Folate intake and the risk of oral cavity and pharyngeal cancer: A pooled analysis within the International Head and Neck Cancer Epidemiology Consortium

Carlotta Galeone[1]*, Valeria Edefonti[2]*, Maria Parpinel[3], Emanuele Leoncini[4], Keitaro Matsuo[5], Renato Talamini[6], Andrew F. Olshan[7], Jose P. Zevallos[8], Deborah M. Winn[9], Vijayvel Jayaprakash[10], Kirsten Moysich[10], Zuo-Feng Zhang[11], Hal Morgenstern[12,13], Fabio Levi[14], Cristina Bosetti[1], Karl Kelsey[15], Michael McClean[16], Stimson Schantz[17], Guo-Pei Yu[18], Paolo Boffetta[19], Yuan-Chin Amy Lee[20], Mia Hashibe[21], Carlo La Vecchia[2]* and Stefania Boccia[4,22]*

[1] Department of Epidemiology, IRCCS—Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy

[2] Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy

[3] Department of Medical and Biological Sciences, Unit of Hygiene and Epidemiology, University of Udine, Udine, Italy

[4] Section of Hygiene, Institute of Public Health, Department of Public Health, Università Cattolica del Sacro Cuore, Rome, Italy

[5] Department of Preventive Medicine, Faculty of Medical Sciences, Kyushu University, Kyushu, Japan

[6] Unit of Epidemiology and Biostatistics, Aviano Cancer Centre, Aviano, Italy

[7] Department of Epidemiology, University of North Carolina School of Public Health, Chapel Hill, NC

[8] Bobby R. Alford Department of Otolaryngology Head and Neck Surgery, Baylor College of Medicine, University of Texas School of Dentistry at Houston, Houston, TX

[9] Division of Cancer Control and Population Sciences, National Cancer Institute, Bethesda, MD

[10] Department of Cancer Prevention and Control, Roswell Park Cancer Institute, Buffalo, NY

[11] Department of Epidemiology, UCLA School of Public Health, Los Angeles, CA

[12] Department of Epidemiology, School of Public Health and Comprehensive Cancer Center, University of Michigan, Ann Arbor, MI

[13] Department of Environmental Health Sciences, School of Public Health and Comprehensive Cancer Center, University of Michigan, Ann Arbor, MI

[14] Cancer Epidemiology Unit, Institute for Social and Preventive Medicine (IUMSP), Lausanne University Hospital, Lausanne, Switzerland

[15] Department of Epidemiology, Brown University, Providence, RI

[16] Department of Environmental Health, Boston University School of Public Health, Boston, MA

[17] Department of Otolaryngology, New York Eye and Ear Infirmary, New York, NY

[18] Medical Informatics Center, Peking University, Beijing, China

[19] The Tisch Cancer Institute and Institute for Translational Epidemiology, Icahan School of Medicine at Mount Sinai, New York, NY

[20] Department of Family and Preventive Medicine, University of Utah School of Medicine, Salt Lake City, UT

[21] Department of Family and Preventive Medicine, Huntsman Cancer Institute, University of Utah School of Medicine, Salt Lake City, UT

[22] Area of Systems Approaches and Non Communicable Diseases, IRCCS San Raffaele Pisana, Rome, Italy

There are suggestions of an inverse association between folate intake and serum folate levels and the risk of oral cavity and pharyngeal cancers (OPCs), but most studies are limited in sample size, with only few reporting information on the source of dietary folate. Our study aims to investigate the association between folate intake and the risk of OPC within the International Head and Neck Cancer Epidemiology (INHANCE) Consortium. We analyzed pooled individual-level data from ten case–control studies participating in the INHANCE consortium, including 5,127 cases and 13,249 controls. Odds ratios (ORs) and the corresponding 95% confidence intervals (CIs) were estimated for the associations between total folate intake (natural, fortification and supplementation) and natural folate only, and OPC risk. We found an inverse association between total folate intake and overall OPC risk (the adjusted OR for the highest *vs.* the lowest quintile was 0.65, 95% CI: 0.43–0.99), with a stronger association for oral cavity (OR = 0.57, 95% CI: 0.43–0.75). A similar inverse association, though somewhat weaker, was observed for folate intake from natural sources only in oral cavity cancer (OR = 0.64, 95% CI: 0.45–0.91). The highest OPC risk was observed in heavy alcohol drinkers with low folate intake as compared to never/light drinkers with high folate (OR = 4.05, 95% CI: 3.43–4.79); the attributable proportion (AP) owing to interaction was 11.1% (95% CI: 1.4–20.8%). Lastly, we reported an OR of 2.73 (95% CI:2.34-3.19) for those ever tobacco users with low folate intake, compared with nevere tobacco users and high folate intake (AP of interaction =10.6%, 95% CI: 0.41-20.8%). Our project of a large pool of case–control studies supports a protective effect of total folate intake on OPC risk.

What's new?

Folate is essential to DNA synthesis and repair, suggesting that folate deficiency, in disrupting normal DNA processes, may facilitate the development of certain cancers, including oral and pharyngeal cancer (OPC). The relationship between folate intake and risk of OPC, however, is unclear. In this analysis of data from the International Head and Neck Cancer Epidemiology (INHANCE) Consortium, high levels of folate intake were found to be inversely associated with overall OPC risk. The association was strongest for cancer of the oral cavity. Risk of OPC was highest among heavy alcohol drinkers with low folate levels.

