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# Legume intake and cancer risk in a network of case-control studies

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## Article

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# Abstract

Evidence on the relationship between legume consumption and risk of specific cancer sites is inconclusive. We used data from a network of case-controls studies, conducted in Italy and in the Swiss Canton of Vaud between 1991 and 2009 to quantify the association between legume consumption and several cancer sites including oral cavity, esophagus, larynx, stomach, colorectum, breast, endometrium, ovary, prostate and kidney. Multiple logistic regression models controlled for sex, age, education, smoking, alcohol, body mass index and consumption of fruit, vegetables, processed meat and total calorie intake were used to estimate the odds ratios (OR) of different cancer sites and their corresponding 95% confidence intervals(CI). For female hormone-related cancers, the models included also adjustments for age at menarche, menopausal status and parity. For all cancer sites considered, except endometrium, the OR for  $\geq$  2 portions of legumes per week *vs* < 1 portion were below unity, and were significant for oral cavity (OR: 0.71, 95% CI: 0.52–0.97), esophagus (OR: 0.50, 95% CI: 0.29–0.86), larynx (OR: 0.55, 95% CI: 0.34–0.89), colorectum (OR: 0.70, 95% CI: 0.58–0.83) and kidney (OR: 0.69, 95% CI: 0.49–0.95). For esophagus, colorectal, ovarian and kidney cancers we found a significant trend (OR for 1 portion per week: 0.82,0.88, 0.89 and 0.88 respectively. The analysis stratified by sex showed that most associations were limited to men. The inverse association found for several cancers suggest a possible role of legumes in preventing cancer risk. The sex-specific pattern of association requires further research.

# Introduction

The burden of cancer incidence and mortality is rapidly growing worldwide due to aging of the world population and an improvement in life expectancy particularly in middle- and high-income countries <sup>(1)</sup>.

Positive behaviour changes can significantly reduce cancer burden <sup>(2, 3)</sup>. In relation to diet, the most recent report by the World Cancer Research Fund (WCRF) and the International Agency for research on cancer (IARC) recommend consuming a diet rich in whole grains, vegetables, fruit, and beans, with a sub goal to consume a diet providing at least 30 g/day of dietary fibre from whole foods <sup>(4)</sup>. Pulses/ legumes (i.e. the dry edible seeds of non-oilseed legumes, like dry beans, chickpeas, dry peas and lentils) are an excellent source of protein, carbohydrates, fatty acids and dietary fibre <sup>(5)</sup>. They also contain several non-nutrients that have been shown to have interactive bioactive properties <sup>(6, 7)</sup>.

Despite the potential benefit of legumes, the evidence on the relationship between legume consumption and risk of specific cancer site is limited and inconclusive <sup>(8-11)</sup>. Most studies published so far used fibre intake (including those from legumes) or an overall dietary pattern, including legumes, as exposure, whereas only a few of them evaluated the association between legume consumption and cancer risk <sup>(11-14)</sup>. This led the 2018 WCRF/IARC report to define the impact of legumes on the risk of the three most common cancers (breast, colorectal and prostate) as "limited-no conclusion" <sup>(4)</sup> indicating a need for more robust studies focusing specifically on legume consumption.

The aim of this study was therefore to quantify the role of legumes on the risk of cancer at several sites using an integrated network of casecontrol studies.

# **Materials And Methods**

This work is based on data from an integrated network of 16 case-control studies conducted between 1991 and 2009 in various areas of northern (the greater Milan area; the provinces of Rome and Latina) and southern (the urban area of Naples) Italy, and in the Canton of Vaud, Switzerland <sup>(15)</sup>. Fourteen studies collecting information on legume consumption were included in this work <sup>(13,16-28)</sup>. The studies enrolled incident, histologically confirmed cases of oral cavity, oesophageal, stomach, colorectal, larynx, breast, endometrial, ovarian, prostate and kidney cancers. Controls were enrolled in the same hospitals among patients admitted for acute and nonneoplastic conditions and were partly overlapping for various studies in this network. Trained interviewers asked participants to report sociodemographic information, height, weight, smoking habit, food and beverages consumption including alcoholic beverages, physical activity, medical history, and familiarity for cancer. Information was collected using a structured questionnaire which included a validated food frequency questionnaire (FFQ) evaluating portion sizes and frequency of consumption of 78 foods, food groups or recipes <sup>(29)</sup>. Consumption of food and beverages were collected over the year preceding the hospital admission.

