RESEARCH
Brief Report

Prebiotics and the Risk of Upper Digestive Tract and Stomach Cancers: The PrebiotiCa Study

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ABSTRACT

Background Fiber intake may lower digestive tract cancer risk, possibly by modulating the composition of gut microbiota. However, no data are available about the role of specific fiber fractions with prebiotic activity (e.g., inulin-type fructans (ITFs), fructo-oligosaccharides (FOSs) and galactooligosaccharides (GOSs)) on the risk lower digestive tract cancers.

Objective The objective was to assess the association between prebiotic intake and the risk of cancers of the upper digestive tract and stomach.

Design Within the PrebiotiCa study, data were derived from a network of Italian case−control studies conducted between 1992 and 2009. Participants' usual diet was assessed using a food frequency questionnaire. ITFs, and selected FOSs (nystose, kestose, and 1F-β-fructofuranosylnystose) and GOSs (raffinose and stachyose) were quantified in several food products via laboratory analyses. Participants' prebiotic intake was calculated by multiplying food frequency questionnaire intake by the prebiotic content of each food item.

Participants/setting Cases were patients admitted to major hospitals with incident histologically confirmed cancers; there were 946 cases of cancer of the oral cavity/pharynx, 198 of the nasopharynx, 304 of the esophagus, 230 of the stomach. More than 4,000 patients admitted to the same hospitals for acute nonneoplastic and not diet-related conditions were selected as control subjects.

Main outcome measures The outcomes were oral and pharyngeal, nasopharyngeal, esophageal, and stomach cancers.

Statistical analyses performed The odds ratios and corresponding 95% CIs of the various cancers were derived using logistic regression models adjusted for major confounders and energy intake.

Results No association was observed between intake of prebiotics and risk of cancers of the oral cavity and pharynx, nasopharynx, and esophagus. High raffinose intake reduced stomach cancer risk (odds ratio for the third vs the first tertile 0.6, 95% CI 0.3 to 0.9); no other prebiotic was associated with stomach cancer.

Conclusions The current study does not support a major role of prebiotic fibers on selected upper digestive tract cancers. The association between high raffinose intake and reduced stomach cancer risk needs further investigation.
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ALTHOUGH VARIOUS DEFINITIONS OF PREBIOTICS have been proposed,[1,2] the most commonly accepted is that of substrates that are selectively utilized by host microorganisms conferring health benefits.[2] Currently established prebiotics are the fiber types galacto-oligosaccharides (GOSs) and inulin-type fructans (ITFs), including fructooligosaccharides (FOSs).[3] Prebiotics improve the integrity and permeability of the gastrointestinal barrier, prevent pathogen colonization by raising competitive pressure and by producing compounds with antibiotic or immunomodulating effects, favorably influence immune function, and increase mineral absorption.[4] Most of these effects are attributed to short-chain fatty acids (SCFAs), produced from the anaerobic fermentation of prebiotics by intestinal bacteria. SCFAs, primarily acetate, propionate, and butyrate, have potent antineoplastic properties.[5] Although the strongest evidence for the protective role of SCFAs is for colorectal cancer, other neoplasms may be influenced, including bladder, breast, stomach, liver, lung, pancreatic, and prostate cancers.[5] In particular, a study showed that butyrate and propionate induce apoptosis and necrosis in gastric cancer cells in vitro.[6] In addition, prebiotics have dietary fiber effects; for example, bulking effects and favorable effects on glucose and lipid metabolism.

Several studies showed that fiber intake is associated with reduced risks of cancers of the oral cavity and pharynx,[7-9] esophagus,[10] and stomach.[11] Limited evidence also exists for the association of dietary fiber from fresh food items with nasopharyngeal cancer.[12] To our knowledge, no study has assessed the association between the intake of specific fibers classified as prebiotics with the risk of upper digestive tract and stomach cancers.

The prebiotics FOSs and GOSs occur naturally in diverse plant products, but food composition data on these prebiotic molecules and estimates of prebiotic consumption in individuals are limited.[13-18]

The PrebiotiCa study was established to quantify prebiotics in commonly consumed foods and associate their intake with cancer development using data from a network of Italian case-control studies on various cancer sites; the study collected detailed dietary information through a reproducible[19] and valid[20] food frequency questionnaire (FFQ).

