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Original Contribution

Fiber-type prebiotics and gynecological and breast cancers risk: the PrebiotiCa study

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Abstract

Prebiotics may influence the risk of hormone-related female cancers by modulating the gut microbiota involved in estrogen metabolism. We evaluated the association of fiber-type prebiotic intake with breast, endometrial, and ovarian cancers. Data derived from a network of Italian hospital-based case-control studies (1991-2006), including 2560 cases of cancer of the breast ($n = 2588$ control participants), 454 of the endometrium ($n = 908$ control participants), and 1031 of the ovary ($n = 2411$ control participants). Inulin-type fructans and selected fructo-oligosaccharides (namely, nystose, kestose, and 1F-β-fructofuranosylnystose) and galacto-oligosaccharides (namely, raffinose and stachyose) were quantified in food products *via* laboratory analyses. Prebiotic intake was estimated by multiplying intake according to food frequency questionnaire responses by the foods' prebiotic content. Odds ratios (ORs) and the corresponding 95% CIs were derived by multiple logistic regression models. Nystose intake was marginally directly associated with breast (for quartile 4 vs quartile 1: OR = 1.20; 95% CI, 1.00-1.45), ovarian (OR = 1.39; 95% CI, 1.04-1.84), and endometrial (OR = 1.32; 95% CI, 0.85-2.03) cancer risk. High amounts of 1F-β-fructofuranosylnystose intake were inversely associated with ovarian cancer (OR = 0.67; 95% CI, 0.52-0.85). Inulin-type fructans, kestose, raffinose, and stachyose were not associated with the 3 cancers. The intake of most fiber-type prebiotics was not appreciably and consistently associated with breast, endometrial, and ovarian cancer risks.

This article is part of a Special Collection on Gynecological Cancer.

Key words: breast cancer; ovarian cancer; endometrial cancer; risk; diet; prebiotics.

Introduction

With an estimated 2.3 million new cases, breast cancer was the most commonly diagnosed cancer worldwide in 2020. In the same year, endometrial and ovarian cancers ranked, respectively, sixth and eighth in cancer incidence, with more 417 000 and 313 000 cases. Together, these cancers account for one-third of all cancer diagnoses in women.[1]

Breast, endometrial, and ovarian cancer risks are, to various degrees, linked with reproductive and hormonal factors. Among them are early age at menarche, late age at menopause, nulliparity, and use of postmenopausal hormone replacement therapy (HRT), which contribute to the lifetime estrogen exposure.[2-4] Use of oral contraceptives (OCs) is associated with a modest, short-term increased risk of breast cancer, but with reduced long-term risks of endometrial and ovarian cancers.[5,6] In addition, excess body weight[7,8] and, possibly, metabolic disorders, including diabetes[9-12] and the metabolic syndrome,[13] are risk factors for these neoplasms.

With regard to diet, various studies indicated that high intakes of fruit and vegetables and related compounds, including fibers, are associated with reduced risks of breast[14,15] and endometrial cancer.[16,17] Evidence on diet and ovarian cancer relationship is in the same direction, though less consistent.[18-20] A role of the gut microbiota in estrogen-modulated diseases has been suggested.[21-23] In the gastrointestinal lumen, estrogens are deconjugated to their free "active" forms through bacterial secretion of β-glucuronidase and partially absorbed.[24] The aggregate of gut bacterial genes responsible for estrogen metabolism is called the estrobolome. Estrabolome enriched in β-glucuronidase–producing bacteria would promote absorption of free estrogens and thus increase levels of circulating estrogens, potentially influencing the development of estrogen-related cancers,[23,24] including breast, endometrial, and ovarian cancers.[25]

Diet influences the gut microbiota.[26,27] In particular, fiber is not digested or absorbed in the small intestine but is fermented by the gut microbiota, potentially modulating the gut bacterial

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composition and the microbial metabolic activities. Diets high in plant foods and fiber have been associated with reduced serum estrogen levels,[28-30] possibly by decreasing the activity of bacterial β-glucuronidase enzymes.