Oral and pharyngeal cancer (OPC) is the seventh most common cancer worldwide, with more than half a million cases and about 300,000 deaths in 2012.[1] Tobacco smoking and alcohol consumption are predominant risk factors for OPC although other factors, including the aspects of diet, may affect the risk.[2] In particular, a high intake of fruit and vegetables has been linked with a lower risk of OPC, whereas a poor nutritional status and unbalanced diet have been related to an elevated risk.[2–4] The association between dietary habits and OPC was investigated in the International Head and Neck Cancer Epidemiology (INHANCE) Consortium.[5] Dietary habits reflecting high fruit/vegetable and low red meat intake were associated with reduced head and neck cancer risk (*per* unit score increment, odds ratio [OR] = 0.90, 95% confidence interval [CI]: 0.84–0.97).

Folate, also known as vitamin B_9, is a water-soluble vitamin and is found naturally in green leafy vegetables, cereals, legumes and fruits. In humans, folate plays the fundamental role of providing methyl groups for *de novo* deoxynucleotide synthesis and for intracellular methylation reactions.[6] Only a few case–control studies, however, addressed the effect of folate on OPC, with inconsistent results.[7–11] Three out of the five studies reported no relationships with risk,[8,9,11] whereas two others found an inverse association.[7,10] However, all these studies provided data on natural folate intake only. Folate, in fact, can be derived from both plant and animal foods (natural folate), from fortified food products and from supplements (synthetic folate also known as folic acid).

Alcohol intake and tobacco consumption are reported to impair folate levels.[12] Alcohol perturbs the folate metabolism by reducing folate absorption, increasing folate excretion or inhibiting methionine synthase,[13,14] whereas tobacco consumption increases the folate turnover in response to the rapid tissue proliferation or DNA repair in aerodigestive tissues among smokers.[15,16]

As alcohol and tobacco consumption are the major risk factors for OPC, it is worth assessing whether the effect of folate intake on OPC risk is modified by alcohol and tobacco,[10,17,18] and whether there is evidence of interaction between variables.

We considered, therefore, the association between folate intake and the risk of OPC in a pooled analysis of case–control studies participating in the INHANCE Consortium, which covers populations from Europe, North America and Japan.

Material and Methods
Studies and participants

The INHANCE Consortium was established in 2004 and to date includes 35 head and neck cancer case–control studies (several of which are multicenter) for a total of 25,478 cases and 37,111 controls (data, version 1.5).[19,20] Cases included patients with invasive tumors of the oral cavity, oropharynx, hypopharynx, larynx, oral cavity or pharynx not otherwise specified or overlapping as defined previously.[21,22] Details on the case–control studies, harmonizing questionnaire data and

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data pooling methods for the INHANCE consortium have been described previously.[19,21] All the studies were performed according to the Declaration of Helsinki and were approved by the local ethics committees, according to the legislations at study conduction.

In our analyses, we excluded laryngeal cancer cases and corresponding controls.

All case–control studies in the INHANCE Consortium were eligible for inclusion in our analysis if information on folate intake was available from the corresponding food frequency questionnaire (FFQ) for at least 80% of the subjects. Folate and energy intakes were estimated using validated study-specific food composition tables.[23–27] The subjects who lacked information or had inconsistent values on folate intake from FFQ were considered as missing. The cases were divided according to the following anatomic sites: (i) oral cavity (including lip, tongue, gum, floor of mouth and hard palate); (ii) oropharynx (including base of tongue, lingual tonsil, soft palate, uvula, tonsil and oropharynx) and hypopharynx (including pyriform sinus and hypopharynx); (iii) oral cavity, pharynx unspecified or overlapping (not otherwise specified, NOS). The main characteristics of the ten eligible studies are summarized in Table 1, including 5,127 cases of oral cavity/pharyngeal cancer (1,613 of the oral cavity, 2,571 of oropharynx/hypopharynx and 943 of oral cavity/pharynx NOS) and 13,249 controls.[28–37]

The estimate of total folate intake was defined in each study and included at least one of the following sources: natural sources of folate, folate-fortified food products and folate supplementation. The study-specific definition of total folate intake represented the most accurate proxy of the real intake of folate in each population considered. In detail, among the ten studies included, six reported folate estimates exclusively from natural sources.[28–31,35,37] Two other studies reported folate estimates from natural sources, as well as from other combined sources (i.e., natural food sources, folate-fortified food products and folate supplementation)[34,36] and two studies reported folate estimates exclusively from natural sources and combined folate supplementation.[32,33]

Statistical analysis

The main analyses were based on total folate intake, defined as the most complete information on folate intake reported in each of the ten studies. A secondary analysis was based on those studies (eight studies) providing information on the natural sources of dietary folate only.[28–31,34–37] For all the analyses, we calculated the study-specific quintiles for folate intake among controls. The study-specific cutoff values are listed in Table 1.