Participants were asked to report the size of the portion consumed (small, medium, large), assuming a medium portion of fresh legumes of 100 grams and of dried legumes of 40 grams. Small and large portions were set to be 0.66 or 1.33 times the medium portion, respectively. Frequency of consumption was collected as number of portions per week. Legume consumption was then expressed as number of medium portions consumed in a week, and used in the analysis as continuous variable or categorized into 3 levels of consumption, i.e. <1, 1 portion or  $\geq$  2 portions per week. The association between legume consumption and different cancer sites was evaluated by the odds ratio (OR) and corresponding 95% confidence intervals (CI) estimated through multiple logistic regression models. Each model was adjusted for sex, age

(continuous), years of education (< 13  $vs \ge$  13 years), smoking (current, ever, never), alcohol intake (study-specific tertiles), body mass index (< 18.5, 18.5–24.9, 25-29.9,  $\ge$  30 kg/m<sup>2</sup>), consumption of raw and cooked vegetables (study-specific tertiles), fruit (study-specific tertiles) and processed meat (study-specific tertiles) and energy intake. Estimates for breast, endometrial, ovarian cancers were also adjusted for age at menarche (continuous), menopausal status (pre, peri and post-menopause) and number of children (none, 1 and  $\ge$  2 children).

Completeness was above 85% for the majority of variables in all studies. For raw vegetables and processed meat in the study of stomach cancer and cooked vegetables in the studies on oral cavity, stomach, esophagus and laryngeal cancers, the percentage of missing values exceeded 5% (**Online Supplement S1**).

A multiple imputation technique using a fully conditional specification (FCS) method was implemented to account for missing values under the missing at random assumption <sup>(30)</sup>. Ten completed data sets were generated for each cancer site and used to obtain ten different estimates and these corresponding standard errors, were then combined using the Rubin's rule <sup>(31)</sup>.

All models included vegetable, fruit and whole bread intakes to control for confounding related to the fact that legume consumers tend to have a healthier diet compared to non-consumers. This also implies that legume consumers have a higher fibre intake, in part because legumes are an important source of fibre and in part because of the high consumption of other fibre-rich foods. Given that most of the health benefit of legumes are related to dietary fibre, we also assessed the differences in fibre intake in cases and controls and across categories of legume consumption. Mean differences were estimated using linear regression models including a group indicator (cases *vs* controls) and the category of legume consumption as predictors.

The main analysis included both sexes. A stratified analysis by sex was also performed to evaluate sex-differences in the association between legumes and cancer risk.

The study was approved by the ethical committees of the hospitals involved, and all participants gave informed consent.

# Results

This work included a total of 10,482 cancer cases (1292 cancers of oral cavity, 488 oesophageal cancers, 225 stomach cancers, 1914 colorectal cancers, 604 laryngeal cancers, 2554 breast cancers, 357 endometrial cancers, 1028 ovarian cancers, 1270 prostate cancers, and 750 kidney cancers). Table 1 gives the sex and age distribution among cases and controls, separately for each cancer site. Around 80-90% of the cases of oral cavity, esophagus and laryngeal cancer were men, whereas the percentage was lower (~ 60%) among cases of stomach, colorectal and kidney cancers. More than 75% of the cases of oral cavity, breast and ovarian cancers were diagnosed at ages below 65 years, while this percentage ranged between 54–66% for the remaining cancer sites, except prostate cancer for which only 40% of cases were diagnosed at ages < 65.

