Title

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

Journal

International Journal of Cancer, 136(4)

ISSN

0020-7136

Authors

Galeone, Carlotta
Edefonti, Valeria
Parpinel, Maria
et al.

Publication Date

2015-02-15

DOI

10.1002/ijc.29044

Peer reviewed

Published in final edited form as:
Int J Cancer. 2015 February 15; 136(4): 904–914. doi:10.1002/ijc.29044.

Folate intake and the risk of oral cavity and pharyngeal cancer: a pooled analysis within the INHANCE 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], **Fabio Levi**[13], **Cristina Bosetti**[1], **Karl Kelsey**[14], **Michael McClean**[15], **Stimson Schantz**[16], **Guo-Pei Yu**[17], **Paolo Boffetta**[18], **Yuan-Chin Amy Lee**[19], **Mia Hashibe**[20], **Carlo La Vecchia**[2,*], and **Stefania Boccia**[4,21,*]

[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]Unit of Hygiene and Epidemiology, Department of Medical and Biological Sciences, University of Udine, Udine, Italy [4]Section of Hygiene, Institute of Public Health, Università Cattolica del Sacro Cuore, Rome, Italy [5]Kyushu University Faculty of Medical Sciences, Kyushu, Japan [6]Aviano Cancer Centre, Aviano, Italy [7]University of North Carolina School of Public Health, Chapel Hill, NC, USA [8]Baylor College of Medicine; University of Texas School of Dentistry at Houston, Houston, TX, USA [9]National Cancer Institute, Bethesda, MD, USA [10]Roswell Park Cancer Institute, Buffalo, NY, USA [11]UCLA School of Public Health, Los Angeles, CA, USA [12]Departments of Epidemiology and Environmental Health Sciences, School of Public Health and Comprehensive Cancer Center, University of Michigan, Ann Arbor, MI, USA [13]Cancer Epidemiology Unit, Institute for Social and Preventive Medicine (IUMSP), Lausanne University Hospital, Lausanne, Switzerland [14]Brown University, Providence, Rhode Island, USA [15]Boston University School of Public Health, Boston, MA [16]New York Eye and Ear Infirmary, New York, NY, USA [17]Medical Informatics Center, Peking University [18]The Tisch Cancer Institute and Institute for Translational Epidemiology, Icahan School of Medicine at Mount Sinai, New York, NY, USA [19]Department of Family & Preventive Medicine, University of Utah School of Medicine, Salt Lake City, UT, USA [20]Department of Family & Preventive Medicine and Huntsman Cancer Institute, University of Utah School of Medicine, Salt Lake City, UT, USA [21]IRCCS San Raffaele Pisana, Rome, Italy

Abstract

There are suggestions of an inverse association between folate intake and serum folate levels and the risk of oral cavity and pharyngeal cancers (OPC), but most studies are limited in sample size, with only few reporting information on the source of dietary folate. This study aims to investigate

Corresponding author: Stefania Boccia, MSc, DSc, PhD, Genetic Epidemiology and Public Health Genomics Unit, Section of Hygiene, Institute of Public Health, Università Cattolica del Sacro Cuore, L.go F. Vito, 1 - 00168 - Rome, Italy, sboccia@rm.unicatt.it, Fax: +39 (0) 6 35001522 –Ph: +39 (0) 6 30154396/35001527.
*Equal contribution

Conflict of interest statement
The authors declare no conflict of interest.

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 10 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 versus 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 (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 due to interaction was 11.1%(95% CI: 1.4–20.8%).

The present project of a large pool of case-control studies supports a protective effect total folate intake on OPC risk.

Medical Subject Headings (MeSH)

Head and Neck Neoplasms; Meta-Analysis [Publication Type]; Folate

INTRODUCTION

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 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 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, OR = 0.90, 95% 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 *denovo* 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 five studies reported no relation with risk, [8, 9, 11] while two others found an inverse association.[7, 10] However, all these studies provided data on natural folate intake only. Folate, in fact, can derive either from plant and animal foods (natural folate), from fortified food products, and 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] while 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 data pooling methods for the INHANCE consortium have been previously described. [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 the present analyses, we excluded laryngeal cancer cases and corresponding controls.