The association between folate intake and OPC risk was assessed by estimating the ORs and the corresponding 95% CIs, using unconditional logistic regression model for each case–control study, adjusted for age (quinquennia, categorically), gender, education level (no formal education, less than junior high school, some high school, high-school graduate, vocational/some college and college graduate/postgraduate), race/ethnicity (non-Hispanic White, Black, Hispanic/Latino, Asian and other), cigarette smoking (never, 1–10, 11–20, 21–30, 31–40, 41–50, >50 pack-years), alcohol drinking (nondrinkers, 0 to <1, ≥1 to <3, ≥3 to <5, ≥5 drinks/day) and total energy intake (continuous).

The pooled effect estimates from all studies were estimated with fixed-effects and random-effects logistic regression models.[38] We tested for heterogeneity between the study-specific ORs by conducting a likelihood ratio test comparing a model that included the product terms between each study (other than the reference study) and the variable of interest and a model without product terms, for the risk of oral cavity and pharyngeal cancers combined and for that of each anatomical subsite. We used the random-effects[38] estimates when heterogeneity was detected ($p < 0.10$), and the fixed-effects estimates otherwise. We quantified inconsistencies across studies and their impact on the analysis by using Cochrane's Q and the I^2 statistic.[39,40]

We also conducted a sensitivity analysis in which each study was excluded one at a time to ensure that the magnitude of the overall estimates was not dependent on any specific study. Subgroup analyses were also conducted by stratifying the results for total folate intake according to age, gender, geographic region, education level, study design, cancer subsite, body mass index, tobacco status and alcohol drinking status.

Effect measure modification was evaluated by testing for deviation from a multiplicative interaction model, using the log-likelihood ratio test to compare the fit of logistic models with and without an interaction term. Biological interaction between alcohol, tobacco smoking and total folate intake was estimated using departure from additivity of effects as the criterion of interaction as proposed by Rothman.[41] To quantify the amount of interaction, the attributable proportion (AP) owing to interaction was calculated as described by Andersson et al.[42] The AP owing to interaction is the proportion of individuals among those exposed to the two interacting factors that is attributable to the interaction per se and it is equal to 0 in the absence of a biological interaction.

Data analyses were conducted using SAS version 9.2 (SAS Institute, Cary, NC) statistical software.

Results

Among the ten studies included, three were conducted in Europe (26% of total cases and 33% of controls), six in North America (65% of total cases and 44% of controls) and one in Japan (9% of total cases and 23% of controls). Three studies were based on cancer registries, whereas the remaining ones were hospital-based case–control studies (Table 1). Table 2 summarizes the characteristics of the study population, which included a total of 13,133 men and 5,233 women (26.7% of cases and 29.2% of all controls were women). In total, more than 78% of cases and 68% of controls were non-Hispanic

Table 1. Characteristics of the ten individual studies on OPC [1] from the INHANCE Consortium pooled analysis and including information on folate intake

Study ID	Study location	Recruitment period	Source (cases/controls)	Participation rate of cases and controls (%)	Sources of folate			Quintile cutoffs of total folate intake[3]	OPC cases	Controls
					Natural food only	Supplements only	All sources together[2]			
Europe										
Italy Multicenter[28]	Aviano, Milan and Latina	1990–2005	Hospital/hospital	>95, >95	Yes	No	No	212.6; 254.6; 291.6; 344.5	801	2,716
Switzerland[29]	Lausanne	1991–1997	Hospital/hospital	>95, >95	Yes	No	No	192.1; 238.2; 282.2; 347.2	392	883
Italy[30]	Milan	2006–2009	Hospital/hospital	>95, >95	Yes	No	No	198.3; 236.4; 273.8; 322.7	142	755
North America										
USA (Buffalo)[31]	Buffalo	1982–1998	Hospital/hospital	>50, >50	Yes	No	No	267.0; 341.8; 425.5; 539.9	441	1,256
USA Multicenter[32]	US Multicenter	1983–1984	Cancer Registry/Random digit dialing and health care rosters	75, 76	No	No	Yes	193.8; 254.3; 311.7; 391.6	1,114	1,268
USA (MSKCC)[33]	MSKCC, New York	1992–1994	Hospital/blood donors	>95, >95	No	No	Yes	167.2; 225.2; 284.4; 374.7	103	176
USA (Boston)[34]	Boston	1999–2003	Hospital/neighborhood	88.7, 48.7	Yes	No	Yes	344.1; 456.5; 641.7; 815.8	473	659
USA (Los Angeles)[35]	Los Angeles	1999–2004	Cancer Registry/Neighborhood	49,68	Yes	No	No	125.8; 163.2; 207.3; 258.1	338	1,040
USA (North Carolina)[36]	North Carolina	2002–2006	Cancer registry/DMV files	88, 61	Yes	Yes	Yes	245.4; 324.9; 410.8; 530.1	887	1,396
Asia										
Japan[37]	Japan	2001–2005	Hospital/hospital	97, 97	Yes	No	No	232.4; 284.6; 335.3; 403.4	436	3,102
Total subjects									5,127	13,249

[1]Oral and Pharyngeal cancer.
[2]Two studies reported folate estimates exclusively from natural sources and combined folate supplementation,[32,33] and two studies from natural sources, folate-fortified food products and combined folate supplementation .[34,36]
[3]Calculation of cutoffs for quintile of the most complete information on folate intake reported in each study was based on the distribution of controls.

