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Prebiotics and the risk of upper digestive tract and stomach cancers: the *PrebiotiCa* study

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FT, CLV, FC and MP designed the research; FT defined the methodology and drafted the manuscript; PB and FT performed the statistical analyses; FC, FF and MP contributed substantially to nutritional analysis and results interpretation; WG, AC, ML, EN, DS and CLV collected data; CLV, EN and DS defined study design for the case-control studies. All authors reviewed and commented on subsequent drafts of the manuscript.

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Prebiotics and the risk of upper digestive tract and stomach cancers: 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 over 1600 cancer patients 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.

Prebiotics and the risk of upper digestive tract and stomach cancers: the PrebiotiCa study

Abstract

Background

Fiber intake may lower digestive tract cancer risk, possibly by modulating the composition of gut microbiota. However, no data is 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 cancer.

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 (FFQ). 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 FFQ 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. Over 4,000 patients admitted to the same hospitals for acute non-neoplastic nor diet-related conditions were selected as controls.

Main outcome measures

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

Statistical analyses performed

The odds ratios (OR) and corresponding confidence intervals (CI) of the various cancers were derived using logistic regression models adjusted for major confounders and energy intake.

Results

No association was observed between the intake of prebiotics and the risk of cancers of the oral cavity and pharynx, nasopharynx and esophagus. High raffinose intake reduced stomach cancer risk (OR for the third *versus* the first tertile 0.6, 95% confidence interval, CI: 0.3-0.9); 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 in future studies.

Prebiotics and the risk of upper digestive tract and stomach cancers: the PrebiotiCa study

Introduction

Although various definitions of prebiotics have been proposed [1, 2], the most commonly accepted is that of a substrate that is 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, impact favorably on the 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 anti-neoplastic properties[5]. While the strongest evidence for the protective role of SCFAs is for colorectal cancer, other neoplasms may be effected, 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, e.g., 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, however, 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, i.e., 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.

Methods

Study design and data collection

Data for the *PrebiotiCa* study derived from a network of case–control studies on various neoplasms conducted between the 1990's and the 2000's 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 2492 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, i.e., 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 controls 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 of each study conduction, and all participants gave informed consent.

Cases and controls were interviewed by centrally trained interviewers using the same structured questionnaire, which included socio-demographic 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 non-alcoholic 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 wholegrain 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 17 May to 24 June 2021) and analysis were conducted in a certified laboratory (for food analysis) by Neotron SpA, Modena.

ITFs were determined using an internal analytical method based on AOAC 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 (LOD) of the methodology was 0.005g / 100g. ITFs content ranged from 25.1 g/100g in garlic to 1 g/100g 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. Sunnyvale, CA, 2015). The following molecules were quantified: raffinose (GOS), stachyose (GOS), nystose (FOS), kestose (FOS) and 1f-fructofuranosylnystose (FOS). The LOD was 0.002-0.02g/100g, based on the food matrix. The principal contributor of FOSs was Jerusalem artichoke (4.45 g/100g), with other foods containing less than 1 g/100 g, and was represented principally as kestose. Total FOSs was calculated as the sum of nystose, kestose and 1F-β-fructofuranosylnystose. The primary contributor of GOSs content was pulses, excluded green beans, with a mean content of 1.17±0.87 g/100g. In particular, raffinose was particularly abundant in dried peas (0.498 g/100g) and chickpeas (0.463 g/100 g) and stachyose in dried beans (1.905 g/100g) and peas (1.814 g/100g).

Statistical analysis

The odds ratios (OR) and the corresponding 95% confidence intervals (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 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 one standard deviation, 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, 7-11, ≥12), 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 (BMI) (in categories, <20, 20-24.9, 25-29.9, ≥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 covariate. No multicollinearity was observed between dietary variables derived from the same FFQ (all Pearson's 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. 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 [IQR: 368-1201, esophageal cancer study database] and 946 mg/day [IQR: 479-1970, nasopharyngeal cancer study database]. For kestose, the median daily intake ranged between 163 [IQR: 127-210, oral and pharyngeal cancer study database] and 175 mg/day [IQR: 135-232, nasopharyngeal cancer study database]. For nystose, the median daily intake ranged between 15 [IQR: 11-19 stomach cancer study database] and 16 mg/day [IQR: 13-20, nasopharyngeal cancer

study database]. For raffinose, the median daily intake ranged between 91 [IQR: 72-117, stomach cancer study database] and 96 mg/day [IQR: 86-115, nasopharyngeal cancer study database]. For stachyose, the median daily intake ranged between 175 [IQR: 98-262, esophageal cancer study database] and 200 mg/day [IQR: 127-310, 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]. Kestose intake was the largest contributor of total FOSs intake (~90%); nystose (7.5-8%) and 1F-β-fructofuranosylnystose intakes (2-2.5%) 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 and esophagus. The OR of stomach cancer for the third *versus* the first tertile of raffinose intake showed a decreased risk, 0.6 (95% CI: 0.3-0.9). In sensitivity analyses, further adjustment for total fiber intake reduced the strength of the association (OR for the third *versus* the first tertile: 0.7, 95% CI: 0.4-1.2), while 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: 0.3-0.9). The association between raffinose intake and stomach cancer was similar in strata of age, sex, education, BMI, smoking, and adherence to the Mediterranean diet (Figure 1). No other prebiotic was significantly associated with the neoplasm.