	Cases		Controls		
	Sex				
Cancer site	Men	Women	Men	Women	
Oral cavity	1064 (82.4)	228 (17.6)	2308 (70.4)	972 (29.6)	
Esophagus	421 (86.3)	67 (13.7)	905 (72.9)	337 (27.1)	
Stomach	141 (62.7)	84 (37.3)	270 (51.8)	251 (48.2)	
Colorectum	1094 (57.2)	820 (42.8)	2029 (49.6)	2060 (50.4)	
Larynx	547 (90.6)	57 (9.4)	1141 (79.8)	289 (20.2)	
Breast	•	2554 (100)	•	2572 (100)	
Endometrium	•	357 (100)	•	785 (100)	
Ovary	•	1028 (100)	•	2379 (100)	
Prostate	1270 (100)	•	1416 (100)	•	
Kidney	479 (63.9)	271 (36.1)	962 (64.2)	536 (35.8)	
	Age				
	< 65 years	$\geq$ 65 years	< 65 years	$\geq$ 65 years	
Oral cavity	972 (75.2)	320 (24.8)	2375 (72.4)	905 (27.6)	
Esophagus	316 (64.8)	172 (35.2)	806 (64.9)	436 (35.1)	
Stomach	121 (53.8)	104 (46.2)	278 (53.4)	243 (46.6)	
Colorectum	1154 (60.3)	760 (39.7)	2953 (72.2)	1136 (27.8)	
Larynx	397 (65.7)	207 (34.3)	950 (66.4)	480 (33.6)	
Breast	2028 (79.4)	526 (20.6)	1955 (76.0)	617 (24.0)	
Endometrium	234 (65.5)	123 (34.5)	500 (63.7)	285 (36.3)	
Ovary	792 (77)	236 (23)	1756 (73.8)	623 (26.2)	
Prostate	511 (40.2)	759 (59.8)	767 (54.2)	649 (45.8)	
Kidney	450 (60)	300 (40)	900 (60.1)	598 (39.9)	

Table 1 Sex and age distribution of cases and controls by cancer site

Table 2 shows the frequency distribution of legume consumption among cases and controls. Most cases (60-75%) were in the lowest category of consumption (i.e. < 1 portion per week), 20-30% consumed at least one portion in a week and only a minority (6-13%) consumed 2 or more portions. The adjusted ORs indicated an inverse association between consumption of 2 or more portions of legumes per week and cancers of oral cavity (OR: 0.71, 95% CI: 0.52-0.97), esophagus (OR: 0.50, 95% CI: 0.29-0.86), colorectum (OR: 0.70, 95% CI: 0.58-0.83), larynx (OR: 0.55, 95% CI: 0.34-0.89) and kidney (OR: 0.69, 95% CI: 0.49-0.95). No significant associations were found for cancers of stomach, breast, endometrium, ovary and prostate, although for breast, ovarian and prostate cancers the point estimates indicated a reduced risk, but the Cl included 1. An inverse association was also found for consumption of 1 portion of legume per week and colorectal (OR: 0.79, 95% CI: 0.69-0.91) and ovarian cancers (OR: 0.79, 95% CI: 0.66-0.95). A significant reducing risk with increasing level of consumption was observed for cancer of esophagus (OR per portion: 0.82, *p* for trend: 0.049), colorectum (OR per portion: 0.88, *p* for trend < 0.001), ovary (OR per portion: 0.89, *p* for trend: 0.017), and kidney (OR per portion: 0.88, *p* for trend: 0.041).

### Table 2

Legume consumption among cancer cases and controls and odds ratio for cancer according to categories of legume consumption by cancer

