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Citrus fruit intake and gastric cancer: The stomach cancer pooling (StoP) project consortium

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Abbreviations: CI: confidence intervals; EPIC: European Prospective Investigation into Cancer and Nutrition cohort; FFQ: food frequency questionnaire; OR: odds ratio; StoP: Stomach cancer pooling project

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Diets rich in vegetables and fruit have been associated with reduced risk of gastric cancer, and there is suggestive evidence that citrus fruits have a protective role. Our study aimed at evaluating and quantifying the association between citrus fruit intake and gastric cancer risk. We conducted a one-stage pooled analysis including 6,340 cases and 14,490 controls from 15 case–control studies from the stomach cancer pooling (StoP) project consortium. Odds ratios (ORs) and the corresponding 95% confidence intervals (CIs) of gastric cancer across study-specific tertiles of citrus fruit intake (grams/week) were estimated by generalized linear mixed effect models, with logistic link function and random intercept for each study. The models were adjusted for sex, age, and the main recognized risk factors for gastric cancer. Compared to the first third of the distribution, the adjusted pooled OR (95% CI) for the highest third was 0.80 (0.73–0.87). The favourable effect of citrus fruits increased progressively until three servings/week and leveled off thereafter. The magnitude of the association was similar between cancer sub-sites and histotypes. The analysis by geographic area showed no association in studies from the Americas. Our data confirm an inverse association between citrus fruits and gastric cancer and provide precise estimates of the magnitude of the association. However, the null association found in studies from America and in some previous cohort studies prevent to draw definite conclusions on a protective effect of citrus fruit consumption.

What's new?

The association between citrus fruit intake and cardia cancer was classified as limited/suggestive, whereas no conclusions could be drawn on non-cardia cancer. Our pooled analysis within a global consortium of case-control studies indicates and quantifies a protective effect of citrus fruits on both cardia and non-cardia cancers.

Introduction

Gastric cancer is the fifth most common cancer worldwide and the third cancer-related cause of death.[1] It can originate from two different anatomic areas of the organ, i.e. the proximal part of the stomach (cardia cancers) or the mid and distal part (noncardia cancers), with distinct etiology and epidemiology. Gastroesophageal reflux and obesity have been identified as risk factors for cardia cancers, while *Helicobacter pylori* (*H. pylori*) infection, low socio-economic status, smoking, heavy alcohol drinking, consumption of food preserved by salt and processed meat are the main risk factors for noncardia cancers.[2]

Cardia cancer incidence has been stable or increasing, while noncardia cancers have been substantially decreasing,[3–6] likely as a consequence of a reduced prevalence of *H. pylori* infection, improvements in diet, and advances in food preservation technology.[7]

Healthy dietary patterns, rich in vegetables and fruit, have been associated with reduced risk of gastric cancer[8,9] and there is suggestive evidence that citrus fruits could have a protective role.[2,10,11] Such a favorable effect has been related to bioactive compounds contained in citrus fruits, including, among others, vitamin C and flavonoids. Vitamin C, an enzymatic cofactor and scavenger of reactive oxygen species, inhibits nitrosamine formation in the stomach, thus reducing oxidative damage of the gastric mucosa.[12,13] Flavonoids are aromatic secondary plant metabolites, which have antioxidant, radical scavenging and immunomodulatory activity.[14]

Our study aims at verifying and quantifying the strength of the association between citrus fruit intake and the risk of gastric cancer through the analysis of data from the stomach cancer pooling (StoP) project consortium.[15]

Materials and Methods

Study population

Our study is based on the second data release of the StoP project consortium (http://www.stop-project.org/), which included 31 case–control studies of gastric cancer conducted worldwide. Detailed information on the aims and methods of the StoP project has been given elsewhere.[15,16] Participating studies were involved through personal contacts of participating investigators. Principal investigators provided a signed data transfer agreement and, thereafter, the complete original data set of the study. We collected and harmonized all data according to a pre-specified format. For these analyses, we selected 15 case–control studies with data on citrus fruit intake including one study from Greece,[17] three from Italy,[18–20] one from Portugal,[21] one from Russia,[22] one from Spain,[23] two from Iran,[24,25] one from Japan,[26] one from Canada,[27] one from the USA[28] and three from Mexico.[29–31]

Studies' quality was assessed by the Newcastle-Ottawa quality assessment scale for case–control studies. The scale evaluates the study quality on the basis of three different categories: selection, exposure and comparability. A study can be awarded a maximum of nine stars that indicates the highest quality.