Discussion

In the present investigation within the *PrebiotiCa* study based on over 1600 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, i.e., the other member of the GOSs family, such association needs to be interpreted with caution since 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. While stachyose, i.e., 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], wholegrain cereals[38] and wholegrain fiber[9] have been associated with reduced risk of stomach cancer risk; their consumption, however, cannot fully explain the association between raffinose and stomach cancer, in particular since legumes and wholegrain 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 wholegrain 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 the 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 (i.e., confounding which remains despite adjustment) cannot be excluded. Adjustment for Human papillomavirus (in oral and pharyngeal cancer) and *Helicobacter pylori* (in stomach cancer analyses) could not be made as 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 over-adjustment that can bias results towards 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. In particular, 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, soya beans) nor did it distinguish whole-grain from non-wholegrain 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 ITF content. Since 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 ITF intake, may therefore be not accurate. In general, although individual estimates of prebiotics intakes may be misestimated, misclassification should be balanced between cases and controls. Another limitation

relates to the application of results from food content analyses conducted in 2021 to dietary intakes collected in the 1990s' and 2000's, since 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 nutritional studies[44].

Conclusions

In conclusion, the current study does 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.

References

1. Bindels LB, Delzenne NM, Cani PD, Walter J. Towards a more comprehensive concept for prebiotics. *Nat Rev Gastroenterol Hepatol.* 2015;12:303-310.
2. Gibson GR, Hutkins R, Sanders ME, et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol.* 2017;14:491-502.
3. Carlson JL, Erickson JM, Lloyd BB, Slavin JL. Health Effects and Sources of Prebiotic Dietary Fiber. *Curr Dev Nutr.* 2018;2:nzy005.
4. Verspreet J, Damen B, Broekaert WF, Verbeke K, Delcour JA, Courtin CM. A Critical Look at Prebiotics Within the Dietary Fiber Concept. *Annu Rev Food Sci Technol.* 2016;7:167-190.
5. Mirzaei R, Afaghi A, Babakhani S, et al. Role of microbiota-derived short-chain fatty acids in cancer development and prevention. *Biomed Pharmacother.* 2021;139:111619.
6. Matthews GM, Howarth GS, Butler RN. Short-chain fatty acid modulation of apoptosis in the Kato III human gastric carcinoma cell line. *Cancer Biol Ther.* 2007;6:1051-1057.
7. Kawakita D, Lee YA, Gren LH, Buys SS, La Vecchia C, Hashibe M. Fiber intake and the risk of head and neck cancer in the prostate, lung, colorectal and ovarian (PLCO) cohort. *Int J Cancer.* 2019;145:2342-2348.
8. Kawakita D, Lee YA, Turati F, et al. Dietary fiber intake and head and neck cancer risk: A pooled analysis in the International Head and Neck Cancer Epidemiology consortium. *Int J Cancer.* 2017;141:1811-1821.
9. Kasum CM, Jacobs DR, Jr., Nicodemus K, Folsom AR. Dietary risk factors for upper aerodigestive tract cancers. *Int J Cancer.* 2002;99:267-272.
10. Sun L, Zhang Z, Xu J, Xu G, Liu X. Dietary fiber intake reduces risk for Barrett's esophagus and esophageal cancer. *Crit Rev Food Sci Nutr.* 2017;57:2749-2757.
11. Zhang Z, Xu G, Ma M, Yang J, Liu X. Dietary fiber intake reduces risk for gastric cancer: a meta-analysis. *Gastroenterology.* 2013;145:113-120 e113.
12. Mai ZM, Ngan RK, Kwong DL, et al. Dietary fiber intake from fresh and preserved food and risk of nasopharyngeal carcinoma: observational evidence from a Chinese population. *Nutr J.* 2021;20:14.
13. Muir JG, Shepherd SJ, Rosella O, Rose R, Barrett JS, Gibson PR. Fructan and free fructose content of common Australian vegetables and fruit. *J Agric Food Chem.* 2007;55:6619-6627.
14. Biesiekierski JR, Rosella O, Rose R, et al. Quantification of fructans, galacto-oligosacharides and other short-chain carbohydrates in processed grains and cereals. *J Hum Nutr Diet.* 2011;24:154-176.
15. Moshfegh AJ, Friday JE, Goldman JP, Ahuja JK. Presence of inulin and oligofructose in the diets of Americans. *J Nutr.* 1999;129:1407S-1411S.
16. Campbell JM, Bauer LL, Fahey GC, Hogarth A, Wolf BW, Hunter DE. Selected fructooligosaccharide (1-kestose, nystose, and 1F-β-fructofuranosylnystose) composition of foods and feeds. *Journal of agricultural and food chemistry.* 1997;45:3076-3082.