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Table 2. Distribution of OPC cases and controls according to the selected variables[1] in the ten studies included in the INHANCE Consortium

	OPC cases		Controls	
	n	%	*n*	%
Age (years)				
<40	237	4.6	739	5.6
40–44	228	4.5	625	4.7
45–49	526	10.3	1,043	7.9
50–54	785	15.3	1,879	14.2
55–59	953	18.6	2,261	17.1
60–64	814	15.9	2,148	16.2
65–69	734	14.3	2,087	15.7
70–74	542	10.5	1,644	12.4
≥75	308	6.0	821	6.2
p (χ^2 test)		<0.0001		
Sex				
Men	3,753	73.3	9,380	70.8
Women	1,369	26.7	3,864	29.2
p (χ^2 test)		0.001		
Race/ethnicity				
Non-Hispanic white	4,006	78.3	9,064	68.6
Black	484	9.5	627	4.8
Hispanic/Latino	122	2.4	308	2.3
Asian	466	9.1	3,166	24.0
Other	37	0.7	48	0.3
p (χ^2 test)	<0.0001			
Education				
No formal	235	4.6	716	5.4
Less than junior high school	1,117	21.8	4,088	30.9
Some high school	1,064	20.8	2,003	15.1
High-school graduate	764	14.9	1,638	12.4
Vocational school, some college	1,317	25.7	2,749	20.8
College graduate/ postgraduate	627	12.2	2,046	15.4
p (χ^2 test)		<0.0001		
Cigarette smoking (pack-years)				
Never smokers	919	18.2	5,239	40.2
1–10	356	7.1	1,788	13.7
11–20	406	8.0	1,422	10.9
21–30	583	11.6	1,248	9.6
31–40	633	12.6	1,136	8.6
41–50	594	11.8	778	6.0
>50	1,546	30.7	1,436	11.0
p (χ^2 test)		<0.0001		
Alcohol intake (drinks/die)				
Nondrinkers	646	13.0	3,303	25.6

Table 2. Distribution of OPC cases and controls according to the selected variables in the ten studies included in the INHANCE Consortium (Continued)

	OPC cases		Controls	
	n	%	*n*	%
>0 to <1	1,143	22.9	4,300	33.4
≥1 to <3	1,051	21.1	3,035	23.5
≥3 to <5	710	14.3	1,255	9.7
≥5	1,425	28.7	1,001	7.8
p (χ^2 test)		<0.0001		
Body mass index (kg/m^2)				
<25	2,942	59.4	6,436	48.9
≥25	2,014	40.6	6,721	51.1
p (χ^2 test)		<0.0001		
Total energy intake (kcal/die)				
Mean ± SD	1,584 ± 1,232		1,283 ± 939	
p (*t*-test)		<0.0001		

[1]The sum does not add up to the total because of some missing values.

white. Cases were more likely cigarette smokers and alcohol drinkers than controls (Table 2).

The associations between total folate and folate from natural sources only and OPC risk are summarized in Table 3. Considering the ten studies included in the total folate intake analysis, the overall ORs of OPC were 0.78 (95% CI: 0.67–0.91) for the second quintile, 0.77 (95% CI 0.61–0.96) for the third quintile, 0.72 (95% CI: 0.51–1.01) for the fourth quintile and 0.65 (95% CI: 0.43–0.99) for the fifth quintile compared to the first quintile, with a significant *p*-value for trend and heterogeneity between the studies. When the results were stratified by anatomic subsite, the ORs for the highest *versus* the lowest quintile of total folate intake were 0.57 (95% CI: 0.43–0.75) and 0.58 (95% CI: 0.42–0.81) for oral cavity and NOS, respectively, with no evidence of heterogeneity across studies. The OR for the highest *versus* the lowest quintile of total folate intake was 0.74 (95% CI: 0.42–1.30) for oropharynx/hypopharynx combined, with heterogeneity across studies (*p* = 0.06).

Considering the eight studies included in the folate intake from natural sources only, the overall ORs of OPC were 0.75 (95% CI: 0.57–1.00) for the second quintile, 0.74 (95% CI: 0.50–1.10) for the third quintile, 0.70 (95% CI: 0.46–1.06) for the fourth quintile and 0.72 (95% CI: 0.46–1.14) for the fifth quintile compared to the first quintile, with heterogeneity across studies (*p* < 0.01). When the results were stratified by anatomic subsite, the ORs for the highest *versus* the lowest quintile of natural folate intake were 0.64 (95% CI: 0.45–0.91), 0.79 (95% CI: 0.44–1.43) and 0.69 (95% CI: 0.36–1.32) for oral cavity, oropharynx/hypopharynx combined and NOS, respectively, with evidence of heterogeneity across the studies for the latter two subsites.