The StoP project received ethical approval from the University of Milan Review Board (reference 19/15 on 01/04/2015).

Citrus fruit intake

Citrus fruit intake was measured through food frequency questionnaires (FFQs) that asked participants to indicate food and beverage consumption before the diagnosis of gastric

cancer, for all the studies. Of the 15 studies included, 9 collected citrus fruit consumption one year before diagnosis/interview, 2 two years, and 4 three to five years. Citrus fruit consumption was expressed in grams per week, by taking into account the serving and frequency of consumption indicated in each study-specific FFQ. When the FFQ did not contain a specific variable for the whole citrus fruit group, we combined the available information on the consumption of single food items, including oranges, lemons, tangerines, grapefruits and citrus fruit juices. Fruit juices containing a mixture of citrus and noncitrus fruits were not considered. When the consumption of the food item was not expressed in grams, we converted the amount of fruit reported into grams by considering the average weight for each fruit: 150 g for oranges and citrus fruit juices, 300 g for grapefruits, 50 g for tangerines, and 30 g for lemons.

Data analysis

We carried out an individual participant data pooled analysis using a one-stage approach.[32] We run generalized linear mixed effect models with logistic link function and random intercept for each study, to estimate the odds ratio (OR) and corresponding 95% confidence intervals (CIs) of gastric cancer across study-specific tertiles of citrus fruit intake. Tertiles were derived from the distribution of citrus fruit intake among controls. ORs and corresponding 95% CIs were estimated also for the number of servings per week that was included in the model as a categorical variable, ranging from 0 to 7 or more servings per week. The number of servings per week was computed by considering an average serving of 150 g. The models were adjusted for sex, 5-year age groups, socioeconomic status (low, intermediate, high), tobacco smoking (never, former, current low, current intermediate, current high), alcohol drinking (never, low: ≤ 12 g/day, intermediate: > 12 to <48 g/day, high: ≥48 g/day), study-specific salt, other fruit and vegetable intake (low, intermediate, high), and family history of gastric cancer. Information on these covariates were collected by structured questionnaires, self-administered or administered by trained interviewers. Subjects with missing values for a given covariate were retained in the model by including them in a separate category of the variable.

A dose-risk relationship was modeled using polynomial models. This flexible class of models allowed to evaluate the possible nonlinear trends of the dose-risk relationship by fitting several functional forms, including the linear one. The Akaike information criterion was used to select the model that provided the best fit with the data.

We performed stratified analyses by sex, age group, socioeconomic status, geographic area, smoking status, alcohol drinking, total fruit intake, salt intake, family history of gastric cancer, *H. pylori* infection, type of controls, cancer sub-site and histotype. For the strata of *H. pylori* infection, we did not included the Spain 2 study since the information was collected only for cases. For the strata of sub-site and histotype, we used multinomial mixed effect models to estimate the ORs for each type of cancer separately (i.e., cardia and noncardia or intestinal and diffuse). For each stratifying variable, the Q statistics was computed to test the heterogeneity across strata.

We also carried out a series of sensitivity analyses: 1) we excluded citrus fruit juices from the evaluation of citrus fruit consumption in studies that had this item listed in the FFQ, since the fruit content may vary in fruit juices; 2) we estimated the ORs of gastric cancer across thirds of the distribution of citrus fruit intake using a two-stage approach;[32] 3) we restricted the analysis to the studies that scored more than five stars at the Newcastle-Ottawa quality assessment score; 4) we removed from the analysis the studies that evaluated citrus fruit consumption by self-administered FFQ; 5) to evaluate if the time window for dietary information modified our results, we provided two separate estimates for studies who collected citrus fruit consumption 1 year before and 2 to 5 years before diagnosis of gastric cancer.