Specific types of fiber, such as inulin-type fructans (ITFs), fructo-oligosaccharides (FOSs), and galacto-oligosaccharides (GOSs), can be further classified as prebiotics. Prebiotics are defined as "substrates selectively used by host microorganisms conferring health benefits"[31]; they stimulate the growth and activity of beneficial bacteria in the gut, particularly Bifidobacteria and Lactobacilli.[31,32] Although studies have suggested an involvement of gut microbiota on the development of breast,[33] endometrial,[34] and ovarian cancers,[35] a role for those specific bacteria has not been yet established. However, probiotics containing *Lactobacillus acidophilus* may reduce bacterial β-glucuronidase enzymes activity,[36] thus possibly affecting estrogen levels.

ITFs, GOSs, and FOSs are distributed widely in a large variety of plant-based products; however, systematic quantification of these prebiotics in foods has been conducted rarely.[37-40] In addition, very limited data exist on prebiotic intake in populations.[41-43]

The PrebiotiCa ("The role of Prebiotics in the prevention of Cancer, an integrated network of Italian case–control studies") study was designed to assess whether a diet rich in fibers with prebiotic activity may reduce cancer risk.[44-46] It is based on a network of Italian case-control studies on various common neoplasms which collected detailed dietary information through a food frequency questionnaire (FFQ). To derive study participants' prebiotic intake, we quantified ITFs, GOSs, and FOSs in a wide range of raw and processed foods.[47] In the present investigation within the PrebiotiCa study, we investigated the association between the intake of selected fiber-type prebiotics (ie, ITFs; 3 FOSs: nystose, kestose, and 1F-β-fructofuranosylnystose; and 2 GOSs: raffinose and stachyose) and the risk of breast, endometrial, and ovarian cancers.

Methods

Study design and data collection

Data for the present analysis were derived from 3 hospital-based case-control studies conducted between 1991 and 2006 in different areas of Italy. In particular, the study on breast cancer was conducted in the metropolitan area of Milan; the urban area of Genoa; the provinces of Pordenone, Gorizia, Forlì, and Latina; and the urban area of Naples. The study on endometrial cancer was conducted in the metropolitan area of Milan, the provinces of Pordenone and Udine, and the urban area of Naples. And the study on ovarian cancer was conducted in the metropolitan area of Milan; the provinces of Pordenone, Padua, Gorizia, and Latina; and the urban area of Naples. Incident histologically confirmed cases according to the *International Classification of Diseases, Ninth Revision* (code 174 for breast cancer, 182 for endometrial cancer, and 183 for ovarian cancer) and admitted to the major teaching and general hospitals were considered. The control group consisted of women admitted to the same hospital network as the case patients. They were admitted for a wide range of acute, non-neoplastic conditions (ie, trauma; other orthopedic conditions; acute surgical conditions; and miscellaneous conditions, including eye, nose, ear, or skin conditions) not related to hormonal, gynecological, or digestive disorders; nutritional conditions; or other known risk factors for each specific cancer site.

More than 95% of the women contacted agreed to participate. We thus included a total of 2569 case patients with cancer of the breast and a corresponding control group of 2588 participants; 454 case patients with cancer of the endometrium and 908 control participants; and 1031 case patients with cancer of the ovary and a 2411 control participants.

A structured questionnaire was used by centrally trained interviewers to collect data from case patients and control participants during their hospital stay. Data collected included sociodemographic and anthropometric information, including self-reported height and weight 1 year prior to diagnosis or interview, selected lifestyle behaviors (ie, alcohol consumption, tobacco smoking, and physical activity), general clinical history, family history of cancer, reproductive and menstrual factors, and use of OCs and HRT. Dietary habits in the 2 years prior to cancer diagnosis (or prior to hospital admission for control participants) were assessed through an FFQ including 78 items. Satisfactory reproducibility[48,49] and validity[50] of the FFQ have been shown. The participants were asked to recall their usual frequency of intake of each dietary item weekly, taking into account variation in seasonal consumption of fruit and vegetables. Occasional use, defined as less than once weekly but at least once monthly, was coded as 0.5 per week.

Prebiotic determinations in foods

The methodology used to quantify prebiotic fibers in foods is detailed elsewhere.[47] The content of the FOSs (nystose, kestose, and 1F-β-fructofuranosylnystose) and GOSs (raffinose and stachyose) were determined in 78 food sources, including 15 types of fruits; 32 varieties of vegetables, root vegetables, and tubers; 9 types of dried, canned, or fresh legumes; and 22 types of cereals and cereal-based products (both whole-grain and refined products). ITFs were determined in 7 food sources: fresh onion, garlic, banana, leek, Jerusalem artichoke, artichoke, and shallot. Food sampling and analysis were performed by Neotron SpA.