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Table 3. Associations between folate intake and risk of OPC, overall and stratified by anatomic site. INHANCE Consortium

	Controls (n)	OPC		Oral cavity		Oropharynx/hypopharynx		NOS[1]	
		Cases (n)	OR[2] (95% CI)	Cases	OR[2] (95% CI)	Cases	OR[2] (95% CI)	Cases	OR[2] (95% CI)
Total folate intake (ten studies included[3])									
I Quintile[4]	2,425	1,009	1[(Ref)]	342	1[(Ref)]	491	1[(Ref)]	176	1
II Quintile	2,420	796	0.78 (0.67–0.91)	260	0.74 (0.60–0.92)	383	0.77 (0.62–0.96)	153	0.89 (0.69–1.15)
III Quintile	2,429	859	0.77 (0.61–0.96)	255	0.65 (0.52–0.81)	441	0.84 (0.60–1.17)	163	0.84 (0.64–1.10)
IV Quintile	2,435	860	0.72 (0.51–1.01)	266	0.64 (0.50–0.82)	422	0.73 (0.47–1.16)	172	0.87 (0.66–1.15)
V Quintile	2,431	951	0.65 (0.43–0.99)	286	0.57 (0.43–0.75)	516	0.74 (0.42–1.30)	149	0.58 (0.42–0.81)
Missing	1,109	652		204		318		130	
Total	13,249	5,127		1,613		2,571		943	
p-Value for trend			0.04		<0.01		0.28		<0.01
p-Value for heterogeneity between studies			0.04		0.74		0.06		0.24
Folate intake from natural sources only (eight studies included[5])									
I Quintile[3]	2,156	781	1[(Ref)]	241	1[(Ref)]	410	1[(Ref)]	130	1[(Ref)]
II Quintile	2,142	606	0.75 (0.57–1.00)	189	0.73 (0.57–0.94)	298	0.75 (0.55–1.03)	119	0.86 (0.55–1.36)
III Quintile	2,155	626	0.74 (0.50–1.10)	184	0.72 (0.55–0.95)	314	0.74 (0.47–1.17)	128	0.93 (0.48–1.80)
IV Quintile	2,162	621	0.70 (0.46–1.06)	174	0.63 (0.47–0.85)	331	0.71 (0.42–1.20)	116	0.83 (0.45–1.52)
V Quintile	2,160	696	0.72 (0.46–1.14)	195	0.64 (0.45–0.91)	389	0.79 (0.44–1.43)	112	0.69 (0.36–1.32)
Missing	1,030	580		169		293		118	
Total	11,805	3,910		1,152		2,035		723	
p-Value for trend			0.08		<0.01		0.19		0.31
p-Value for heterogeneity between studies			<0.01		0.72		0.02		0.02

[1]NOS, not otherwise specified.
[2]Random-effects estimates were used when heterogeneity was detected ($p < 0.10$) and fixed-effects otherwise. Adjusted for age, gender, race/ethnicity, education, study, cigarette smoking (pack-years), alcohol intake and total energy intake.
[3]Studies included Refs. 28–37.
[4]Calculation of cutoffs for quintile was based on the distribution of controls in each study (study specific).
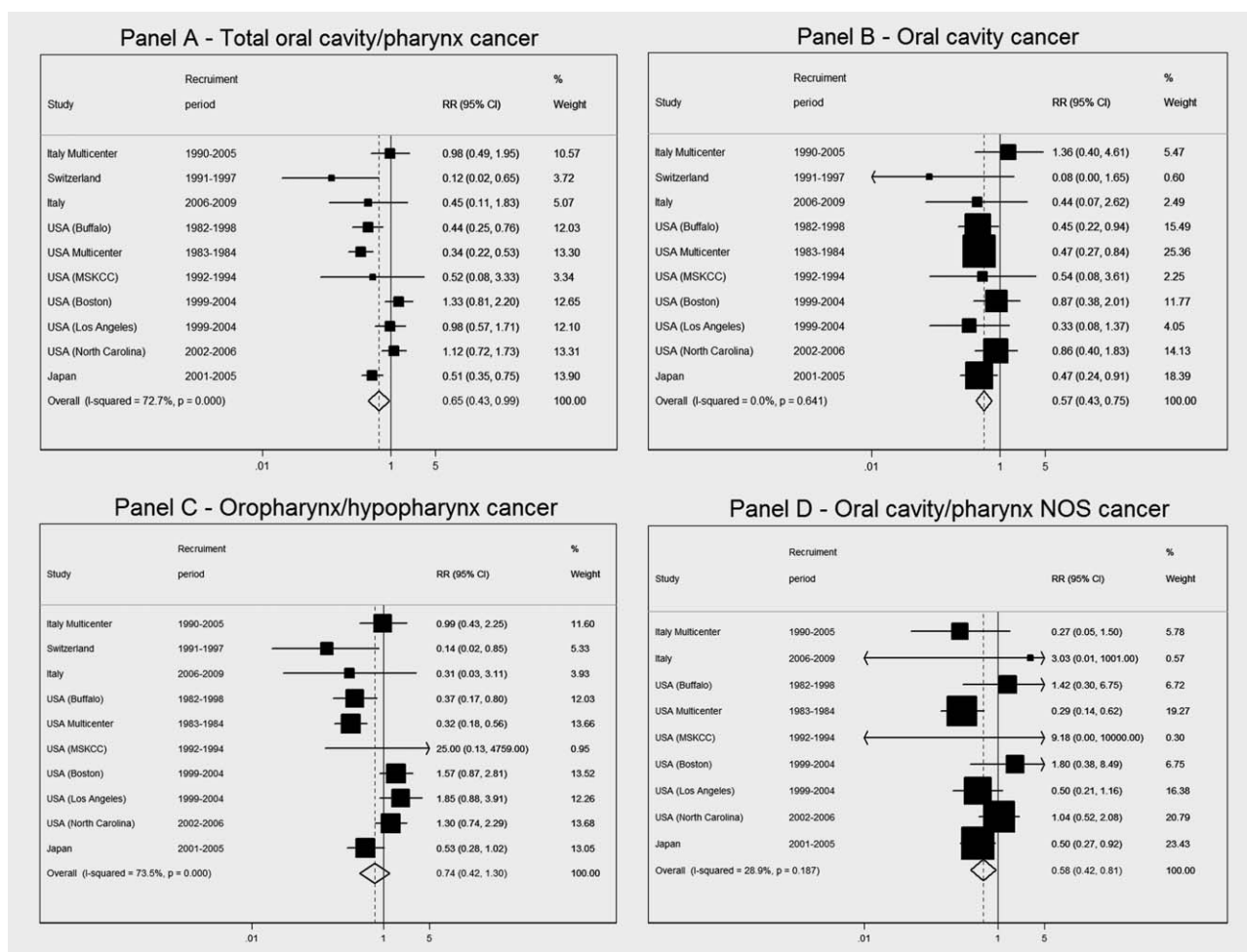[5]Studies included Refs. 28–31,34–37.