Results

Table 1 gives the distribution of the sociodemographic characteristics and the main lifestyle risk factors of the 6,340 gastric cancer cases and 14,490 controls. Almost 60% of cases came from Europe, about one third from the Americas, and the remaining cases from Asia. Cases were more likely than controls to be males, older, of low socioeconomic status, heavy smokers and alcohol drinkers, and to have a positive family history of gastric cancer. Fruit intake was lower among cases as compared to controls.

Table 2 shows the distribution of citrus fruit consumption (including and excluding citrus fruit juices) for cases and controls by study. Most studies showed lower citrus fruit intake in cases than in controls. In cases, median citrus fruit intakes ranged between 56 g per week in the Iran 1 study and 789 g per week in the study from Canada, while in controls they ranged between 200 g per week in the Iran 2 study to over 1 kg per week in the study from Greece. Most of the citrus fruit intake in the studies from the USA and Canada came from citrus fruit juices (around 80%).

The risk of gastric cancer was inversely related to citrus fruit consumption (Table 3). Compared to the 1st third of the distribution of citrus fruit intake, the adjusted pooled ORs (95% CIs) for the 2nd and 3rd third were: 0.80 (0.74–0.86) and 0.80 (0.73–0.87), respectively. Figure 1 shows the ORs for the highest compared to the lowest third of citrus fruit intake for each study along with the pooled estimate. Heterogeneity emerged across studies.

The inverse relationship between citrus fruits and gastric cancer risk increased progressively until three servings per week and leveled off thereafter (Table 3). Figure 2 shows the dose-risk relationship between citrus fruit consumption and gastric cancer risk estimated by a model including the natural logarithm of citrus fruit intake as exposure variable, after allowing for confounders. The best fitting dose-risk

Table 1. Distribution of gastric cases and controls according to study center, sex, age, and other selected covariates. Stomach cancer pooling (StoP) project consortium

	Case		Control	
	N	%	N	%
Total	6,340	100.0	14,490	100.0
Study				
Europe				
Greece	110	1.7	100	0.7
Italy 1	769	12.1	2,081	14.4
Italy 2	230	3.6	547	3.8
Italy 4	1,016	16.0	1,159	8.0
Portugal	692	10.9	1,667	11.5
Russia	450	7.1	611	4.2
Spain 2	401	6.3	455	3.1
Asia				
Iran 1	217	3.4	394	2.7
Iran 2	286	4.5	304	2.1
Japan 3	153	2.4	303	2.1
The Americas				
Canada	1,182	18.6	5,039	34.8
USA 1	132	2.1	132	0.9
Mexico 1	248	3.9	478	3.3
Mexico 2	220	3.5	752	5.2
Mexico 3[31]	234	3.7	468	3.2
Sex				
Men	3,995	63.0	7,747	53.5
Women	2,345	37.0	6,743	46.5
Age (years)				
<40	265	4.2	1,401	9.7
40–44	261	4.1	1,035	7.1
45–49	429	6.8	1,307	9.0
50–54	570	9.0	1,500	10.4
55–59	781	12.3	1,678	11.6
60–64	996	15.7	2,132	14.7
65–69	1,203	19.0	2,364	16.3
70–74	1,209	19.1	2,142	14.8
≥75	626	9.9	931	6.4
Socioeconomic status (study-specific)				
Low	3,533	55.7	5,952	41.1
Intermediate	1,861	29.4	4,853	33.5
High	827	13.0	3,505	24.2
Missing	*119*	*1.9*	*180*	*1.2*
Tobacco smoking				
Never	2,670	42.1	6,522	45.0
Former	1,739	27.4	3,889	26.8
Current				
Low	448	7.1	1,360	9.4
Intermediate	630	9.9	1,389	9.6
High	566	8.9	981	6.8
Missing	*287*	*4.5*	*349*	*2.4*
Alcohol drinking status				
Never	1,885	29.7	4,650	32.1
Ever	4,373	69.0	9,550	65.9
Missing	*82*	*1.3*	*290*	*2.0*
Alcohol drinking (gr/day)[1]				
Low (≤12)	1,349	30.3	4,156	42.3
Intermediate (>12 and <48)	1,915	43.1	3,307	33.6
High (≥48)	932	21.0	1,535	15.6
Missing	*251*	*5.6*	*835*	*8.5*
History of stomach cancer in first degree relatives[2]				
No	2,988	67.1	5,219	67.3
Yes	645	14.5	842	10.9
Missing	*823*	*18.5*	*1,692*	*21.8*
Total fruit intake (study-specific tertiles)[3]				
Low	2,114	34.6	4,063	29.0
Intermediate	2,089	34.2	4,871	34.7
High	1,831	30.0	5,010	35.7
Missing	*72*	*1.2*	*78*	*0.6*
Salt intake (study-specific tertiles)[4]				
Low	2,081	39.9	5,356	40.5
Intermediate	1,679	32.2	3,987	30.1
High	1,244	23.9	3,119	23.6
Intermediate or high	160	3.1	308	2.3
Missing	*50*	*1.0*	*461*	*3.5*