All case-control studies in the INHANCE Consortium were eligible for inclusion in the current 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] Subjects who lacked information or had inconsistent values on folate intake from FFQ were considered as missing. Cases were divided according to the following anatomic sites: 1) oral cavity (including lip, tongue, gum, floor of mouth and hard palate); 2) oropharynx (including base of tongue, lingual tonsil, soft palate, uvula, tonsil and oropharynx) and hypopharynx (including pyriform sinus and hypopharynx); 3) oral cavity, pharynx unspecified or overlapping (not otherwise specified, NOS). The main characteristics of the 10 eligible studies are reported 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 10 studies included, 6 reported folate estimates exclusively from natural sources. [29, 30, 32, 33, 36, 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) [31, 34] and 2 studies reported folate estimates exclusively from natural sources and folate supplementation combined.[28, 35]

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 10 studies. A secondary analysis was based on those studies (8 studies) providing information on the natural sources of dietary folate only.[29–34, 36, 37] For all the analyses, we calculated the study-specific quintiles for folate intake among controls. The study-specific cut-off values are reported in Table 1.

The association between folate intake and OPC risk was assessed by estimating the odds ratios (ORs) and the corresponding 95% confidence intervals (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, 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 (non-drinkers, >0–<1, >=1–<3, >=3–<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 time to ensure that the magnitude of the overall estimates were 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) due to interaction was calculated as described

by Andersson et al.[42] The AP due 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, USA) statistical software.

RESULTS

Among the 10 studies included, 3 were conducted in Europe (26% of total cases and 33% of controls), 6 in North America (65% of total cases and 44% of controls) and 1 in Japan (9% of total cases and 23% of controls). Three studies were based on cancer registry, while the remaining ones were hospital-based case-control studies (Table 1). Table 2 reports 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). Over 78% of cases and 68% of controls were non-Hispanic 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 reported in Table 3. Considering the 10 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 studies. When 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 8 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 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 studies for the latter two subsites.

The forest plots depict the pooled and study-specific OR estimates for the associations between the highest versus the lowest quintile of total folate intake, considering all cancer sites combined and separately (Figure 1). Out of the 10 studies, the ORs of OPC were below unity in 8 studies (significant in 4) and above unity in 2 studies (nonsignificant).

Table 4 reports the ORs of OPC for the highest versus the lowest quintile of total folate intake according to 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) (p for heterogeneity= 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 with subjects with low alcohol and intermediate/high total folate intake (p for interaction=0.75). Using the estimated ORs in Table 5, the attributable proportion (AP) due 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 with subjects with never tobacco users and intermediate/high total folate intake (p for interaction=0.90). The AP due to interaction is (2.73−1.33−2.11+1)/2.73 = 10.6% (95% CI: 0.4%–20.8%), suggesting that 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 10 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 for oropharynx/hypopharynx. When pooling the 8 studies (3,910 OPC cases and 11,805 controls) detailing the solely intake of natural folate from diet, however, the inverse association with OPC was no longer significant.

Only a few case-control studies with limited sample sizes 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 versus 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 of 4.9 ng/mL) compared with control groups of non-smokers (mean value of 9.7 ng/mL, p-value<0.05) and smokers (mean value of 9.1 ng/mL, p-value<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 [30] reported the estimates of natural

folate only, but 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 this 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 to perform any meaningful sensitivity analysis. Interestingly, these studies are the only two that reported an OR above 1 for the highest versus the lowest quintile of total folate intake. Since 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, i.e. 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.

Due 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 relation between OPC and folate intake using absolute cut-offs, 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 subjects carriers of the homozygous C677T variant, which is present in 30% of Caucasians.[49] Subjects with impaired enzyme activity have reduced folate concentrations, higher serum homocysteine levels, and higher DNA hypomethylation compared with 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 results showing the absence of an increased risk of cancer among those carrying the unfavourable 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 variants association studies suggest that even though folate intake are 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 that 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, increase folate excretion, or inhibiting methionine synthase [14], so it is expected that 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 10% of OPC cases among heavy alcohol-drinkers with a low folate intake, and around 10% of OPC among those ever smokers with low folate intake have arisen because of the synergistic interaction amongst the two component causes. Taken together, these result has important implications from a public health point of view, since it shows 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.