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Figure 1. Study-specific and pooled estimates of OPC (*a*), oral cavity (*b*), oropharynx/hypopharynx (*c*) and NOS (*d*) cancer for the highest *versus* the lowest quintile of total folate intake. INHANCE Consortium.

The forest plots show the pooled and study-specific OR estimates for the associations between the highest and the lowest quintile of total folate intake, considering all cancer sites combined and separately (Fig. 1). Out of the ten studies, the ORs of OPC were below unity in eight studies (significant in four) and above unity in two studies (nonsignificant).

Table 4 lists the ORs of OPC for the highest *versus* the lowest quintile of total folate intake according to the selected covariates. There was little evidence of notable effect modification, except for a stronger inverse association in the hospital-based studies (OR = 0.52; 95% CI: 0.40–0.69) compared to the population-based ones (OR = 0.80; 95% CI: 0.63–1.01) (for heterogeneity, $p = 0.02$).

The analysis of interaction between total folate intake and alcohol reported an OR of 4.05 (95% CI: 3.43–4.79) for heavy drinkers with a low intake of folate, compared to subjects with low alcohol and intermediate/high total folate intake (for interaction, $p = 0.75$). Using the estimated ORs listed in Table 5, the AP owing to interaction is $(4.05 -$

$1.32 - 3.28 + 1)/4.05 = 11.1\%$ (95% CI: 1.4–20.8%). Thus, we estimate that 11.1% of OPC cases occurring among heavy drinkers with low folate intake was attributable to biological interaction (synergy). As for the interaction between tobacco smoking and folates, we reported an OR of 2.73 (95% CI: 2.34–3.19) for those ever tobacco users with a low folate intake, compared to subjects with never tobacco users and intermediate/high total folate intake (for interaction, $p = 0.90$). The AP owing to interaction is $(2.73 - 1.33 - 2.11 + 1)/2.73 = 10.6\%$ (95% CI: 0.4–20.8%), suggesting that around 11% of OPC cases occurring among those ever smokers and with low folate levels occurred because of the interaction among the risk factors.

Discussion

This pooled analysis of ten case–control studies including 5,127 OPC cases provided evidence of an inverse association between folate intake and OPC risk. The estimated association was stronger for oral cavity cancer, with more than 40% risk reduction for the highest quintile of folate intake, than

Table 4. Distribution of cases of OPC and controls, and corresponding OR[1] and 95% CI, for the highest quintile of total folate intake *versus* the lowest one in strata of selected covariates. INHANCE Consortium

	OPC			*p*-Value for heterogeneity between studies
	Cases[2] *n:n*	Controls[2] *n:n*	OR (95% CI)	
Age (years)				
<55	350:348	810:751	0.69 (0.40–1.20)	<0.01
≥55	659:603	1,615:1,680	0.70 (0.44–1.12)	0.03
p-Value for heterogeneity between strata	0.97			
Gender				
Men	674:769	1,637:1,820	0.60 (0.37–0.97)	0.03
Women	335:182	788:611	0.80 (0.55–1.16)	0.23
p-Value for heterogeneity between strata	0.36			
Geographic region[3]				
Europe	319:233	828:811	0.67 (0.37–1.19)	0.98
North America	577:667	1,010:1,020	0.73 (0.58–0.90)	0.22
Asia	113:51	587:600	0.51 (0.35–0.75)	–
p-Value for heterogeneity between strata	0.29			
Education				
<high school graduate	325:235	898:908	0.57 (0.40–0.80)	0.24
≥high school graduate	684:716	1,527:1,523	0.71 (0.57–0.87)	0.21
p-Value for heterogeneity between strata	0.28			
Study design				
Hospital based	551:387	1,663:1,664	0.52 (0.40–0.69)	0.66
Population based	457:564	761:767	0.80 (0.63–1.01)	0.46
p-Value for heterogeneity between strata	0.02			
Body mass index (kg/m^2)[4]				
<25	638:542	1,222:1,156	0.61 (0.48–0.79)	0.59
≥25	339:394	1,186:1,262	0.61 (0.33–1.13)	0.03
p-Value for heterogeneity between strata	1.00			
Tobacco consumption[4,5]				
Never tobacco users	141:134	834:874	1.05 (0.48–2.28)	<0.01
Light tobacco users	129:140	527:592	0.74 (0.48–1.14)	0.94
Heavy tobacco users	696:644	914:813	0.55 (0.43–0.71)	0.47
p-Value for heterogeneity between strata	0.19			
Alcohol consumption[6]				
Never drinkers	140:88	670:570	0.51 (0.32–0.82)	0.24
Light drinkers	438:359	1,266:1,300	0.71 (0.35–1.44)	0.08
Heavy drinkers	431:504	489:561	0.59 (0.39–0.90)	<0.01
p-Value for heterogeneity between strata	0.74			