ITFs were determined using an internal analytical method based on AOAC International's 997.08 procedure.[51] Enzymatic hydrolysis and high-performance anion exchange chromatography coupled to pulsed amperometric detection (HPAE-PAD) were used. The limit of detection was < 0.5 g per 100 g. FOSs and GOSs in fresh samples were determined by the method of Aggrawal and Rohrer based on alkaline hydrolysis and HPAE-PAD detection.[52] From the homogenized sample, 1 g was extracted with 200 mL of sodium hydroxide 0.0025 M and then analyzed using HPAE-PAD method. The limit of detection was between < 0.002 and < 0.010 g per 100 g.

GOSs were abundant in legumes.[47] In particular, stachyose was detected in appreciable amounts (per 100 g) in legumes only, with amounts highest in dried beans, peas, and chickpeas (∼ 1.6-1.9 g), followed by fresh beans (1.27 g), lentils, fresh peas, and fresh chickpeas (∼ 0.27-0.41 g). Raffinose was abundant in legumes, especially dried peas and chickpeas (∼ 0.46-0.5 g) and found also in whole-meal flour (0.3 g), selected whole-grain based products (∼ 0.2), and barley (0.22 g), as well as in white wheat flour and wheat products, but in lower amounts (0.04 g). As for dietary FOSs, kestose was abundant in shallot, garlic, whole-wheat pasta, whole-meal biscuits, banana, and barley (range, ∼0.54-0.15 g per 100 g) and was found in low or undetectable amounts in legumes. Nystose and, in particular, 1F-β-fructofuranosylnystose were detected in small concentrations in a few foods (eg, shallot, garlic, barley).[47]

Prebiotic and nutrient intake

Total energy and nutrient intake were calculated by multiplying the reported frequency of consumption of food items by standard

portion size and nutrient content per 100 g based on an Italian food composition database.[53,54] For FOSs, GOSs, and ITFs intake, we used laboratory data obtained as previously described.

Accounting for amount consumed, 42% of raffinose intake came from cereal-based products, 15% from fruit, and 11% from legumes. The major sources of stachyose were legumes (34%), soups (31%), and cereal-based products (31%). As for FOSs, kestose derived mainly from cereal-based products and fruit, in similar proportions (35%-37%); 64% of nystose intake came from cereal-based products (55% from pasta), and fruit and vegetables contributed, respectively, to 9% and 7% of the intake; fruit (almost exclusively banana) accounted for 57% of ITFs intake. The major source of 1F-β-fructofuranosylnystose intake was desserts (70%), with vegetables accounting for almost the remaining 30%.

Statistical analysis

Unconditional multiple logistic regression models were fitted to calculate the odds ratios (ORs) and the corresponding 95% CIs of breast, ovarian, and endometrial cancers according to quartiles (calculated among control participants) of prebiotic intake. Tests for trends across quartiles were performed by including the examined variable as ordinal. The models included terms for study areas; year of interview; age (in 5-year groups); years of education (<7, 7–11, ≥12); alcohol drinking (0, 0.1-7, ≥7 drinks per day); tobacco smoking status (never, former, current smokers); body mass index (< 20, 20-24.9, 25-29.9, ≥30 kg/m^2); total energy intake (in quartiles); family history of breast, endometrial, or ovarian cancer; age at menarche (<12, 12-13,14-15, ≥16 years); menopausal status; parity (0, 1, 2, ≥3 children); and use of OCs and HRT. All the analyses were conducted with the SAS software, version 9.4 (SAS Institute).

Results

The distribution of breast, endometrial, and ovarian cancer case patients and corresponding control participants by selected factors is provided in Table 1. Patients with breast or ovarian cancer had a higher educational level than the control participants; endometrial cancer case patients reported higher body mass index and were less frequently OCs users than their corresponding control participants; for all cancer types, the prevalence of family history was higher in case patients than in control participants.

Table 2 shows the distribution of fiber-type prebiotics in case patients and control participants in the 3 studies. The median values (per day) ranged from 0.714 to 0.867 g for ITFs, from 0.151 to 0.164 g for kestose, from 0.013 to 0.015 g for nystose, from 0.002 to 0.003 g for 1F-β-fructofuranosylnystose, from 0.085 to 0.092 g for raffinose, and from 0.176 to 0.181 g for stachyose.