While the present 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. Firstly, we were unable to dissect the effect of folate on OPC risk according to the intake of supplements or fortified foods. Secondly, the investigation might be affected by limitations of case-control studies, including recall bias that generally lead 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. Further, we were able to adjust for energy intake in all the studies, 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 allow 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 only. If anything, however, the inverse association with folate was stronger for other OPC sites.

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.

Acknowledgments

The authors would like to thank all of the participants who took part in this research for providing us very insightful and constructive comments, which helped improve this manuscript.

Funding

The INHANCE core data pooling was supported by NIH grants (NCI R03CA113157 & NIDCR R03DE016611). The individual studies were supported by the following grants: Milan study (2006–2009): Italian Association for Research on Cancer (AIRC, grant n. 10068) and Italian Ministry of Education (PRIN 2009 X8YCBN). Italy Multicenter study: Italian Association for Research on Cancer (AIRC), Italian League Against Cancer and Italian Ministry of Research. Swiss study: Swiss League against Cancer and the Swiss Research against Cancer/ Oncosuisse (KFS-700, OCS-1633). Boston study: National Institutes of Health (NIH) US (R01CA078609, R01CA100679). Los Angeles study: National Institute of Health (NIH) US (P50CA090388, R01DA011386, R03CA077954, T32CA009142, U01CA096134, R21ES011667) and the Alper Research Program for Environmental Genomics of the UCLA Jonsson Comprehensive Cancer Center. MSKCC study: NIH (R01CA051845). North Carolina (1994–1997):National Institutes of Health (NIH) US (R01CA061188), and in part by a grant from the National Institute of Environmental Health Sciences (P30ES010126). US Multicenter study: The Intramural Program of the NCI, NIH, United States. Japan (2001–2005): Scientific Research grant from the Ministry of Education, Science, Sports, Culture and Technology of Japan (17015052) and grant for the Third-Term Comprehensive 10-Year Strategy for Cancer Control from the Ministry of Health, Labor and Welfare of Japan (H20-002). The work of SB was supported by Italian Association for Research on Cancer (AIRC, grant n. 10491 – 2010/2013). The work of CG and EL was supported by Fondazione Veronesi.

References

1. Ferlay, J.; Soerjomataram, I.; Ervik, M., et al., editors. IARC Cancer Base No 10. Lyon, France: International Agency for Research on Cancer; 2013. Globocan 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet].
2. (AICR) WCRFWaAIfCR. Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspectiveed. Washington, DC: American Institute for Cancer Research; 2007. Mouth, pharynx, and larynx; p. 245-9.
3. La Vecchia C, Franceschi S, Levi F, Lucchini F, Negri E. Diet and human oral carcinoma in Europe. Eur J Cancer B Oral Oncol. 1993; 29B:17–22. [PubMed: 8180571]
4. Winn DM. Diet and nutrition in the etiology of oral cancer. Am J Clin Nutr. 1995; 61:437S–45S. [PubMed: 7840089]
5. Chuang SC, Jenab M, Heck JE, Bosetti C, Talamini R, Matsuo K, Castellsague X, Franceschi S, Herrero R, Winn DM, La Vecchia C, Morgenstern H, et al. Diet and the risk of head and neck cancer: a pooled analysis in the INHANCE consortium. Cancer Causes Control. 2012; 23:69–88. [PubMed: 22037906]
6. Blount BC, Mack MM, Wehr CM, MacGregor JT, Hiatt RA, Wang G, Wickramasinghe SN, Everson RB, Ames BN. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. Proc Natl Acad Sci U S A. 1997; 94:3290–5. [PubMed: 9096386]
7. Aune D, Deneo-Pellegrini H, Ronco AL, Boffetta P, Acosta G, Mendilaharsu M, De Stefani E. Dietary folate intake and the risk of 11 types of cancer: a case-control study in Uruguay. Ann Oncol. 2011; 22:444–51. [PubMed: 20647222]
8. De Stefani E, Ronco A, Mendilaharsu M, Deneo-Pellegrini H. Diet and risk of cancer of the upper aerodigestive tract--II. Nutrients Oral Oncol. 1999; 35:22–6.
9. McLaughlin JK, Gridley G, Block G, Winn DM, Preston-Martin S, Schoenberg JB, Greenberg RS, Stemhagen A, Austin DF, Ershow AG, et al. Dietary factors in oral and pharyngeal cancer. J Natl Cancer Inst. 1988; 80:1237–43. [PubMed: 3418729]
10. Pelucchi C, Talamini R, Negri E, Levi F, Conti E, Franceschi S, La Vecchia C. Folate intake and risk of oral and pharyngeal cancer. Ann Oncol. 2003; 14:1677–81. [PubMed: 14581278]
11. Weinstein SJ, Gridley G, Harty LC, Diehl SR, Brown LM, Winn DM, Bravo-Otero E, Hayes RB. Folate intake, serum homocysteine and methylenetetrahydrofolate reductase (MTHFR) C677T genotype are not associated with oral cancer risk in Puerto Rico. J Nutr. 2002; 132:762–7. [PubMed: 11925474]