[1]Data not available for the study Iran 2.
[2]Data not available for the following studies: Canada, Mexico 1, Mexico 2, and Mexico 3.
[3]Data not available for the study Mexico 3.
[4]Data not available for the studies Italy 4 and Greece.

relationship between citrus consumption and gastric cancer risk was: ln(OR) = −0.05535. ln(citrus fruit consumption in grams per week).

The stratified analysis showed similar effects of citrus fruit intake among strata of sex, age group, smoking status, alcohol drinking, total fruit intake, salt intake, family history of gastric cancer, *H. pylori* infection, type of controls, cancer sub-site and histotype, while the protective effect was greater in people of low socio-economic status (Q = 4.6, $p = 0.032$). There were also significant differences across geographic areas (Q = 18.6, $p < 0.0001$) with no association in studies from America (Fig. 3).

The results of the sensitivity analysis excluding citrus fruit juices from the estimation of citrus fruit consumption did not materially differ from those of the main analysis. The pooled OR for the highest compared to the lowest citrus fruit intake (excluding juices) was 0.81 (0.74–0.89). Similarly, using the

Table 2. Characteristics of the case–control studies included and distribution of citrus fruit intake, by study

Study	Study period	Controls[1]	Citrus fruit intake, gr/week, median (33th – 66th centile)		Citrus fruit intake (excluding juices), gr/week, median (33th – 66th centile)	
			Cases	Controls	Cases	Controls
Europe						
Greece	1981–1984	Hospital-based	545 (360; 1,110)	1,160 (487; 1,360)	-	-
Italy 1	1985–1997	Hospital-based	225 (75; 300)	300 (150; 525)	-	-
Italy 2	1997–2007	Matched, hospital-based	525 (375; 675)	525 (300; 750)	-	-
Italy 4	1985–1987	Population-based	280 (143; 454)	380 (222; 565)	-	-
Portugal	1999–2006	Matched, population-based	98 (0; 179)	228 (98; 390)	-	-
Russia	1996–1997	Hospital-based	298 (213; 420)	315 (240; 465)	215 (170; 320)	235 (170; 350)
Spain 2	1995–1999	Matched, hospital-based	423 (363; 726)	666 (363; 847)		
Asia						
Iran 1	2004–2005	Matched, population-based	56 (56; 225)	225 (225; 525)	-	-
Iran 2	2005–2007	Population-based	156 (107; 194)	200 (135; 313)	-	-
Japan 3	1998–2002	Matched, hospital-based	344 (244; 550)	379 (273; 523)	-	-
The Americas						
Canada	1994–1997	Matched, population-based	789 (410; 1,050)	789 (450; 1,050)	150 (70; 450)	150 (70; 450)
USA 1	1992–1994	Hospital-based	750 (369; 1,143)	675 (351; 1,200)	188 (58; 421)	150 (41; 375)
Mexico 1	2004–2005	Matched, population-based	286 (171; 469)	257 (164; 343)	158 (116; 285)	89 (72; 148)
Mexico 2	1989–1990	Matched, population-based	450 (171; 512)	471 (171; 512)	-	-
Mexico 3	1994–1996	Matched, hospital-based	563 (284; 1,050)	610 (420; 1,077)	416 (149; 560)	434 (196; 762)

[1]Frequency matched on age and sex for the Italy 2, Portugal, Iran 1, Canada, Mexico 1, and Mexico 2 studies; on age, sex, and area of residence for the Spain 2, Japan 3 and Mexico 3 studies.