[1]Random-effects estimates were used when heterogeneity was detected, and fixed-effects otherwise. Adjusted for age, sex, race/ethnicity, education, study, cigarette smoking (pack-years), alcohol intake and total energy intake (as appropriate). The reference category was the lowest quintile of folate intake in each stratum. Calculation of cutoffs for quintile was based on the distribution of controls in each study (study specific).
[2]Number of subjects in the lowest quintile (I quintile): Number of subjects in the highest quintile (V quintile).
[3]Europe included two studies from Italy[28,30] and from from Switzerland.[29] North America included six studies.[31–36] Asia included one study from Japan.[37]
[4]The sum does not add up to the total because of some missing values.
[5]Light tobacco users were smokers of ≤20 tobacco-years (combination of pack-years of cigarettes and pack-years of cigars/pipe in cigarette equivalent), or subjects only snuffing tobacco. Heavy tobacco users were smokers of >20 tobacco-years or subjects ever chewing tobacco. Light drinkers were defined as subjects who drank <3 drinks of alcoholic beverages *per* day and heavy drinkers ≥3 drinks *per* day.

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Table 5. ORs[1] and 95% CIs of OPC according to total folate intake and alcohol and tobacco consumption. INHANCE Consortium

	Total folate intake[2]	
	Intermediate to high	Low
Alcohol consumption[3]		
Never and light drinkers	1 (Ref)	1.32 (1.17–1.48)
Cases:controls	*1,545:6,538*	*902:3,286*
Heavy drinkers	3.28 (2.89–3.73)	4.05 (3.43–4.79)
Cases:controls	*1,429:1,735*	*680:800*
Tobacco consumption		
Never tobacco users	1(Ref)	1.33 (1.09–1.61)
Cases:controls	*429:3,059*	*241:1,414*
Ever tobacco users	2.11 (1.84–2.42)	2.73 (2.34–3.19)
Cases:controls	*2,471:4,799*	*1,299:2,435*

[1]Adjusted for age, sex, race/ethnicity, education, study, cigarette smoking (pack-years) and total energy intake.
[2]Based on the tertiles of intake. Calculation of cutoffs for tertile of total folate intake was based on the distribution of controls in each study (study specific).
[3]Light drinkers were defined as subjects who drank <3 drinks of alcoholic beverages *per* day and heavy drinkers ≥3 drinks *per* day.

for oropharynx/hypopharynx. When pooling the eight studies (3,910 OPC cases and 11,805 controls) detailing the intake of natural folate from diet only, however, the inverse association with OPC was no longer significant.

Only a few case–control studies with limited sample sizes were considered on the association between (natural) folate intake estimated from FFQ and OPC risk.[7–11] Little or no association was found in three epidemiological studies on this issue conducted in the USA (OR = 0.7 for the highest *vs.* lowest level of intake, in both men and women),[9] Central America (OR = 1.1, 95% CI: 0.6–2.2)[11] and Uruguay (OR = 1.3, 95% CI: 0.8–2.2).[8] Two subsequent case–control studies, one conducted in Italy and Switzerland from 1992 to 1997[10] and one in Uruguay from 1996 to 2004,[7] found an inverse association between folate intake and OPC risk, with ORs, respectively, of 0.53 (95% CI: 0.40–0.69) and 0.49 (95% CI, 0.24–0.98) for the highest *versus* lowest level of intake. Another Italian study reported lower serum folate levels in patients with head and neck squamous cell carcinoma (mean value, 4.9 ng/mL) compared to control groups of nonsmokers (mean = 9.7 ng/mL and $p < 0.05$) and smokers (mean = 9.1 ng/mL and $p < 0.05$).[43]

The results of our study suggest that total folate intake, including fortified food and supplements, is inversely related to OPC risk. Apart from UCLA study, the study-specific definition of total folate intake represented the most accurate proxy of the real intake of folate in each population considered. In fact, these estimates take into account if supplements and/or folate-fortified food products were commonly used in each population during the enrollment study period. The

UCLA Study[35] reported the estimates of natural folate only, but it was conducted in a time and in a place where folate fortification in staple foods was mandated (after January 1998) and dietary supplement use was popular. For this reason, we performed a sensitivity analysis by excluding that study. The pooled OR for the highest *versus* the lowest intake of total folate was 0.62 (95% CI: 0.39–0.98) and was similar to the pooled OR when considering all the ten studies (pooled OR = 0.65; 95% CI: 0.43–0.99).