The present investigation assessed the association between the intake of selected fiber-type prebiotics, that is, ITFs, nystose (FOS), kestose (FOS), 1F-β-fructofuranosylnystose (FOS), raffinose (GOS), and stachyose (GOS), and the risk of cancers of the upper digestive tract and the stomach within the PrebiotiCa study.

RESEARCH SNAPSHOT

Research Question: Does a diet rich in prebiotics reduce the risk of upper digestive tract and stomach cancers?

Key Findings: In the present investigation within the PrebiotiCa study, including more than 1,600 patients with cancer from a network of Italian case–control studies, a lack of association was observed between the intake of fibers with recognized prebiotic activity and the risk of cancers of the oral cavity and pharynx, nasopharynx, and esophagus. A high intake of raffinose, a galacto-oligosaccharides, was associated with reduced stomach cancer risk.

METHODS

Study Design and Data Collection

Data for the PrebiotiCa study were derived from a network of case–control studies on various neoplasms conducted between the 1990s and the 2000s in various Italian areas. The present analysis focused on cancers of the upper digestive tract and stomach, and included a total of 946 cases of cancer of the oral cavity and pharynx (with corresponding 2,492 controls),[21] 198 of the nasopharynx (594 controls),[22] 304 of the esophagus (743 controls),[23] and 230 of the stomach (547 controls)[24,25] (Table 1). Each cancer study has its own database; that is, four distinct databases. Briefly, all studies included incident cases, identified in the major teaching and general hospitals of the study areas. Controls were patients admitted to the same network of hospitals of cases for a wide spectrum of acute, nonneoplastic conditions unrelated to smoking, alcohol consumption, or long-term diet modification. Controls were frequency matched with cases by age and sex in the study on stomach cancer; by age, sex, period of interview, and study area in the studies on nasopharyngeal and esophageal cancers; by study center and age in the study on oral and pharyngeal cancer (to compensate for the rarity of oral and pharyngeal cancer in women, an overrepresentation of female vs male control subjects was adopted). The participation rate was >95% for cases and controls in all the studies. The study protocols were revised and approved by the ethical committees of the hospitals involved, according to the regulations at the time each study was conducted, and all participants gave informed consent.

Cases and controls were interviewed by centrally trained interviewers using the same structured questionnaire, which included sociodemographic characteristics (e.g., education and occupation), lifetime smoking habits, physical activity, anthropometric measures at various ages, a problem-oriented personal medical history, and family history of cancer. Participants' usual diet in the two years preceding diagnosis (for cases) or hospital admission (for controls) was assessed using a reproducible[19] and valid[20] FFQ. The FFQ asked for the average weekly consumption of 78 items, including foods, food groups, recipes, and nonalcoholic beverages; an additional section of the questionnaire addressed the consumption of alcoholic beverages typical of the Italian tradition. Intakes lower than once per week, but at least once per month, were coded as 0.5 per week. Energy and nutrient intakes were computed by combining FFQ data on frequency of consumption with Italian food composition databases[26,27] using standard methodology.[28]

Prebiotic Determinations in Foods

The methodology used for the quantification of prebiotic fibers was described in detail elsewhere.[29] Briefly, the content of GOSs and FOSs was determined in 78 foods, most of which were assessed by the FFQ used in the present network of studies: 15 types of fruits; 32 varieties of vegetables, root vegetables, and tubers; nine types of dried or fresh legumes; and 22 types of cereals and cereals-based products (both whole-grain and refined products). ITFs were determined in seven foods: fresh onion; garlic; banana; leek, Jerusalem artichoke, artichoke, and shallot (all but Jerusalem artichoke were assessed in the FFQ). Food sampling (from supermarkets located in Modena from May 17 to June 24, 2021) and analysis were conducted in a certified laboratory (for food analysis) by Neotron SpA.

ITFs were determined using an internal analytical method based on AOAC International 997.08 procedure, based on an enzymatic hydrolysis and a high-performance anion-exchange chromatography coupled to pulsed amperometric detection (HPAE-PAD). The limit of detection of the methodology was 0.005 g/100 g. ITFs content ranged from 25.1 g/100 g in garlic to 1 g/100 g in onion and leek.

