetables and fruit for the risk of ovarian cancer, and their results are inconsistent with some of them showing no association (10–13), an inverse association (3, 13, 14) or a positive association (15, 45). In addition, we found no association between risk of ovarian cancer and intake of total carbohydrates, total cereals, or total vegetables. Thus, the results in this study and current literature do not provide any strong or consistent evidence for that phytoestrogen-rich foods play a major role in ovarian cancer development, at least among women with a low bean/soy intake.

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Comparison between studies of dietary phytoestrogen intakes and disease is limited by differences in phytoestrogen databases used in various studies. Databases often differ in calculation methods, chosen references, analytical methods, and origin of analysis of phytoestrogens in food items. A strength of this study is that we used a phytoestrogen database designed for a Swedish population (28) and that most of the phytoestrogen content in the foods were analyzed by 2 gas chromatography–mass spectrometric methods and carried out in the same laboratory (20).

Strengths of our study include its prospective design, large size, and complete follow-up. The ethnic homogeneity of our study population (only 3% of the women were born outside the northern countries) reduces the risk of confounding by unmeasured factors. Cancer registration in Sweden is obligatory, making the assessment of cases almost complete. We adjusted for several known nutritional and nonnutritional risk factors for ovarian cancer. Also, we were able to take use of antibiotics into count, because antibiotics affect the bacterial microflora in the gut (22) that transforms plant lignans into mammalian lignans (46); this could have an effect of phytoestrogens metabolism. However, the information on antibiotics was not available for the whole cohort, leading to small sample size and unstable estimates. Furthermore, information about antibiotic use was based on life-time consumption and we do not have any information on the specific time of consumption. If antibiotic use is distant, in time, from ovarian cancer development, antibiotic treatment may not have an impact on the possible effect of phytoestrogens on ovarian cancer. However, a large proportion (19%) of the women had used antibiotics more than 10 times in life and lignan metabolism could be affected by antibiotic use up to more than a year after consumption (22). Also, equol production is reduced by antibiotics (23). Thus, at least for those women with frequent antibiotic use, the antibiotic treatment could have influenced the production of bioactive phytoestrogens in the gut and also influenced the metabolism of the phytoestrogens. Even if we do not have the power to conclude, our results for crisp and whole-grain bread indicates that lignan containing foods are protective only among low consumers of antibiotics.

We have only baseline dietary information, and misclassification of dietary intake maybe greater than if we had measured the diet at several points through the follow-up time. However, the latency time for ovarian cancer is probably decades and our baseline measures may reflects diet in the past, which may be the exposure of interest.

In conclusion, we found no association between overall ovarian cancer risk and intake of isoflavonoids, lignans, and coumestrol, specific food items rich in phytoestrogens or fiber. Our overall results suggest that phytoestrogens does not play a major etiologic role in ovarian cancer, at least in this predominantly Caucasian women cohort with low bean/soy intake. However, the differences in the effect of diet between invasive and borderline ovarian cancer need to be evaluated in other studies with higher power.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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