Table 3. Distribution of cases and controls according to citrus fruit intake (expressed as study-specific tertiles and servings per week), odds ratios (ORs) and corresponding 95% confidence intervals (CIs) for gastric cancer

	Cases		Controls			
	N	%	N	%	OR (95% CI)[1]	OR (95% CI)[2]
Study-specific tertiles						
[min-T1)	2,654	42.5	4,890	34.0	1[3]	1[3]
[T1-T2)	2,070	33.1	5,436	37.8	0.72 (0.67–0.77)	0.80 (0.74–0.86)
[T3-max]	1,525	24.4	4,046	28.2	0.68 (0.62–0.73)	0.80 (0.73–0.87)
Servings per week						
0	1,964	31.4	3,407	23.7	1[3]	1[3]
1	1,050	16.8	2,156	15.0	0.81 (0.74–0.89)	0.85 (0.77–0.94)
2	756	12.1	1,517	10.6	0.76 (0.69–0.85)	0.85 (0.76–0.95)
3	692	11.1	2,116	14.7	0.61 (0.55–0.68)	0.71 (0.64–0.80)
4	276	4.4	651	4.5	0.62 (0.53–0.72)	0.70 (0.59–0.82)
5	354	5.7	1,024	7.1	0.63 (0.54–0.72)	0.73 (0.63–0.85)
6	227	3.6	674	4.7	0.62 (0.53–0.74)	0.80 (0.67–0.95)
≥7	930	14.9	2,827	19.7	0.67 (0.61–0.74)	0.82 (0.73–0.92)

[1]Estimated by logistic mixed effect model with a random intercept for each study.
[2]Further adjusted for sex, age category, social class, smoking status, salt intake, alcohol intake, other fruit and vegetable intake and family history of gastric cancer.
[3]Reference category.