FOSs and GOSs in fresh samples were determined according to Manali Aggrawal and Jeff Rohrer method based on an alkaline hydrolysis and HPAE-PAD detection (Thermo Scientific, Application Note 1149: Profiling Fructosyloligosaccharides (FOS)-containing samples by HPAE-PAD. 2015). The following molecules were quantified: raffinose (GOS), stachyose (GOS), nystose (FOS), kestose (FOS), and 1f-fructofuranosylnystose (FOS). The limit of detection was 0.002 to 0.02 g/100 g, based on the food matrix. The principal contributor of FOSs was Jerusalem artichoke (4.45 g/100 g), with other foods containing <1 g/100 g, and was represented principally as kestose. Total FOSs was calculated as the sum of

Table 1. Italian case-control studies on cancers of the oral cavity and pharynx, nasopharynx, esophagus, and stomach contributing to the present analysis

			Total	Cases		Controls	
			Cases/controls	Men/women	Age (y)	Men/women	Age (y)
Cancer site	Study period	Italian areas of study conduction	n/n	n/n	Median (IQR[a])	n/n	Median (IQR)
Oral cavity and pharynx	1992-2009	Milan, Pordenone, Rome/Latina	946/2,492	756/190	58 (52-66)	1,497/995	58 (50-66)
Nasopharynx	1992-2008	Milan, Pordenone, Naples, Catania	198/594	157/41	52 (43-62)	471/123	52 (43-63)
Esophagus	1992-1997	Milan, Pordenone, Padua	304/743	275/29	60 (54.5-66)	593/150	60 (54-67)
Stomach	1997-2007	Milan	230/547	143/87	63 (53-69)	286/261	63 (53-69)

[a]IQR = interquartile range.

nystose, kestose, and 1F-β-fructofuranosylnystose. The primary contributors of GOSs content were pulses, excluding green beans, with a mean content of 1.17 $\pm$ 0.87 g/100 g. In particular, raffinose was abundant in dried peas (0.498 g/100 g) and chickpeas (0.463 g/100 g) and stachyose in dried beans (1.905 g/100 g) and peas (1.814 g/100 g).

Statistical Analysis

The odds ratio (OR) and the corresponding 95% CI of cancers of the oral cavity and pharynx, nasopharynx, and esophagus according to the intake of selected prebiotics were estimated using unconditional multiple logistic regression models. For consistency with previous analyses on the same database,[24,30] logistic regression models conditioned on age and sex were used in the study on stomach cancer. On the basis of the study sample, quartiles of intake were used in the study on oral and pharyngeal cancer and tertiles of intake in the other studies. Tertiles or quartiles were calculated based on the distributions of the intakes among controls. Prebiotics were also considered as continuous variables in the models: the OR for an increment of intake equal to 1 SD, calculated after a log-transformation of the prebiotic variables, were estimated. Models for the various cancer sites included the same set of covariates, but these were included using different categorizations based on sample size and covariate distribution in cases and controls in each cancer database. Covariates were sex, age (5- or 10-years age groups, depending on study database), study center (in categories), year of interview (continuous variable), years of education (in categories $<$7 years, 7 to 11 years, and $\geq$12 years), alcohol drinking (in 3, 4 or 5 categories of levels of consumption), tobacco smoking (in categories of never, ex, and current smokers of 2 or 3 levels of tobacco consumption), body mass index (in categories $<$20, 20 to 24.9, 25 to 29.9, or $\geq$30 kg/m^2), and total energy intake (in tertiles/quartiles/quintiles). To account for the overall dietary pattern of study participants, adherence to the Mediterranean diet as measured by the Mediterranean diet score[31] was included in the models (continuous variable) as a covariate. No multicollinearity was observed between dietary variables derived from the same FFQ (all Pearson correlation coefficients were well below 0.8). A few missing values on adjustment factors were replaced by the median value (continuous variables) or mode category (categorical variables) according to case/control status and sex. Tests for trends across quantiles were performed by including the examined variable as ordinal. In case of statistically significant associations between a specific prebiotic and a specific cancer site, stratified analyses were performed; effect modification was assessed using the likelihood ratio test comparing models with and without interaction terms. *P* values were considered significant when $<$ 0.05. All analyses were conducted using SAS version 9.4.[32]