12. Bailey LB. Folate status assessment. J Nutr. 1990; 120 (Suppl 11):1508–11. [PubMed: 2243297]
13. Barak AJ, Beckenhauer HC, Tuma DJ, Badakhsh S. Effects of prolonged ethanol feeding on methionine metabolism in rat liver. Biochem Cell Biol. 1987; 65:230–3. [PubMed: 3580171]
14. Mason JB, Choi SW. Effects of alcohol on folate metabolism: implications for carcinogenesis. Alcohol. 2005; 35:235–41. [PubMed: 16054985]
15. Heimburger DC. Localized deficiencies of folic acid in aerodigestive tissues. Ann N Y Acad Sci. 1992; 669:87–95. discussion -6. [PubMed: 1444061]
16. Piyathilake CJ, Hine RJ, Dasanayake AP, Richards EW, Freeberg LE, Vaughn WH, Krumdieck CL. Effect of smoking on folate levels in buccal mucosal cells. Int J Cancer. 1992; 52:566–9. [PubMed: 1399138]
17. Matsuo K, Rossi M, Negri E, Oze I, Hosono S, Ito H, Watanabe M, Yatabe Y, Hasegawa Y, Tanaka H, Tajima K, La Vecchia C. Folate, alcohol, and aldehyde dehydrogenase 2 polymorphism and the risk of oral and pharyngeal cancer in Japanese. Eur J Cancer Prev. 2012; 21:193–8. [PubMed: 21946912]
18. Shanmugham JR, Zavras AI, Rosner BA, Giovannucci EL. Alcohol-folate interactions in the risk of oral cancer in women: a prospective cohort study. Cancer Epidemiol Biomarkers Prev. 2010; 19:2516–24. [PubMed: 20841386]
19. Conway DI, Hashibe M, Boffetta P, Wunsch-Filho V, Muscat J, La Vecchia C, Winn DM. Enhancing epidemiologic research on head and neck cancer: INHANCE - The international head and neck cancer epidemiology consortium. Oral Oncol. 2009; 45:743–6. [PubMed: 19442571]
20. Leoncini E, Ricciardi W, Cadoni G, Arzani D, Petrelli L, Paludetti G, Brennan P, Luce D, Stucker I, Matsuo K, Talamini R, La Vecchia C, et al. Adult height and head and neck cancer: a pooled analysis within the INHANCE Consortium. Eur J Epidemiol. 2014; 29:35–48. [PubMed: 24271556]
21. Hashibe M, Brennan P, Benhamou S, Castellsague X, Chen C, Curado MP, Dal Maso L, Daudt AW, Fabianova E, Fernandez L, Wunsch-Filho V, Franceschi S, et al. Alcohol drinking in never users of tobacco, cigarette smoking in never drinkers, and the risk of head and neck cancer: pooled analysis in the International Head and Neck Cancer Epidemiology Consortium. J Natl Cancer Inst. 2007; 99:777–89. [PubMed: 17505073]
22. Leoncini E, Ricciardi W, Cadoni G, Arzani D, Petrelli L, Paludetti G, Brennan P, Luce D, Stucker I, Matsuo K, Talamini R, La Vecchia C, et al. Adult height and head and neck cancer: a pooled analysis within the INHANCE Consortium. Eur J Epidemiol. 2013
23. Gnagnarella P, Parpinel M, Salvini S, Franceschi S, Palli D, Boyle P. The update of the Italian Food Composition Database. J Food Compos Anal. 2004; 17:509–22.
24. Gnagnarella, P.; Salvini, S.; Parpinel, M. Food Composition Database for Epidemiological Studies in Italy. Milan, Italy: Istituto Europeo di Oncologia; 2008. Version 2. 2008
25. U.S. Department of Agriculture ARS; Laboratory ND, editor. USDA National Nutrient Database for Standard Reference, Release 11 and following. Home Page, http://www.ars.usda.gov/ba/bhnrc/ndl
26. Agriculture USDo. Handbook Composition of Foods. 1–19.
27. Council R, Agency SaT, Japan tGo. Standard Tables of Food Composition in Japan, 5 th revised versioned. Tokyo: Ministry of Finance Printing Bureau; 2000.
28. Blot WJ, McLaughlin JK, Winn DM, Austin DF, Greenberg RS, Preston-Martin S, Bernstein L, Schoenberg JB, Stemhagen A, Fraumeni JF Jr. Smoking and drinking in relation to oral and pharyngeal cancer. Cancer Res. 1988; 48:3282–7. [PubMed: 3365707]
29. Bosetti C, Gallus S, Trichopoulou A, Talamini R, Franceschi S, Negri E, La Vecchia C. Influence of the Mediterranean diet on the risk of cancers of the upper aerodigestive tract. Cancer Epidemiol Biomarkers Prev. 2003; 12:1091–4. [PubMed: 14578148]
30. Cui Y, Morgenstern H, Greenland S, Tashkin DP, Mao J, Cao W, Cozen W, Mack TM, Zhang ZF. Polymorphism of Xeroderma Pigmentosum group G and the risk of lung cancer and squamous cell carcinomas of the oropharynx, larynx and esophagus. Int J Cancer. 2006; 118:714–20. [PubMed: 16094634]