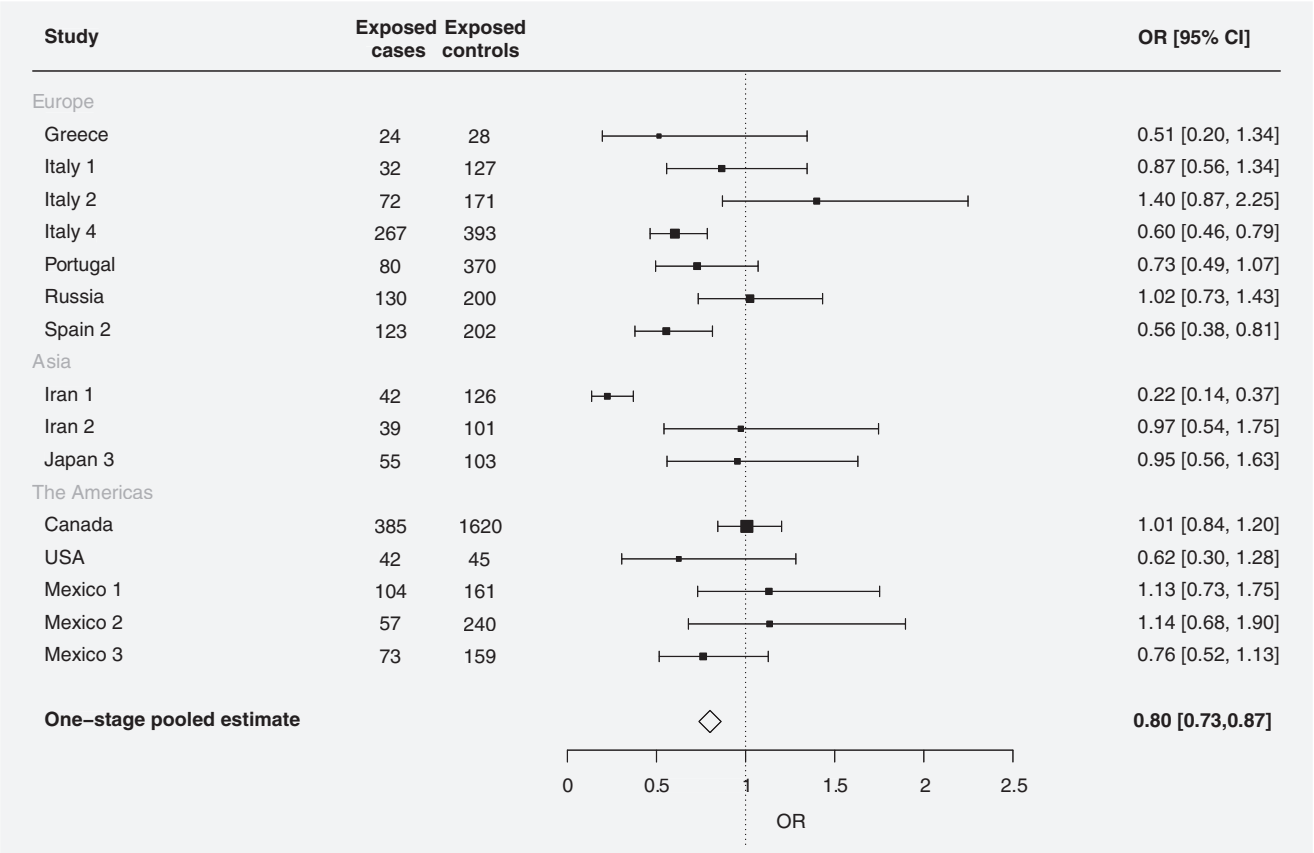

Figure 1. Study-specific and pooled odds ratio of gastric cancer for the highest compared to the lowest study-specific third of the distribution of citrus fruit consumption.

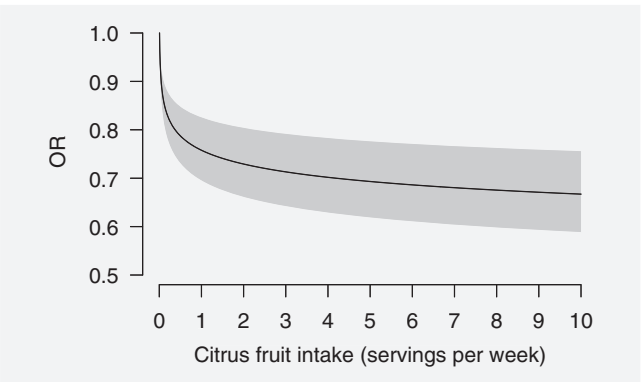

Figure 2. Dose-risk relationship between citrus fruit intake and gastric cancer, obtained by a logistic mixed effects model including the natural logarithm of citrus fruit intake as exposure variable. Citrus fruit consumption was converted in servings per week by considering one serving equal to 150 g of fruit or juice.

two-stage approach the pooled estimate of the OR for the highest compared to the lowest citrus fruit intake was similar to that obtained by the one-stage approach (OR: 0.79, 95% CI: 0.64–0.97).

When applying the Newcastle-Ottawa quality assessment scale to the included studies, four of them was awarded seven stars, nine studies scored six stars, and two studies (Greece and Canada) scored five stars. Removing the latter studies from the analysis did not changed substantively the magnitude of the association (OR for the last compared to the first third of citrus fruit consumption: 0.74, 95% CI: 0.66–0.82). Similar results were obtained when removing the studies (Canada, USA 1 and Russia) that evaluated citrus fruit consumption by self-administered FFQs instead of trained interviewers (OR for the last compared to the first third of citrus fruit consumption: 0.74, 95% CI: 0.67–0.83).

The inverse association was slightly stronger in studies that collected citrus fruit consumption one year before diagnosis as compared to those that collected it between 2 to 5 years before diagnosis (ORs for the last compared to the first third of citrus fruit consumption: 0.74, 95% CI: 0.64–0.85 and 0.82, 95% CI: 0.68–0.86, respectively).

Discussion

In this uniquely large study, we found an inverse association between citrus fruit intake and gastric cancer. The magnitude of the association was similar between cancer sub-sites (cardia and non-cardia) and histotype (intestinal and diffuse), while it was stronger in people of low socio-economic status and in studies from Asia.