RESULTS

Among control subjects, median daily intake of ITFs across study databases ranged between 679 mg/day (interquartile range [IQR] 368-1201 mg/day) (esophageal cancer study database) and 946 mg/day (IQR 479-1970 mg/day) (nasopharyngeal cancer study database). For kestose, the median daily intake ranged between 163 mg/day (IQR 127-210 mg/day) (oral and pharyngeal cancer study database) and

175 mg/day (IQR 135-232 mg/day) (nasopharyngeal cancer study database). For nystose, the median daily intake ranged between 15 mg/day (IQR 11-19 mg/day) (stomach cancer study database) and 16 mg/day (IQR 13-20 mg/day) (nasopharyngeal cancer study database). For raffinose, the median daily intake ranged between 91 mg/day (IQR 72-117 mg/day) (stomach cancer study database) and 96 mg/day (IQR 86-115 mg/day) (nasopharyngeal cancer study database). For stachyose, the median daily intake ranged between 175 mg/day (IQR 98-262 mg/day) (esophageal cancer study database) and 200 mg/day (IQR 127-310 mg/day) (stomach cancer study database); and for 1F-β-fructofuranosylnystose, the median daily intake was 2 mg/day (IQRs in all cancer databases of ~1 - 6 mg/day). Kestose intake was the largest contributor of total FOSs intake (~90%); nystose (7.5% to 8%), and 1F-β-fructofuranosylnystose intakes (2% to 2.5%) accounted for a small fraction of total FOSs intake (data not shown).

Table 2 gives the OR of cancers of the upper digestive tract and of the stomach according to the intakes of the various prebiotic fibers. No association was observed between the intake of prebiotics and the risk of cancers of the oral cavity and pharynx, nasopharynx, or esophagus. The OR of stomach cancer for the third vs the first tertile of raffinose intake showed decreased risk (0.6, 95% CI 0.3 to 0.9). In sensitivity analyses, further adjustment for total fiber intake reduced the strength of the association (OR for the third vs the first tertile: 0.7, 95% CI 0.4 to 1.2), whereas results were nearly identical to those from the main analysis with the exclusion of extreme values (i.e., observations whose distances from the IQR are greater than 1.5 times the size of the IQR) (OR 0.6, 95% CI 0.3 to 0.9). The association between raffinose intake and stomach cancer was similar in strata of age, sex, education, body mass index, smoking, and adherence to the Mediterranean diet (Figure). No other prebiotic was significantly associated with the neoplasm.

DISCUSSION

In the present investigation within the PrebiotiCa study based on more than 1,600 cancer patients, no association was observed between the intake of fibers with recognized prebiotic activity and the risk of cancers of the upper digestive tract. For stomach cancer, a reduced risk for high intake of raffinose was found; in the absence of consistent associations with the other prebiotics, in particular stachyose,the other member of the GOSs family, such an association needs to be interpreted with caution because it may be a chance finding of multiple comparisons.

This is the first study to investigate the association of dietary prebiotics with the risk of cancers. Within the same PrebiotiCa study, a high intake of GOSs was associated with a reduced risk of colorectal[33] and laryngeal cancer.[34]

According to laboratory analyses conducted within the PrebiotiCa study, GOSs were abundant in legumes. Dried peas, dried chickpeas, and beans were foods with the highest raffinose content. Although stachyose, the other member of the GOSs family, was found in significant amounts almost exclusively in legumes, raffinose-rich foods also include whole-meal flour, selected whole-grain–based products, and barley. Raffinose was also found in white-wheat flour and refined-wheat products, but in lower amounts than their whole counterparts. The Italian diet is rich in cereals and cereal products[35]; accounting for the amount of foods consumed, the largest contributors of raffinose intake in the study population were cereal-based products, followed by legumes.