Table 3 provides the ORs and the corresponding 95% CIs of breast, ovarian, and endometrial cancers according to prebiotic intake. The intake of nystose was directly associated with breast and ovarian cancers, with ORs for the fourth quartile vs the first quartile of intake of 1.20 (95% CI, 1.00-1.45) and 1.39 (95% CI, 1.04-1.84), respectively. The OR was nonsignificantly above unity (OR = 1.32; 95% CI, 0.85-2.03) for endometrial cancer. A high intake of 1F-β-fructofuranosylnystose was inversely associated with the risk of ovarian cancer (OR = 0.67; 95% CI, 0.52-0.85). Kestose (an FOS), ITFs, raffinose (a GOS), and stachyose (a GOS) were not associated with the 3 cancer sites. Similar results were obtained with further adjustment for total fiber intake (data not shown).

Discussion

In the present investigation, based on a network of Italian case-control studies, the intake of fibers with widely accepted prebiotic activity was not appreciably and consistently associated with the risk of breast, endometrial, and ovarian cancers. The moderate direct association between nystose intake and breast, ovarian, and endometrial cancers is of uncertain interpretation, given the lack of association between the other prebiotics of the FOSs family and breast cancer, the apparent inverse association between the 1F-β-fructofuranosylnystose (an FOS) and ovarian cancer, and concerns with the validity of estimates of the intake of such prebiotics. In addition, the 1 significant direct and the 1 significant inverse association may be due simply to multiple testing.

To our knowledge, this is the first study evaluating dietary prebiotics consumption in relation to hormone-related female cancers. Previous analyses from the same network of studies found an inverse association between the intake of GOSs and the risk of colorectal[46] and laryngeal cancers,[45] and suggested an inverse association between the intake of raffinose (a GOS) and stomach cancer[44]; prebiotic fibers, however, were not associated with upper digestive tract cancers.[44]

Almost two-thirds of nystose intake derived from cereal-based products, mainly pasta. Although the FFQ distinguished between whole-grain and non–whole-grain cereals only for bread, cereals products are consumed largely in the form of refined grains in our population. Indeed, although almost 90% of control women in the breast cancer study database reported consuming white bread, and 76% to eat at least 1 portion of white bread per day, consumption of whole-grain bread was reported by less than 20% of women, with only 13% declaring to eat at least 1 portion per day. Similar proportions were found in the ovarian cancer study database.

Along this line, we previously reported that a high intake of starch[55,56] and of starchy foods (eg, bread; cereal dishes, including pasta)[57,58] was associated with increased risks of breast and ovarian cancers. Other studies suggested that high consumption of dietary carbohydrates, cereals, and refined grains increase the risk of breast, ovarian, and endometrial cancer,[59-62] but the evidence is largely inconclusive.[63-67] Of note, an estrogen-related dietary pattern developed within the Prostate, Lung, Colorectal and Ovarian Screening Trial and characterized by high consumption of non-whole or refined grains was directly related with postmenopausal breast cancer.[68] Nystose was positively correlated with glycemic load in our study (ie, correlation coefficients of 0.48, 0.52, and 0.53, respectively, in the breast, ovarian, and endometrial cancer study databases), and diets with high glycemic load have been associated with increased risks of endometrial, ovarian, and breast cancers.[69-71] The effect could be linked to hyperinsulinemia, which may increase the expression of insulin-like growth factor, a promoter of the process of carcinogenesis.[72]

Our investigation is based on a network of case-control studies, and potential weaknesses are selection and information biases. However, participants admitted to hospitals for conditions related to chronic and gynecologic conditions or diseases related to diet modifications or known risk factors for breast, ovarian, and endometrial cancers were not eligible as control participants; case ascertainment in the catchment areas was almost complete; and participation was satisfactory. Information bias was minimized by the direct interview of case patients and control participants by the same trained interviewers in similar hospital conditions. We collected dietary data 2 years before participant interviews to reflect lifelong dietary habits before potential changes related

Table 1. Distribution of cases of breast, endometrial, and ovarian cancers and corresponding controls according to selected characteristics: Italy, 1991-2006