It was not possible, however, to determine how much of this association was due to natural or synthetic folate, as information on the intake of the two aforementioned sources was detailed only in two studies, with no chance, therefore, of performing any meaningful sensitivity analysis. Interestingly, these studies are the only two that reported an OR of >1 for the highest *versus* the lowest quintile of total folate intake. As information on natural folate intake only was available, we calculated the pooled OR for the highest *versus* the lowest quintile of this folate source. This was 1.25 (95% CI: 0.86–1.83), and thus not substantially different from the corresponding pooled OR for total folate intake in these two studies, that is 1.21 (95% CI: 0.87–1.68). Even if it is possible that folic acid may exert a different effect than folate in its natural form[44] and it is known that the bioavailability of folic acid from supplements is higher than the dietary one,[45] the few available data did not show important differences in risks between the two sources of folate.

Owing to potential between-countries variations in folate intake, we decided *a priori* to calculate study-specific quintiles of folate intake. However, we also considered the relationship between OPC and folate intake using absolute cutoffs, based on the distribution of all controls combined. Using this approach, the ORs for subsequent quintiles, as compared to the lowest one, were 0.69, 0.69, 0.65 and 0.63 for all OPC, and the trend in risk was significant. The results were consistent for oral cavity and oropharynx.

Mechanistic evidence provides support for an inverse association between folate intake and cancer risk. Folate deficiency may increase the risk of various type of cancers, particularly of the gastrointestinal tract,[46] through impaired DNA synthesis and disruption of DNA methylation that may lead to protoconcogene activation.[47] The folate pathway is led by the 5,10-methylenetetrahydrofolate reductase gene (MTHFR), which converts the 5,10 methylenetetrahydrofolate to 5-methyltetrahydrofolate, the primary circulating form of folate and a cosubstrate for homocysteine methylation to methionine.[48] A less active form of MTHFR is present among the subject carriers of the homozygous C677T variant, which is present in 30% of Caucasians.[49] The subjects with impaired enzyme activity have reduced folate concentrations, higher serum homocysteine levels, and higher DNA hypomethylation compared to those carrying the wild-type allele.[50] In line with the principle of Mendelian randomization, it is expected that subjects with reduced MTHFR activity are at higher risk of OPC in view of the reduced serum folate levels.

The distribution of alleles in a population is expected to be unrelated to the confounders that may distort observational epidemiologic studies because of the random assignment of alleles at the time of gamete formation.[51] As such, if a functional genetic variant such as C677T of the *MTHFR* is strongly associated with a modifiable exposure (folic acid intake), it can be used to retrieve an unbiased estimate of the association of such exposure (*e.g.*, dietary folate) with a disease (*e.g.*, OPC). Two meta-analyses on the association between *MTHFR* and OPC have been published so far, with the results showing the absence of an increased risk of cancer among those carrying the unfavorable gene variants which is associated with low serum folate levels.[52,53] Taken together, the results of our study and those from the functional genetic variant association studies suggest that although folate intake is, in principle, beneficial toward the risk of OPC, this effect might be differential according to the exact source of folate.

In our study, we reported an additional excess risk of OPC among those with low folate intake who are also heavy drinkers, which is in line with previous findings.[10,17,18] It has been reported that alcohol perturbs folate metabolism by reducing folate absorption, increasing folate excretion or inhibiting methionine synthase,[14] and hence an additional risk of OPC might be present among heavy drinkers with low folate intake. Additionally, our results suggest the presence of biological interaction between cigarette tobacco smoke and folates, which is in line with previous studies and the biological significance of tobacco in inducing cellular proliferation in aerodigestive tissues as a result of the tissue damage.[16] Assuming that the relationships studied are causal and based on the definition of biological interaction between two component causes,[41,54] our results suggest that more than 11% of OPC cases among heavy alcohol drinkers with a low folate intake, and around 11% of OPC among those ever smokers with low folate intake have arisen because of the synergistic interaction among the two component causes. Taken together, these results have important implications from a public health point of view as they show that by increasing folate intake at the population level, even in the presence of harmful lifestyle behaviors (alcohol and tobacco), a relevant proportion of OPC cancer might be prevented.

Although our study has its strengths, including its very large size, its capacity to explore effect modification by several characteristics and the stratified analyses according to cancer subsites, it is not without limitations. First, we were unable to dissect the effect of folate on OPC risk according to the intake of supplements or fortified foods. Second, the investigation might be affected by limitations of case–control studies, including recall bias that generally leads to stronger associations between factors and OPC cancer than in cohort studies. On the other hand, changes in dietary habits after interview could dilute the risks in cohort investigations. Furthermore, we were able to adjust for energy intake in all the studies, and thus reducing the effect of possible systematic under- or over-reporting. Selection bias in case–control studies, especially hospital-based studies, is also a methodological limitation. Therefore, the weaker association observed in population-based studies may be more valid. Nevertheless, hospital-based case–control studies have the advantage over population-based investigations of a higher comparability of information of cases and controls.[55]

With reference to confounding, we were able to adjust for major recognized risk factors for OPC as well as for total energy intake, but no information was available in the INHANCE data, version 1.5, on HPV, which is a relevant risk factor for oropharyngeal cancer. If anything, however, the inverse association with folate was stronger for other OPC sites.

Conclusions

In conclusion, findings from this large pooled analysis suggest that high levels of folate intake may protect against the risk of OPC, after controlling for potential confounding factors, though we cannot rule out selection bias in the hospital-based case–control studies.

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