Legumes,[36,37] whole-grain cereals,[38] and whole-grain fiber[9] have been associated with reduced risk of stomach cancer risk; however, their consumption cannot fully explain the association between raffinose and stomach cancer, in particular because legumes and whole-grain cereals have been associated with lower risks of other cancers of the upper digestive tract as well, including esophageal[36,38,39] and oral and pharyngeal cancer,[9,36,39-41] and no association was observed between raffinose intake and these cancer sites. In addition to fiber, the association between higher whole-grain and legume intake and lower risk of various cancers of the digestive tract is likely related to the presence of antioxidants and bioactive compounds with anticarcinogenic properties.[42,43]

The present study has limitations and strengths. All studies included in the present analysis are retrospective and hospital-based. To limit selection bias, cases and controls were identified in the major teaching and general hospitals of the areas under surveillance and patients admitted to hospital for chronic conditions or digestive tract diseases were excluded from the control group. Participation of cases and controls was satisfactory, and results were consistent across study areas. The similar interview setting for cases and controls reduced information bias, and, although recall bias is possible, this should not have been different based on disease status. In addition, the FFQ used in the network of studies was reproducible[19] and valid.[20] As for possible confounding, estimates were adjusted for major risk factors for the neoplasms as well as for total energy intake; in any case, a certain degree of residual confounding (ie, confounding that remains despite adjustment) cannot be excluded. Adjustment for human papillomavirus (in oral and pharyngeal cancer) and *Helicobacter pylori* (in stomach cancer) could not be made because data were not available. In a sensitivity analysis adjusting further for total fiber intake, the strength of the association between raffinose and stomach cancer declined. However, prebiotics are types of fiber, and adjustment for total fiber intake is an overadjustment that can bias results toward the null.

Estimating individual intake of prebiotics from questionnaires data is challenging and there is no standard methodology for the determination of the prebiotic content of foods. In addition, the definition of ITFs is not universally agreed upon. The FFQ used in the current study was not specifically designed to assess the intake of ITFs, GOSs, and FOSs. In particular, it did not include items on specific dietary products reported to contain prebiotic fibers (e.g., rye products, spelt, Jerusalem artichoke, breakfast cereal products, oats, and soya beans) nor did it distinguish whole-grain from non–whole-grain items, apart from bread. It was therefore not possible to derive participants' prebiotic intake from such foods. However, intake of those foods is uncommon in the Italian population, and hence their contribution to participants' daily prebiotic intake and to the prebiotic–cancer associations is likely to be minimal. Because of methodological difficulties, ITFs were determined in only six foods assessed by the FFQ. Garlic had by far the highest ITFs content. Because

Table 2. Number of cancer cases, adjusted odds ratios[a] (OR) and corresponding 95% CI of cancers of the upper digestive tract and stomach according to quantile of prebiotic fiber intake[b] in the Italian network of case-control studies. Italy, 1992-2009