17. Anderson JL, Hedin CR, Benjamin JL, et al. Dietary intake of inulin-type fructans in active and inactive Crohn's disease and healthy controls: a case-control study. *J Crohns Colitis.* 2015;9:1024-1031.

18. van Loo J, Coussement P, de Leenheer L, Hoebregs H, Smits G. On the presence of inulin and oligofructose as natural ingredients in the western diet. *Crit Rev Food Sci Nutr.* 1995;35:525-552.

19. Franceschi S, Negri E, Salvini S, et al. Reproducibility of an Italian food frequency questionnaire for cancer studies: results for specific food items. *Eur J Cancer.* 1993;29A:2298-2305.

20. Decarli A, Franceschi S, Ferraroni M, et al. Validation of a food-frequency questionnaire to assess dietary intakes in cancer studies in Italy. Results for specific nutrients. *Ann Epidemiol.* 1996;6:110-118.

21. Bravi F, Polesel J, Garavello W, et al. Adherence to the World Cancer Research Fund/American Institute for Cancer Research recommendations and head and neck cancers risk. *Oral Oncol.* 2017;64:59-64.

22. Turati F, Bravi F, Polesel J, et al. Adherence to the Mediterranean diet and nasopharyngeal cancer risk in Italy. *Cancer Causes Control.* 2017;28:89-95.

23. Bosetti C, La Vecchia C, Talamini R, et al. Food groups and risk of squamous cell esophageal cancer in northern Italy. *Int J Cancer.* 2000;87:289-294.

24. Pelucchi C, Tramacere I, Bertuccio P, Tavani A, Negri E, La Vecchia C. Dietary intake of selected micronutrients and gastric cancer risk: an Italian case-control study. *Ann Oncol.* 2009;20:160-165.

25. Lucenteforte E, Scita V, Bosetti C, Bertuccio P, Negri E, La Vecchia C. Food groups and alcoholic beverages and the risk of stomach cancer: a case-control study in Italy. *Nutr Cancer.* 2008;60:577-584.

26. Salvini S, Parpinel M, Gnagnarella P, Maisonneuve P, Turrini A. Banca di composizione degli alimenti per studi epidemiologici in Italia. Milano, Italia: Istituto Europeo di Oncologia; 1998.

27. Gnagnarella P, Parpinel M, Salvini S, Franceschi S, Palli D, Boyle P. The update of the of the Italian food composition database. *J Food Comp Anal.* 2004;17:502-5022.

28. Westenbrink S, Oseredczuk M, Castanheira I, Roe M. Food composition databases: the EuroFIR approach to develop tools to assure the quality of the data compilation process. *Food chemistry.* 2009;113:759-767.

29. Fiori F, Concina F, Turati F, et al. Quantification of naturally occurring prebiotic fiber in Italian foods. *Journal of Food Composition and Analysis.* 2022;112:104678.

30. Bertuccio P, Edefonti V, Bravi F, et al. Nutrient dietary patterns and gastric cancer risk in Italy. *Cancer Epidemiol Biomarkers Prev.* 2009;18:2882-2886.

31. Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean diet and survival in a Greek population. *N Engl J Med.* 2003;348:2599-2608.

32. SAS, version 9.4. SAS Institute Inc., Cary, NC, USA.

33. Turati F, Concina F, Rossi M, et al. Association of prebiotic fiber intake with colorectal cancer risk: the PrebiotiCa study. *Eur J Nutr.* 2022.

34. Turati F, Concina F, Bertuccio P, et al. Intake of prebiotic fibers and the risk of laryngeal cancer: the PrebiotiCa study. *Eur J Nutr.* 2022:*In press*.

35. Leclercq C, Arcella D, Piccinelli R, et al. The Italian National Food Consumption Survey INRAN-SCAI 2005-06: main results in terms of food consumption. *Public Health Nutr.* 2009;12:2504-2532.

36. Aune D, De Stefani E, Ronco A, et al. Legume intake and the risk of cancer: a multisite case-control study in Uruguay. *Cancer Causes Control.* 2009;20:1605-1615.