Variable	Breast[a]		Endometrium[a]		Ovary[a]	
	Case patients (*n* = 2569)	Control participants (*n* = 2588)	Cases patients (*n* = 454)	Control participants (*n* = 908)	Cases patients (*n* = 1031)	Control participants (*n* = 2411)
Age (years)						
<50	839 (32.7)	769 (29.7)	67 (14.8)	134 (14.8)	297 (28.8)	720 (29.9)
50-59	809 (31.5)	808 (31.2)	140 (30.8)	280 (30.8)	341 (33.1)	694 (28.8)
≥60	921 (35.9)	1011 (39.1)	247 (54.4)	494 (54.4)	393 (38.1)	997 (41.4)
Education (years)[b]						
<7	1259 (49.3)	1569 (61.2)	263 (57.9)	553 (60.9)	577 (56.0)	1442 (59.8)
7-11	714 (28.0)	642 (25.0)	119 (26.2)	225 (24.8)	227 (22.0)	620 (25.7)
≥12	582 (22.8)	354 (13.8)	72 (15.9)	130 (14.3)	227 (22.0)	349 (14.5)
Body mass index[b]						
<20	198 (7.7)	201 (7.8)	11 (2.4)	58 (6.4)	79 (7.7)	184 (7.7)
20-24.9	1194 (46.6)	1144 (44.3)	115 (25.3)	355 (39.3)	470 (46.0)	1080 (45.1)
25-29.9	824 (32.2)	844 (32.7)	160 (35.2)	351 (38.8)	299 (29.3)	810 (33.8)
≥30	346 (13.5)	391 (15.2)	168 (37.0)	140 (15.4)	173 (16.9)	320 (13.4)
Family history of cancer						
No	2288 (89.1)	2440 (94.3)	416 (91.6)	852 (93.8)	897 (87.0)	2286 (94.8)
Yes	281 (10.9)	148 (5.7)	38 (8.4)	56 (6.2)	134 (13.0)	125 (5.2)
Smoking habit[b]						
Never	1684 (65.7)	1756 (68.1)	331 (72.9)	646 (71.4)	724 (70.3)	1657 (68.9)
Former	344 (13.4)	252 (9.8)	48 (10.6)	104 (11.5)	123 (11.9)	240 (10.0)
Current	536 (20.9)	571 (22.1)	75 (16.5)	155 (17.1)	183 (17.8)	509 (21.2)
Weekly alcohol units[b]						
0	769 (30.0)	911 (35.3)	137 (30.3)	271 (30.0)	288 (27.9)	830 (34.5)
0.1-7	830 (32.3)	787 (30.5)	159 (35.2)	337 (37.4)	380 (36.9)	785 (32.6)
>7	968 (37.7)	886 (34.3)	156 (34.5)	294 (32.6)	363 (35.2)	791 (32.9)
Parity						
Nulliparous	401 (15.6)	380 (14.7)	68 (15.0)	126 (13.9)	184 (17.9)	381 (15.8)
1	592 (23.0)	509 (19.7)	93 (20.5)	154 (17.0)	196 (19.0)	473 (19.6)
≥2	1576 (61.4)	1699 (65.7)	293 (64.5)	628 (69.1)	651 (63.1)	1557 (64.6)
Menopausal status[b]						
No	989 (38.5)	842 (32.5)	88 (19.6)	175 (19.4)	347 (33.7)	805 (33.4)
Yes	1579 (61.5)	1746 (67.5)	360 (80.4)	726 (80.6)	683 (66.3)	1603 (66.6)
Age at menarche[b] (years)						
<13	1123 (43.8)	1068 (41.3)	206 (45.6)	337 (37.4)	446 (43.4)	1003 (41.7)
≥13	1442 (56.2)	1517 (58.7)	246 (54.4)	564 (62.6)	582 (56.6)	1400 (58.3)
Use of oral contraceptive						
No	2208 (86.0)	2298 (88.8)	408 (89.9)	790 (87.0)	921 (89.3)	2142 (88.8)
Yes	361 (14.0)	290 (11.2)	46 (10.1)	118 (13.0)	110 (10.7)	269 (11.2)
Hormone replacement therapy						
No	2375 (92.5)	2396 (92.6)	405 (89.2)	830 (91.4)	969 (94.0)	2260 (93.7)
Yes	194 (7.5)	192 (7.4)	49 (10.8)	78 (8.6)	62 (6.0)	151 (6.3)

[a]Data are reported as no. (%).
[b]The sum does equal the total number because of missing values.