	Oral cavity and pharynx		Nasoopharynx		Esophagus		Stomach	
	n (%)	Adjusted OR (95% CI)	n (%)	Adjusted OR (95% CI)	n (%)	Adjusted OR (95% CI)	n (%)	Adjusted OR (95% CI)
Inulin-type fructans								
I	227 (24.0)	1	58 (29.3)	1	85 (28.0)	1	67 (29.1)	1
II	223 (23.6)	1.1 (0.8-1.4)	58 (29.3)	0.9 (0.6-1.4)	119 (39.1)	1.4 (0.9-2.0)	73 (31.7)	1.0 (0.6-1.4)
III	227 (24.0)	1.2 (0.9-1.5)	82 (41.4)	1.1 (0.7-1.7)	100 (32.9)	1.3 (0.9-2.0)	90 (39.1)	1.1 (0.7-1.6)
IV	269 (28.4)	1.3 (0.97-1.7)	—	—	—	—	—	—
P for trend		0.071		0.662		0.207		0.700
1-SD[c] increase in log-transformed variable		1.1 (0.98-1.2)		1.1 (0.9-1.3)		1.2 (1.04-1.4)		0.9 (0.8-1.1)
Kestose, FOS[d]								
I	265 (28.0)	1	48 (24.2)	1	133 (43.8)		63 (27.4)	1
II	204 (21.6)	0.8 (0.6-1.06)	67 (33.8)	1.2 (0.7-1.9)	79 (26.0)	0.6 (0.4-0.97)	81 (35.2)	1.0 (0.6-1.5)
III	216 (22.8)	1.0 (0.7-1.3)	83 (41.9)	1.4 (0.8-2.3)	92 (30.3)	0.8 (0.5-1.2)	86 (37.4)	0.9 (0.5-1.4)
IV	261 (27.6)	1.0 (0.8-1.4)	—	—	—	—	—	—
P for trend		0.651		0.260		0.220		0.609
1-STD[c] increase in log-transformed variable		1.0 (0.9-1.1)		1.2 (0.98-1.5)		1.0 (0.8-1.2)		0.8 (0.7-1.0)
Nystose, FOS[d]								
I	208 (22.0)	1	45 (22.7)	1	106 (34.9)		60 (26.1)	1
II	228 (24.1)	1.1 (0.8-1.4)	67 (33.8)	1.2 (0.8-2.0)	93 (30.6)	1.0 (0.6-1.4)	83 (36.1)	1.0 (0.6-1.6)
III	244 (25.8)	1.2 (0.9-1.6)	86 (43.4)	1.6 (0.9-2.5)	105 (34.5)	0.9 (0.6-1.3)	87 (37.8)	0.9 (0.6-1.5)
IV	266 (28.1)	1.1 (0.8-1.4)	—	—	—	—	—	—
P for trend		0.450		0.084		0.483		0.626
1-SD[c] increase in log-transformed variable		1.1 (0.95-1.2)		1.2 (0.9-1.4)		0.9 (0.8-1.1)		0.9 (0.7-1.1)
1F-β-fructofuranosylnystose, FOS[d]								
I	318 (33.6)	1	66 (33.3)	1	130 (42.8)	1	58 (25.2)	1
II	235 (24.8)	1.0 (0.8-1.3)	66 (33.3)	0.9 (0.6-1.3)	97 (31.9)	1.0 (0.7-1.4)	87 (37.8)	1.4 (0.9-2.1)
III	153 (16.2)	0.8 (0.6-1.0)	66 (33.3)	0.8 (0.5-1.2)	77 (25.3)	0.8 (0.6-1.2)	85 (37.0)	1.2 (0.8-1.8)

(*continued on next page*)