	Cases			Controls						
	Portions	ions of legumes per week OR (95% CI) <sup>a</sup>							<i>p</i> -value for linear trend <sup>b</sup>	
Cancer site	< 1	1	≥2	< 1	1	≥2	1 vs<1	$\geq$ 2 vs < 1	Per portion	
	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)				
Oral cavity	919 (71.1)	294 (22.8)	79 (6.1)	2214 (67.5)	770 (23.5)	296 (9.0)	0.95 (0.78- 1.15)	0.71 (0.52- 0.97)	0.94 (0.84– 1.05)	0.252
Esophagus	363 (74.4)	97 (19.9)	28 (5.7)	862 (69.4)	273 (22.0)	107 (8.6)	0.78 (0.55- 1.09)	0.50 (0.29- 0.86)	0.82 (0.67- 1.00)	0.049
Stomach	133 (59.1)	70 (31.1)	22 (9.8)	293 (56.2)	171 (32.8)	57 (10.9)	0.92 (0.62- 1.37)	0.96 (0.53– 1.75)	0.92 (0.74– 1.15)	0.466
Colorectum	1205 (63.0)	457 (23.9)	252 (13.2)	2234 (54.6)	1166 (28.5)	689 (16.9)	0.79 (0.69– 0.91)	0.70 (0.58– 0.83)	0.88 (0.82– 0.94)	< 0.001
Larynx	427 (70.7)	142 (23.5)	35 (5.8)	918 (64.2)	369 (25.8)	143 (10.0)	0.90 (0.68- 1.20)	0.55 (0.34– 0.89)	0.93 (0.79– 1.09)	0.392
Breast	1510 (59.1)	716 (28.0)	328 (12.8)	1477 (57.4)	740 (28.8)	355 (13.8)	0.91 (0.80- 1.04)	0.83 (0.70- 1.00)	0.96 (0.90- 1.03)	0.235
Endometrium	208 (58.3)	114 (31.9)	35 (9.8)	487 (62.0)	224 (28.5)	74 (9.4)	1.21 (0.88– 1.65)	1.18 (0.72– 1.93)	1.10 (0.89– 1.34)	0.381
Ovary	611 (59.4)	282 (27.4)	135 (13.1)	1323 (55.6)	726 (30.5)	330 (13.9)	0.79 (0.66- 0.95)	0.81 (0.64– 1.04)	0.89 (0.81– 0.98)	0.017
Prostate	797 (62.8)	351 (27.6)	122 (9.6)	849 (60.0)	407 (28.7)	160 (11.3)	0.99 (0.82- 1.19)	0.81 (0.61– 1.07)	0.95 (0.85– 1.05)	0.294
Kidney	497 (66.3)	187 (24.9)	66 (8.8)	907 (60.5)	413 (27.6)	178 (11.9)	0.83 (0.66- 1.03)	0.69 (0.49- 0.95)	0.88 (0.77– 0.99)	0.041

CI: Confidence Interval. OR: Odds ratio.

<sup>a</sup> ORs and 95% CI were obtained from a logistic regression models adjusted for sex, age (continuous), years of education (< 13  $vs \ge 13$  years), smoking (current, ever, never), alcohol drinking (study-specific tertiles), body mass index (< 18.5, 18.5–24.9, 25-29.9,  $\ge 30$  kg/m<sup>2</sup>), consumption of raw and cooked vegetables (study-specific tertiles), fruit (study-specific tertiles), processed meat (study-specific tertiles) and energy intake. Estimates for breast, endometrial, ovarian cancers were also adjusted for age at menarche (continuous), menopausal status (pre, peri and post-menopause) and number of children (none, 1 and  $\ge 2$  children).

<sup>b</sup> Obtained from a likelihood ratio test comparing the model with *vs* the model without the variable legume consumption, included as portions per week in continuous (without categorization).

The analysis stratified by sex showed that the association with cancers of for oral cavity, esophagus, colorectum, larynx and kidney was limited to the male sex (Fig. 1).

Legume consumption was associated with high intake of dietary fibre in all studies (Table 3). Consumers of 1 portion of legumes per week had 2.6 to 5.0 more grams of fibre per day than individuals who had less than 1 portion per week, while consumers of 2 or more portions had 4.6 to 7.5 more grams of fibre per day. After controlling for legume consumption, cases of oral cavity, esophageal, laryngeal, colorectal and ovarian cancers had lower fibre intake than controls, whereas no significant differences were found for other cancer sites.

Table 3

Relationship between legumes consumption and fibre intake among cancer cases and controls by cancer site.