37. Stojanovic J, Giraldi L, Arzani D, et al. Adherence to Mediterranean diet and risk of gastric cancer: results of a case-control study in Italy. *Eur J Cancer Prev.* 2017;26:491-496.

38. Zhang XF, Wang XK, Tang YJ, et al. Association of whole grains intake and the risk of digestive tract cancer: a systematic review and meta-analysis. *Nutr J.* 2020;19:52.

39. De Stefani E, Deneo-Pellegrini H, Mendilaharsu M, Ronco A. Diet and risk of cancer of the upper aerodigestive tract--I. Foods. *Oral Oncol.* 1999;35:17-21.

40. La Vecchia C, Chatenoud L, Negri E, Franceschi S. Session: whole cereal grains, fibre and human cancer wholegrain cereals and cancer in Italy. *Proc Nutr Soc.* 2003;62:45-49.

41. Giraldi L, Panic N, Cadoni G, Boccia S, Leoncini E. Association between Mediterranean diet and head and neck cancer: results of a large case-control study in Italy. *Eur J Cancer Prev.* 2017;26:418-423.

42. Gaesser GA. Whole Grains, Refined Grains, and Cancer Risk: A Systematic Review of Meta-Analyses of Observational Studies. *Nutrients.* 2020;12.

43. Messina MJ. Legumes and soybeans: overview of their nutritional profiles and health effects. *Am J Clin Nutr.* 1999;70:439S-450S.

44. Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. *Am J Epidemiol.* 1986;124:17-27.

367 **Figure legend**

368

369 **Figure 1.** Odds ratios[a] (OR) of stomach cancer, and corresponding 95% confidence intervals (CI),
370 for the highest (T3) compared to the lowest tertile (T1) of raffinose intake in strata of age, sex,
371 education, body mass index (BMI), smoking status, and adherence to the Mediterranean diet among
372 230 cases of stomach cancer and 547 controls.

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376 Footnotes of Figure 1.

377 [a] Adjusted for sex, age, study center, year of interview, education, alcohol drinking, tobacco
378 smoking, body mass index, adherence to the Mediterranean diet, and total energy intake unless the
379 variable was the stratification factor. Tests for effect modification were performed considering all
380 the tertiles of raffinose intake.

381 [b] The sum may do not add up to the total because of missing values.

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

Cancer site	Study period	Italian areas of study conduction	Total		Cases		Controls	
			N cases/N controls	N cases/N controls	N (M[a]/W[b])	Age (yrs[c]), median [IQR[d]]	N (M[a]/W[b])	Age (yrs[c]), median [IQR[d]]
Oral cavity and pharynx	1992-2009	Milan, Pordenone, Rome/Latina	946/2492	946/2492	756/190	58 [52-66]	1497/995	58 [50-66]
Nasopharynx	1992-2008	Milan, Pordenone, Naples, Catania	198/594	198/594	157/41	52 [43-62]	471/123	52 [43-63]
Esophagus	1992-1997	Milan, Pordenone, Padua	304/743	304/743	275/29	60 [54.5-66]	593/150	60 [54-67]
Stomach	1997-2007	Milan	230/547	230/547	143/87	63 [53-69]	286/261	63 [53-69]

[a] M=men.
[b] W=women.
[c] yrs=years.
[d] IQR=interquartile range.

Table 2. Number of cancer cases, adjusted odds ratios[a] (OR) and corresponding 95% confidence intervals (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-STD[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-STD[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)
IV	240 (25.4)	1.0 (0.8-1.3)	-	-	-	-	-	-
P for trend		*0.755*		*0.250*		*0.365*		*0.589*
1-STD[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 FOS[d,e]								

	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)
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-STD[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-STD[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-STD[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] STD, standard deviation. The Table provides the OR for an increment of prebiotic intake equal to 1, 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=galactooligosaccharides.

Subgroups	Cases/Controls[b]	OR (T3 vs T1)	95% CI		p effect modification
Age (years)					
<65	125:297	0.5	0.2	1.1	
≥65	105:250	0.7	0.3	1.4	*0.493*
Sex					
Men	143:286	0.6	0.3	1.1	
Women	87:261	0.5	0.2	1.1	*0.662*
Education (years)					
<7	95:236	0.9	0.4	2.0	
≥7	133:307	0.4	0.2	0.7	*0.761*
BMI (kg/m^2)					
<25	118:249	0.6	0.3	1.3	
≥25	108:296	0.6	0.3	1.2	*0.758*
Smoking status					
Never	96:261	0.4	0.2	1.0	
Ever	133:285	0.6	0.3	1.3	*0.156*
MDS					
<4	96:224	0.4	0.2	1.0	
≥4	134:323	0.6	0.3	1.3	*0.882*