31. Divaris K, Olshan AF, Smith J, Bell ME, Weissler MC, Funkhouser WK, Bradshaw PT. Oral health and risk for head and neck squamous cell carcinoma: the Carolina Head and Neck Cancer Study. Cancer Causes Control. 2010; 21:567–75. [PubMed: 20049634]
32. Jayaprakash V, Rigual NR, Moysich KB, Loree TR, Nasca MA, Menezes RJ, Reid ME. Chemoprevention of head and neck cancer with aspirin: a case-control study. Arch Otolaryngol Head Neck Surg. 2006; 132:1231–6. [PubMed: 17116820]
33. Levi F, Pasche C, La Vecchia C, Lucchini F, Franceschi S, Monnier P. Food groups and risk of oral and pharyngeal cancer. Int J Cancer. 1998; 77:705–9. [PubMed: 9688303]
34. Peters ES, McClean MD, Liu M, Eisen EA, Mueller N, Kelsey KT. The ADH1C polymorphism modifies the risk of squamous cell carcinoma of the head and neck associated with alcohol and tobacco use. Cancer Epidemiol Biomarkers Prev. 2005; 14:476–82. [PubMed: 15734975]
35. Schantz SP, Zhang ZF, Spitz MS, Sun M, Hsu TC. Genetic susceptibility to head and neck cancer: interaction between nutrition and mutagen sensitivity. Laryngoscope. 1997; 107:765–81. [PubMed: 9185733]
36. Suzuki T, Wakai K, Matsuo K, Hirose K, Ito H, Kuriki K, Sato S, Ueda R, Hasegawa Y, Tajima K. Effect of dietary antioxidants and risk of oral, pharyngeal and laryngeal squamous cell carcinoma according to smoking and drinking habits. Cancer Sci. 2006; 97:760–7. [PubMed: 16800818]
37. Bravi F, Bosetti C, Filomeno M, Levi F, Garavello W, Galimberti S, Negri E, La Vecchia C. Foods, nutrients and the risk of oral and pharyngeal cancer. Br J Cancer. 2013; 109:2904–10. [PubMed: 24149181]
38. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials. 1986; 7:177–88. [PubMed: 3802833]
39. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ. 2003; 327:557–60. [PubMed: 12958120]
40. Higgins JP, Thompson SG, Spiegelhalter DJ. A re-evaluation of random-effects meta-analysis. J R Stat Soc Ser A Stat Soc. 2009; 172:137–59.
41. Rothman, KJ. Measuring interactions. In: Rothman, KJ., editor. Epidemiology: an introductioned. New York: Oxford University Press; 2002. p. 168-90.
42. Andersson T, Alfredsson L, Kallberg H, Zdravkovic S, Ahlbom A. Calculating measures of biological interaction. Eur J Epidemiol. 2005; 20:575–9. [PubMed: 16119429]
43. Almadori G, Bussu F, Galli J, Cadoni G, Zappacosta B, Persichilli S, Minucci A, Giardina B, Maurizi M. Serum levels of folate, homocysteine, and vitamin B12 in head and neck squamous cell carcinoma and in laryngeal leukoplakia. Cancer. 2005; 103:284–92. [PubMed: 15593092]
44. Lucock M. Folic acid: nutritional biochemistry, molecular biology, and role in disease processes. Mol Genet Metab. 2000; 71:121–38. [PubMed: 11001804]
45. Hannon-Fletcher MP, Armstrong NC, Scott JM, Pentieva K, Bradbury I, Ward M, Strain JJ, Dunn AA, Molloy AM, Kerr MA, McNulty H. Determining bioavailability of food folates in a controlled intervention study. Am J Clin Nutr. 2004; 80:911–8. [PubMed: 15447898]
46. Tio M, Andrici J, Cox MR, Eslick GD. Folate Intake and the Risk of Upper Gastrointestinal Cancers: A Systematic Review and Meta-Analysis. J Gastroenterol Hepatol. 2013
47. Duthie SJ. Folic acid deficiency and cancer: mechanisms of DNA instability. Br Med Bull. 1999; 55:578–92. [PubMed: 10746348]
48. Rozen R. Genetic predisposition to hyperhomocysteinemia: deficiency of methylenetetrahydrofolate reductase (MTHFR). Thromb Haemost. 1997; 78:523–6. [PubMed: 9198208]
49. Botto LD, Yang Q. 5,10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: a HuGE review. Am J Epidemiol. 2000; 151:862–77. [PubMed: 10791559]
50. Graziano F, Kawakami K, Ruzzo A, Watanabe G, Santini D, Pizzagalli F, Bisonni R, Mari D, Floriani I, Catalano V, Silva R, Tonini G, et al. Methylenetetrahydrofolate reductase 677C/T gene polymorphism, gastric cancer susceptibility and genomic DNA hypomethylation in an at-risk Italian population. Int J Cancer. 2006; 118:628–32. [PubMed: 16094648]
51. Smith GD, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? Int J Epidemiol. 2003; 32:1–22. [PubMed: 12689998]