Leg con (por < 1 Oral cavity 17.8 Esophagus 18.2 Stomach 21.0	gume isumption rtions per v 1 8 22.7 2 23.1	week) ≥ 2 25.5 26.8	Legun consu (portic < 1 19.5	ne mption ons per v 1 24.6	week) ≥2	Estimated Cl) <sup>a</sup> Cases <i>vs</i> Controls	mean difference 1 <i>vs</i> < 1	es, g/day (95% 2 <i>vs</i> < 1	Comparison: case <i>vs</i> controls <i>p</i> -value <sup>b</sup>	Comparisor legume consumptio categories <i>p</i> -value <sup>b</sup>
(por <1 Oral cavity 17.8 Esophagus 18.2 Stomach 21.0	rtions per v 1 8 22.7 2 23.1	<ul> <li>week)</li> <li>≥ 2</li> <li>25.5</li> <li>26.8</li> </ul>	(portic < 1 19.5	1 24.6	veek) ≥2	Cases <i>vs</i> Controls	1 <i>vs</i> <1	2 <i>vs</i> <1	controls <i>p</i> -value <sup>b</sup>	legume consumptio categories <i>p</i> -value <sup>b</sup>
< 1 Oral cavity 17.8 Esophagus 18.2 Stomach 21.0	1 8 22.7 2 23.1	≥ 2 25.5 26.8	< 1 19.5	1 24.6	≥2	Cases <i>vs</i> Controls	1 <i>vs</i> <1	2 <i>vs</i> <1	<i>p</i> -value <sup>b</sup>	<i>p</i> -value <sup>b</sup>
Oral cavity 17.8 Esophagus 18.2 Stomach 21.0	8 22.7 2 23.1	25.5 26.8	19.5	24.6		Controls	logumo			
Oral cavity 17.8 Esophagus 18.2 Stomach 21.0	8 22.7 2 23.1	25.5 26.8	19.5	24.6			portion/week	legume portion/week		
Esophagus 18.2 Stomach 21.0	2 23.1	26.8			27.0	-1.8 (-2.3 to -1.3)	5.0 (4.5 to 5.6)	7.5 (6.7 to 8.3)	< 0.0001	< 0.0001
Stomach 21.0			19.6	24.8	26.8	-1.4 (-2.2 to -0.6)	5.0 (4.2 to 5.9)	7.5 (6.1 to 8.8)	0.0005	< 0.0001
	0 23.9	23.6	20.2	23.5	26.0	0.3 (-0.8 to 1.4)	3.1 (2.1 to 4.2)	4.9 (3.2 to 6.5)	0.57	< 0.0001
Colorectum 22.8	8 24.6	30.1	22.2	24.9	28.8	0.5 (0.1 to 0.9)	2.5 (2.0 to 2.9)	6.8 (6.2 to 7.3)	0.0191	< 0.0001
Larynx 19.7	7 23.9	27.3	20.8	24.8	28.4	-1.0 (-1.7 to -0.3)	4.2 (3.2 to 4.8)	7.6 (6.4 to 8.8)	0.0077	< 0.0001
Breast 22.6	6 24.9	27.0	21.8	25.0	27.6	0.3 (-0.1 to 0.7)	2.8 (2.3 to 3.3)	5.1 (4.7 to 5.7)	0.1305	< 0.0001
Endometrium 21.4	4 23.2	24.4	20.7	24.1	26.0	-0.1 (-0.9 to 0.8)	2.9 (2.0 to 3.8)	4.6 (3.2 to 6.0)	0.91	< 0.0001
Ovary 22.8	8 23.8	27.3	20.7	24.1	27.2	1.1 (0.6 to 1.7)	2.6 (2.0 to 3.2)	5.9 (5.1 to 6.7)	< 0.0001	< 0.0001
Prostate 23.0	0 25.7	29.1	22.5	25.5	27.4	0.5 (-0.01 to 1.1)	2.9 (2.2 to 3.5)	5.4 (4.5 to 6.3)	0.053	< 0.0001
Kidney 22.0	0 25.7	27.7	21.9	25.2	27.0	0.3 (-0.3 to 0.9)	3.4 (2.7 to 4.1)	5.3 (4.3 to 6.3)	0.37	< 0.0001

<sup>9</sup> Likelihood ratio test (model with *vs* model without the predictor

## Discussion

Our findings indicate that a high consumption of legumes is associated with a decreased risk of several cancers including those of the upper aerodigestive tract, colorectal, ovary and kidney cancers. When stratified for sex our results showed that the association with cancers of the upper aerodigestive tract, colorectum and kidney cancer was greater in males.

The strongest inverse associations between legume consumption and cancer were observed for esophagus and larynx cancer with OR of 0.50 and 0.55, respectively, for consumption of 2 or more portions per week. These results are consistent with previous case-control studies which have reported OR of 0.54–0.62 for esophagus and larynx cancer with the highest intake of legumes <sup>(32, 33)</sup>. A case-control study of 11 cancer sites conducted in Uruguay between 1996 and 2004 and including 3,539 cancer cases and 2,032 hospital controls reported an OR of 0.54 for esophagus and 0.55 for laryngeal cancer among the highest as compared to the lowest tertile of consumption <sup>(34)</sup>. Other studies from the United States (Connecticut and Los Angeles) looking at associations between legumes and esophageal cancer reported significant inverse

associations between legume intake and risk of esophageal cancer (particularly a decreased risk of esophageal squamous cell carcinoma), although the legume group within these studies included beans and nuts <sup>(35, 36)</sup>.