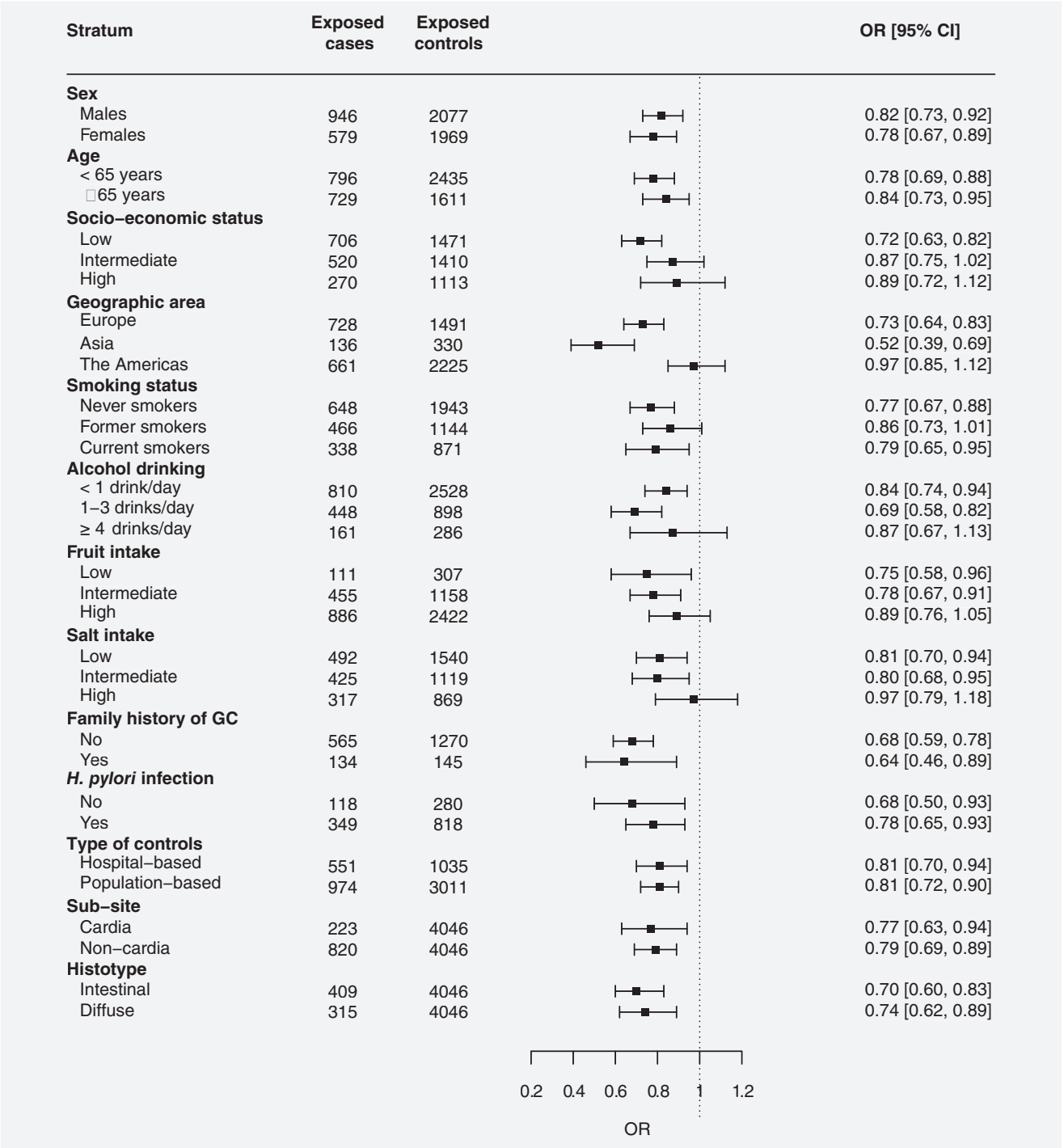

Figure 3. Pooled odds ratios of gastric cancer (GC) for the highest compared to the lowest study-specific third of the distribution of citrus fruit consumption, according to strata of selected variables. Abbreviations: GC, gastric cancer.