Table 2. Distribution of prebiotic fibers intake in the 3 studies on breast, endometrial and ovarian cancers, separately for cases and controls. Italy, 1991-2006

	Percentiles of distribution: 25°, 50°, 75° (g per day)					
	Breast cancer study		Endometrial cancer study		Ovarian cancer study	
	Case patients (*n* = 2569)	Control participants (*n* = 2588)	Case patients (*n* = 454)	Control participants (*n* = 908)	Case patients (*n* = 1031)	Control participants (*n* = 2411)
Inulin-type fructans	0.418, 0.772, 1.369	0.386, 0.719, 1.273	0.386, 0.742, 1.288	0.404, 0.714, 0.132	0.464, 0.867, 1.424	0.405, 0.749, 1.301
Kestose (an FOS)	0.129, 0.164, 0.209	0.122, 0.157, 0.201	0.122, 0.153, 0.195	0.118, 0.151, 0.193	0.132, 0.163, 0.210	0.118, 0.154, 0.200
Nystose (an FOS)	0.011, 0.014, 0.018	0.011, 0.014, 0.018	0.011, 0.014, 0.017	0.010, 0.013, 0.017	0.011, 0.015, 0.018	0.010, 0.014, 0.017
1F-β-fructofuranosylnystose (an FOS)	0.001, 0.003, 0.006	0.001, 0.002, 0.006	0.001, 0.002, 0.006	0.001, 0.002, 0.005	0.001, 0.003, 0.006	0.001, 0.002, 0.006
Raffinose (a GOS)	0.072, 0.092, 0.113	0.068, 0.089, 0.111	0.070, 0.087, 0.105	0.068, 0.085, 0.105	0.074, 0.091, 0.110	0.067, 0.085, 0.109
Stachyose (a GOS)	0.101, 0.176, 0.267	0.097, 0.176, 0.268	0.120, 0.177, 0.267	0.102, 0.179, 0.268	0.113, 0.181, 0.272	0.098, 0.176, 0.267

Abbreviations: FOS, fructo-oligosaccharide; GOS, galactooligosaccharide.

Table 3. Odds ratios[a] and corresponding 95% CIs of cancers of the breast, endometrium, and ovary according to quartiles[b] of prebiotic fibers intake among control participants: Italy, 1991-2006