Table 2. Number of cancer cases, adjusted odds ratios[a] (OR) and corresponding 95% CI of cancers of the upper digestive tract and stomach according to quantile of prebiotic fiber intake[b] in the Italian network of case-control studies. Italy, 1992-2009 (*continued*)

	Oral cavity and pharynx		Nasoopharynx		Esophagus		Stomach	
	n (%)	Adjusted OR (95% CI)	n (%)	Adjusted OR (95% CI)	n (%)	Adjusted OR (95% CI)	n (%)	Adjusted OR (95% CI)
IV	240 (25.4)	1.0 (0.8-1.3)	—	—	—	—	—	—
P for trend		0.755		0.250		0.365		0.589
1-SD[c] increase in log-transformed variable		1.0 (0.9-1.1)		1.0 (0.8-1.2)		1.0 (0.8-1.2)		1.2 (1.0-1.4)
Total FOSs[de]								
I	199 (21)	1	45 (22.7)	1	132 (43.4)	1	60 (26.1)	1
II	220 (23.3)	1.9 (0.7-1.2)	71 (35.9)	1.4 (0.9-2.3)	75 (24.7)	0.6 (0.4-0.9)	86 (37.4)	1.1 (0.7-1.7)
III	228 (24.1)	0.9 (0.7-1.3)	82 (41.4)	1.4 (0.8-2.5)	97 (31.9)	0.8 (0.5-1.2)	84 (36.5)	0.9 (0.5-1.4)
IV	299 (31.6)	1.01 (0.8-1.4)	—	—	-	—	-	—
P for trend		0.857		0.233		0.230		0.472
1-SD[c] increase in log-transformed variable		1.0 (0.9-1.1)		1.2 (0.98-1.6)		1.0 (0.8-1.2)		0.8 (0.7-1.0)
Raffinose, GOS[f]								
I	266 (28.1)	1	47 (23.7)	1	133 (43.8)	1	66 (28.7)	1
II	233 (24.6)	1.0 (0.7-1.2)	73 (36.9)	1.4 (0.9-2.2)	84 (27.6)	0.7 (0.5-1.0)	93 (40.4)	1.0 (0.6-1.5)
III	205 (21.7)	0.9 (0.7-1.2)	78 (39.4)	1.3 (0.8-2.2)	87 (28.6)	0.8 (0.5-1.2)	71 (30.9)	0.6 (0.3-0.9)
IV	242 (25.6)	1.1 (0.8-1.5)	—	—	—	—	—	—
P for trend		0.768		0.334		0.193		0.019
1-SD[c] increase in log-transformed variable		1.0 (0.9-1.1)		1.2 (0.96-1.5)		0.9 (0.8-1.1)		0.8 (0.6-0.99)
Stachyose, GOS[f]								
I	237 (25.1)	1	64 (32.3)	1	100 (32.9)		76 (33.0)	1
II	249 (26.3)	1.1 (0.9-1.4)	75 (37.9)	1.1 (0.7-1.7)	97 (31.9)	1.1 (0.8-1.6)	83 (36.1)	1.1 (0.7-1.6)
III	232 (24.5)	1.0 (0.8-1.4)	59 (29.8)	0.9 (0.5-1.4)	107 (35.2)	1.2 (0.8-1.8)	71 (30.9)	0.8 (0.5-1.2)
IV	228 (24.1)	1.1 (0.9-1.5)	—	—	—	—	-	—
P for trend		0.422		0.481		0.320		0.342
1-SD[c] increase in log-transformed variable		1.1 (0.98-1.2)		1.0 (0.8-1.2)		1.2 (0.98-1.4)		1.0 (0.8-1.2)