A recent meta-analysis[11] including 4,907 cases of gastric cancer from 6 cohort studies (two from the USA, two from Japan, one from China, one from the Netherlands and one from the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort) did not find a significant association between citrus fruit intake and gastric cancer. Three of the included studies reported cardia cancer incidence and two of them (one from the Netherlands and the other one from the EPIC cohort) found a protective effect, while the study from the USA did not show any association. However, a decreased risk of gastric cancer with increasing citrus fruit intake was also reported in hospital- and community-based case–control studies.[10,33]

The mechanisms underneath this potential protective effect were investigated in studies based on gastric cancer cell lines and animal-models. These showed anticancer effects of flavanones, a class of flavonoids contained almost exclusively in citrus fruits and juices.[34–38] Hesperitin and naringenin, two of the major flavanones compounds contained in oranges and mandarins, inhibit human gastric cancer cell proliferation, migration and invasion in a dose- and time-dependent manner.[34,36] Moreover, naringenin showed a combinative effect on human gastric cell lines when administered in combination with ABT-737, an inhibitor of the antiapoptotic protein B-cell lymphoma.[37] These findings were confirmed in a study based on albino rats, in which the administration of naringenin simultaneously with and subsequently to the gastric carcinogen N-methyl-*N′-nitroce*-nitroso-guanidine reduced the tumor mass *via* its antioxidant potential.[35] However, the plasma concentrations used in these studies (from 20 to 400 μmol/L) are far higher than those reached by humans even in cases of very high citrus fruit consumption.[39,40] Dietary intake of flavanones varies according to population and dietary habits, and some studies reported mean intakes ranging between 15 and 40 mg/day.[40–43] In a group of 37 Finnish women,[39] mean plasma concentrations of hesperitin was 0.48 μmol/L during their habitual diet and reached 3.26 μmol/L after 5-week diet containing high amounts of vegetables and fruit, including citrus fruit. Corresponding figures for naringenin were 0.05 μmol/L during habitual diet, and 1.13 μmol/L after the 5-week high vegetables and fruit diet.

In a Greek case–control study, flavanones from citrus fruit were inversely associated with gastric cancer risk.[17] Moreover, citrus fruit are also a good source of vitamin C and high levels of plasma vitamin C were associated with reduced gastric cancer risk in a case–control study nested within the EPIC cohort.[44]

Some between-study heterogeneity emerged and the lack of significant association when pooling the studies from the Americas is attributable to the remarkable high contribution of the study from Canada, which enrolled 70% of all participants from the Americas. In that study, the FFQ was mailed to cancer cases and controls, while in most of the studies included in this pooled analysis the investigators used trained interviewers to collect dietary information. This could result in a less accurate assessment of citrus fruit consumption. Low socio-economic status is a well-recognized risk factor for gastric cancer partly as consequence of an unfavorable distribution of risk factors including *H. pylori* infection, tobacco smoking, alcohol drinking and poor diet.[45] In our study, the stronger inverse association between citrus fruit intake and gastric cancer in people of low socio-economic status suggests that a diet rich in citrus fruits could counteract the negative effects of the lifestyle risk factors related to low social class.

The main limitations of our study lie in the potential inaccurate measure of citrus consumption in a case–control design and the challenging separation of the effect of citrus fruit from that of other dietary factors.

The multicenter nature of our study entailed the evaluation of citrus fruit consumption through different FFQs with different lists of food items. This could have resulted in an underestimation of citrus fruit intake in some studies, which did not collect information on different types of citrus fruits. However, the results were consistent between hospital- and population-based studies, as well as across strata of sex, age, and other major covariates.

The inverse association between citrus fruit consumption and gastric cancer risk can be at least partially attributable to a generally healthier diet associated to high consumption of fruit and vegetables. In fact, high citrus fruit consumers have also a high consumption of other fruits and vegetables that contain dietary components with potential anticarcinogenic effects. However, our results were virtually unmodified after adjustment for other fruit and vegetable intake. With reference to other potential confounders, we considered the dietary factors most strongly correlated to citrus fruit consumption, such as salt and alcohol. We also evaluated the additional inclusion of meat and pickled vegetables (whenever available), but these did not materially modify any of the estimates.

Our study provides more precise and valid evidence than previously available of an inverse relationship between citrus fruit consumption and gastric cancer obtained from a large consortium of case–control studies.

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