Quartile	Breast		Endometrium		Ovary	
	No. of case patients, %	OR (95% CI)	No. of case patients, %	OR (95% CI)	No. of case patients, %	OR (95% CI)
Inulin-type fructans						
1	558 (21.7)	1	120 (26.4)	1	212 (20.6)	1
2	634 (24.7)	1.07 (0.90, 1.26)	98 (21.6)	0.77 (0.53, 1.11)	235 (22.8)	0.86 (0.67, 1.10)
3	652 (25.4)	1.06 (0.90, 1.26)	125 (27.5)	0.97 (0.68, 1.38)	286 (27.7)	1.06 (0.83, 1.35)
4	725 (28.2)	1.10 (0.93, 1.31)	111 (24.5)	0.92 (0.64, 1.33)	298 (28.9)	1.05 (0.82, 1.35)
Kestose (an FOS)						
1	535 (20.8)	1	106 (23.4)	1	173 (16.8)	1
2	628 (24.5)	1.07 (0.90, 1.28)	116 (25.6)	1.07 (0.73, 1.57)	277 (26.9)	1.16 (0.89, 1.51)
3	660 (25.7)	1.05 (0.87, 1.27)	111 (24.5)	1.08 (0.72, 1.63)	285 (27.6)	1.19 (0.89, 1.58)
4	746 (29.0)	1.18 (0.97, 1.45)	121 (26.7)	1.13 (0.73, 1.74)	296 (28.7)	1.17 (0.86, 1.59)
Nystose (an FOS)						
1	564 (22.0)	1	96 (21.2)	1	179 (17.4)	1
2	637 (24.8)	1.10 (0.93, 1.30)	112 (24.7)	1.31 (0.88, 1.93)	250 (24.3)	1.16 (0.90, 1.51)
3	651 (25.3)	1.12 (0.92, 1.31)	125 (27.5)	1.44 (0.97, 2.15)	292 (28.3)	1.34 (1.03, 1.75)
4	717 (27.9)	1.20 (1.00, 1.45)	121 (26.7)	1.32 (0.85, 2.03)	310 (30.1)	1.39 (1.04, 1.84)*
1F-β-fructofuranosylnystose (an FOS)						
1	577 (22.5)	1	108 (23.8)	1	253 (24.5)	1
2	591 (23.0)	0.94 (0.80, 1.14)	97 (21.4)	0.88 (0.61, 1.29)	239 (23.2)	0.67 (0.52, 0.85)
3	688 (26.8)	1.06 (0.90, 1.25)	118 (26.0)	1.03 (0.72, 1.49)	293 (28.4)	0.78 (0.62, 0.99)
4	713 (27.8)	1.10 (0.93, 1.30)	131 (28.9)	1.13 (0.78, 1.62)	246 (23.9)	0.67 (0.52, 0.85)*
Raffinose (a GOS)						
1	527 (20.5)	1	100 (22.0)	1	173 (16.8)	1
2	650 (25.3)	1.15 (0.97, 1.36)	106 (23.4)	1.04 (0.71, 1.54)	256 (24.8)	1.14 (0.87, 1.49)
3	699 (27.2)	1.15 (0.95, 1.38)	134 (29.5)	1.34 (0.90, 2.01)	330 (32.0)	1.32 (0.99, 1.74)
4	693 (27.0)	1.13 (0.92, 1.39)	114 (25.1)	1.13 (0.72, 1.78)	272 (26.4)	1.10 (0.80, 1.51)
Stachyose (a GOS)						
1	601 (23.4)	1	96 (21.2)	1	222 (21.5)	1
2	686 (26.7)	1.07 (0.91, 1.26)	139 (30.6)	1.48 (1.04, 2.11)	253 (24.5)	1.01 (0.80, 1.29)
3	646 (25.2)	0.99 (0.84, 1.17)	108 (23.8)	1.13 (0.77, 1.64)	289 (28.0)	1.08 (0.85, 1.38)
4	636 (24.8)	0.94 (0.80, 1.12)	111 (24.5)	1.09 (0.75, 1.59)	267 (25.9)	1.03 (0.80, 1.32)

Abbreviations: FOS, fructo-oligosaccharide; GOS, galactooligosaccharide; OR, odds ratio.
[a]Adjusted for study areas, year of interview, age, education, alcohol drinking, tobacco smoking status, body mass index, total energy intake, family history for breast, endometrial or ovarian cancer, age at menarche, menopausal status, parity, and use of oral contraceptives and hormone replacement therapy.
[b]The cutpoints for quartiles were those reported in Table 2 in the "control participants" columns.
*P for trend $< .05$.

to the disease diagnosis in the participants interviewed. The use of a satisfactorily reliable and valid FFQ is an additional strength of our study. However, measurement error in dietary assessment using an FFQ is difficult to avoid. In particular, the FFQ did not include questions about certain foods rich in prebiotic fibers, such as rye products, spelt, Jerusalem artichoke, breakfast cereal products, oats, and soya beans, which are, however, infrequently consumed in Italy. In addition, the FFQ distinguished between whole-grain and non–whole-grain only for bread. Of note, nystose and especially 1F-β-fructofuranosylnystose were detectable, in very small amounts, in a few foods or ingredients; some of these (eg, shallots, garlic, barley) were not directly assessed by the FFQ, but their consumption was derived based on their standard amounts in recipes. Thus, due caution is required in the interpretation of the associations. Possible misclassification should not be unbalanced, in any case, between case patients and control participants. Finally, as for confounding, we were able to adjust for a number of correlates of the cancers under investigation, including several reproductive and hormonal factors, as well as for total energy intake. However, residual confounding cannot be excluded.

In conclusion, this is the first study attempting to evaluate whether a diet rich in prebiotic fibers may lower the risk of selected hormone-related female cancers. Despite the acknowledged limitations in the estimation of the intakes of the considered prebiotics, our results indicate a lack of consistent and appreciable associations with cancers of the breast, ovary, and endometrium. Future epidemiologic studies with an FFQ designed ad hoc to assess prebiotic consumption may contribute to further exploration of the issue.

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Conflict of interest

The authors declare no conflicts of interest.

Data availability

Data are available upon reasonable request.

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