[a]Adjusted for sex, age, study center, year of interview, education, alcohol drinking, tobacco smoking, body mass index, adherence to the Mediterranean diet, and total energy intake.
[b]Derived among controls.
[c]The Table provides the OR for an increment of prebiotic intake equal to 1 SD, calculated after a log-transformation of the prebiotic variables.
[d]FOS = fructooligosaccharide.
[e]Total FOSs was calculated as the sum of nystose, kestose, and 1F-β-fructofuranosylnystose.
[f]GOS = galactooligosaccharide.

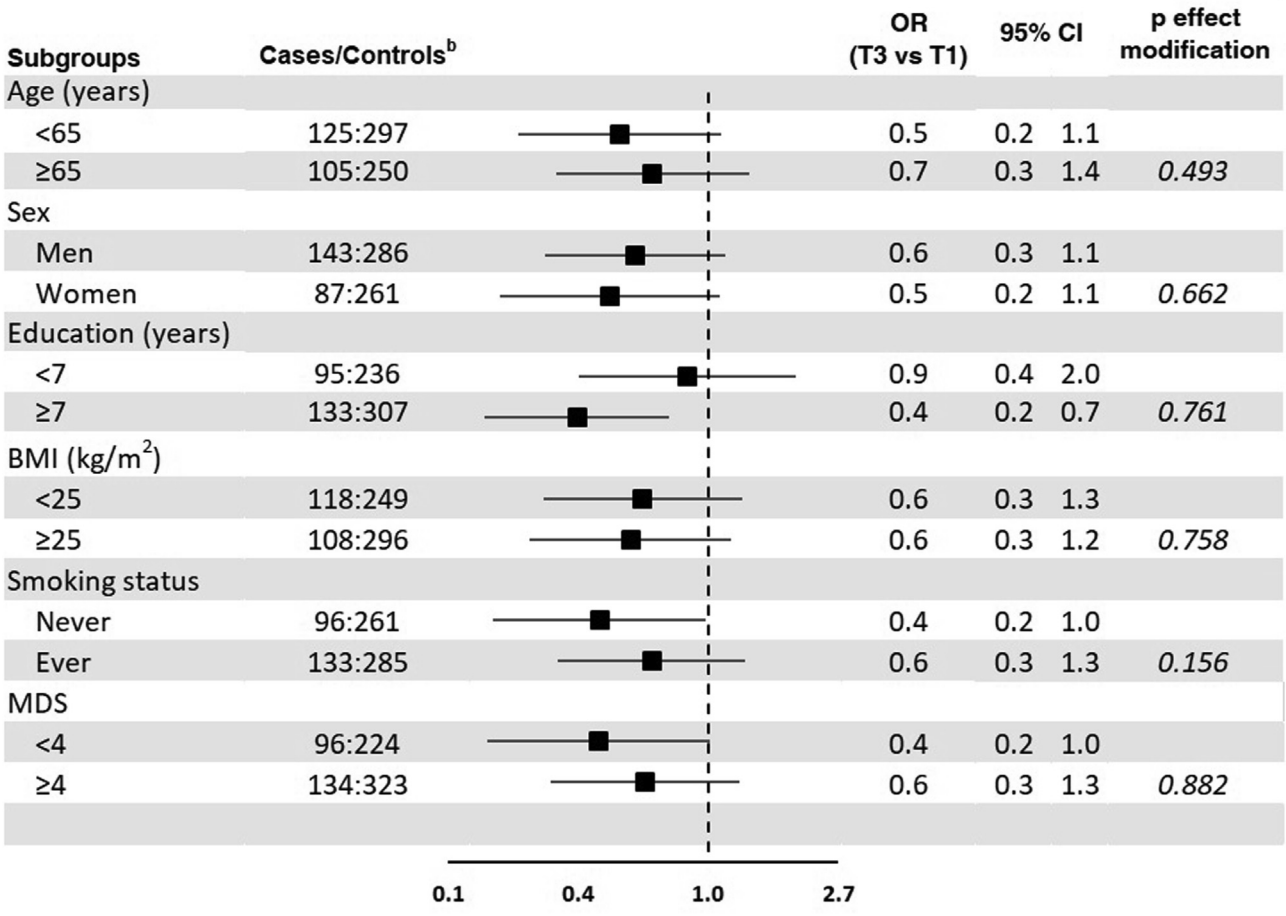

Figure. Odds ratios[a] (OR) of stomach cancer, and corresponding 95% CI, for the highest (T3) compared with the lowest tertile (T1) of raffinose intake in strata of age, sex, education, body mass index (BMI), smoking status, and adherence to the Mediterranean diet (Mediterranean diet score [MDS]) among 230 patients with stomach cancer and 547 control subjects. [a]Adjusted for sex, age, study center, year of interview, education, alcohol drinking, tobacco smoking, body mass index, adherence to the Mediterranean diet, and total energy intake, unless the variable was the stratification factor. Tests for effect modification were performed considering all the tertiles of raffinose intake. [b]The sum may do not add up to the total because of missing values.

the FFQ did not ask a specific question about garlic intake, it was estimated based on the standard amount of garlic in recipes. Assessment of garlic consumption, and hence of ITFs intake, may therefore not be accurate. In general, although individual estimates of prebiotics intake may be misestimated, misclassification should be balanced between cases and controls. Another limitation relates to the application of results from food content analyses conducted during 2021 to dietary intakes collected in the 1990s and 2000s because the contents of ITFs, FOSs, and GOSs in food sources might have changed. However, comprehensive food composition data regarding these prebiotics contemporary to the time of study data collection were not available, and no prior data existed for Italian food sources. The few available databases were scattered across studies conducted outside Europe, which applied heterogeneous methodologies for the quantification of prebiotic molecules, and showed wide variation in prebiotic food composition.[29] Further, the application of food composition data to dietary data collected at a different time point, when contemporary data are not available, is common in nutrition studies.[44]

CONCLUSIONS

The results of the current study do not support a major role of fiber-type prebiotics on the risk of selected upper digestive tract cancers. The association between high raffinose intake and reduced stomach cancer needs independent confirmation by larger studies.

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STATEMENT OF POTENTIAL CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

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AUTHOR CONTRIBUTIONS

F. Turati, C. La Vecchia, F. Concina, and M. Parpinel designed the research; F. Turati defined the methodology and drafted the manuscript; P. Bertuccio and F. Turati performed the statistical analyses; F. Concina, F. Fiori, and M. Parpinel contributed substantially to nutritional analysis and results interpretation; W. Garavello, A. Crispo, M. Libra, E. Negri, D. Serraino, and C. La Vecchia collected data; C. La Vecchia, E. Negri, and D. Serraino defined study design for the case-control studies. All authors reviewed and commented on subsequent drafts of the manuscript.