We found that even a moderate consumption of legumes (i.e. 1 portion a week) is associated with a significant reduction of colorectal cancer risk (OR: 0.70). In line with our findings a recent meta-analysis of observational studies (n = 14: 3 cohort studies, 11 case-control studies) found a decreased risk of colorectal adenoma for the highest versus lowest intake of legumes (OR = 0.83) <sup>(11)</sup>. However, other studies provided mixed results with some indicating an inverse association or no association <sup>(10)</sup>. In the Polyp Prevention Trial, an increased consumption of legumes was associated with a reduced risk of advanced adenoma recurrence. The OR in individuals in the highest quartile of change in dry bean intake from baseline (median change: +41.5 g/day) versus the lowest quartile (-5.7 g/day) was 0.35 <sup>(9)</sup>.

In our study, legume intake was also linked to a reduced risk of kidney and ovarian cancer. Diet has been related to kidney cancer although the role of specific foods or nutrients is still controversial <sup>(12)</sup>. A limited number of studies have specifically examined the association between legume consumption and kidney cancer <sup>(12)</sup> <sup>(37)</sup> <sup>(38)</sup>. Consistent with our findings, a case-control study in Uruguay reported a significant inverse association between legume intake and kidney cancer (OR = 0.41) <sup>(34)</sup>. In addition, a large US cohort (N = 1816) showed a dose-response relationship with a 12% reduced risk of kidney cancer per 2.5g/day increment of dietary legume fibre<sup>(12, 38)</sup>.

To date, a few studies have considered dietary patterns in relation to ovarian cancer risk and none, to our knowledge, has looked specifically at legumes <sup>(14, 39)</sup>. Some studies have examined the intake of plant-based foods and fibre and showed inconsistent results <sup>(14, 40)</sup>.

We found inverse but not significant associations between legume consumption and risk of stomach, breast, endometrium, and prostate cancer. Previous studies for these cancer sites reported mixed results, some reporting weak/moderate associations (OR ranging from 0.42– 0.84) or null associations (<sup>39,41-44</sup>).

As to the mechanisms that could explain a possible protective effect of legume intake on cancer risk there are several possible explanations <sup>(45-48)</sup>. Legumes are recognised as a protein source but are often overlooked as a source of fibre, with 100g of cooked legumes containing, at a minimum, 5g of dietary fibre <sup>(5)</sup>. Our results showed that those who consumed at least 1 portion of legumes per week had 6–8 more grams of fibre per week than individuals who had less than 1 portion per week. Consumers of 2 or more portions per week had up to 7.5 more grams of fibre per day. This represents half of the recommended 14g/1000Kcal/day to reduce chronic disease risk<sup>(7)</sup>. Thus, the beneficial effects related to legume consumption are likely related to their fibre content and this is particularly true for colorectal cancer. When entering the large bowel, fibre increases stool weight, dilutes colonic contents and stimulates bacterial anaerobic fermentation. This process reduces contact between the intestinal contents and mucosa and leads to the production of short chain fatty acids (SCFA) through the fermentation of fibre by gut bacteria. SCFAs reduce cell proliferation, the first biological mechanism promoting carcinogenesis. SCFA reduce colonic pH thereby inhibiting the histone deacetylase enzyme and decreasing the conversion of primary to secondary bile acids (deoxycholic acid and lithocholic acid) which are cytotoxic to colonocytes <sup>(6)</sup>. Furthermore, dietary fibre is a substrate for the gut microbiota affecting amount and composition favouring anti-inflammatory strains which have local and systemic health benefits via modulation of the immune system, production of microbial metabolites, conversion of polyphenols into biologically active forms, and modifying also distant organ tissue-specific strains <sup>(6, 45)</sup>. Beyond fibre, other bioactive compounds in legumes, such as phenolics, may also play a role in inhibition of colorectal cancer <sup>(45)</sup>.

Dietary fibre and proteins from legumes also contribute to lower the glycaemic load of the diet  $^{(6, 21)}$  thus preventing hyperglycaemia and hyperinsulinemia are both sustained by excess body fat and consequential changes in hormonal status, growth factors, inflammatory markers, and oxidative stress – all contributing factors in the development of chronic diseases, including cancers  $^{(6-8)}$ . Pulses have been linked to improvements in these markers  $^{(45)}$ .

In addition to fibre, legumes are also rich in vitamins (i.e. B vitamins), minerals (i.e. iron, folate, calcium and zinc) and a series of biological active compounds, known as phytochemicals which also have antitumor effects <sup>(47)</sup>. These compounds include tannins, flavonols, isoflavones, phenolic acids and phytic acids <sup>(45)</sup>. For example, phytate are excreted in the urine where they inhibit the formation of kidney stones <sup>(37)</sup>, which have been related to kidney cancer <sup>(49)</sup>. Legumes are also a good source of folate, which may protect against cancers of the esophagus and colon <sup>(13, 33)</sup>.

In addition to the direct cancer preventative effects of legume intake, indirect effects may also be at work as well. Higher intake of legumes may replace other sources of protein such as meat or high glycaemic index carbohydrates, both of which have been shown to be linked to several cancers<sup>(48)</sup>.

The sex-specific pattern of association for oral cavity, larynx, esophagus, colorectum and kidney cancers is difficult to explain, but could be related to the differences in dietary habits between men and women. In the studies included in this work, men tended to eat less vegetables

and fruit than women (**Online Supplement Table S2**), thus legumes in men can be an important source of dietary fibre that compensate for the low fibre intake from other sources <sup>(29, 39, 42)</sup>. The observed sex differences may also be linked to a greater effect in men than in women of dietary changes on microbial composition reported in some studies <sup>(50)</sup>. However, whether the microbial composition is involved in the development of cancer remains to be determined.

# Strength And Weakness

In this work, we quantified the association between legume consumption and several cancer sites using a network of large case-control studies. In these studies, the same validated and reproducible questionnaires have been used to collect information on legume consumption and to measure potential confounders. Several confounders have been considered including age, education, overweight/obesity, smoking, alcohol, consumption of fruit, vegetables and processed meat, energy intake and for female hormone-related cancers also age at menarche, menopausal status and number of children.

The study has also some limitations. The first lies in the potential inaccurate measure of legume consumption in a case control design. In addition, the inverse association between legume consumption and various cancers can at least be partially attributable to a generally healthier diet of legume consumers who also had high intake of fibre from other dietary sources. Finally, although the majority of studies included more than 1000 cases, for some cancer sites only a few cases were in the highest category of consumption (i.e.  $\geq$ 2 portions). This should be considered when interpreting the significance of the estimates.

# Conclusions

Our results indicate an inverse association between legume intake and risk of several cancers including those of the upper aerodigestive tract, colon, ovary and kidney. Recommendations to include two portions or 200g of legumes per week (80 gram of dried legumes) could contribute to lower the risk of cancer.

# Declarations

Acknowledgements: The raw data used in this paper were taken from data from an integrated network of 16 case control studies that were conducted in Italy and in the Swiss Canton of Vaud.

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## Conflicts of interest: None

Authors' Contributions: Linia Patel and Gianfranco Alicandro conceptualised the study, Gianfranco Alicandro and Linia Patel designed the study, Gianfranco Alicandro performed the data analysis, Linia Patel, Gianfranco Alicandro wrote the original draft and all authors reviewed and edited drafts. Carlo La Vecchia was responsible for overall supervision. All authors read and approved the final manuscript.

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## **Figures**

## **Oral cavity**

## **Esophagus**





Colorectum

## Stomach



### Larynx



Kidney

No. of legume portions per week	OR [95% CI]	No. of legume portions per week	OR [95% CI]
1 vs <1	0.92 [0.69, 1.24]	1 vs <1	0.88 [0.67, 1.16]
1 vs <1 0	0.84 [0.29, 2.41]	1 vs <1 -0	0.74 [0.49, 1.10]
>=2 vs <1	0.51 [0.31, 0.84]	>=2 vs <1	0.55 [0.36, 0.83]
>=2 vs <1	1.22 [0.26, 5.58]	>=2 vs <1	1.16 [0.65, 2.05]
r <del>rii</del>		r	
0.2 0.5 1 2		0.2 0.5 1 2	

## Figure 1

Sex differences in the odds ratio for cancer of oral cavity, esophagus, stomach, colorectum, larynx and kidney according to categories of legume consumption.

Men = full circle. Women = open circle.

## **Supplementary Files**

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