52. Boccia S, Boffetta P, Brennan P, Ricciardi G, Gianfagna F, Matsuo K, van Duijn CM, Hung RJ. Meta-analyses of the methylenetetrahydrofolate reductase C677T and A1298C polymorphisms and risk of head and neck and lung cancer. Cancer Lett. 2009; 273:55–61. [PubMed: 18789576]

53. Zhuo X, Ling J, Zhou Y, Zhao H, Song Y, Tan Y. Polymorphisms of MTHFR C677T and A1298C association with oral carcinoma risk: a meta-analysis. Cancer Invest. 2012; 30:447–52. [PubMed: 22536935]

54. Rothman KJ, Greenland S. Causation and causal inference in epidemiology. Am J Public Health. 2005; 95 (Suppl 1):S144–50. [PubMed: 16030331]

55. D'Avanzo B, La Vecchia C, Katsouyanni K, Negri E, Trichopoulos D. An assessment, and reproducibility of food frequency data provided by hospital controls. Eur J Cancer Prev. 1997; 6:288–93. [PubMed: 9306076]

Novelty and impact

There are suggestions of an inverse association between folate and the risk of oral and pharyngeal cancer (OPC), but most studies are limited in sample size with only few of them reporting information on the source of dietary folate. Using data from INHANCE Consortium on over 5000 cases and 13000 controls, we provide convincing evidence that folate intake may protect against the risk of OPC, after controlling for recognized confounding factors.

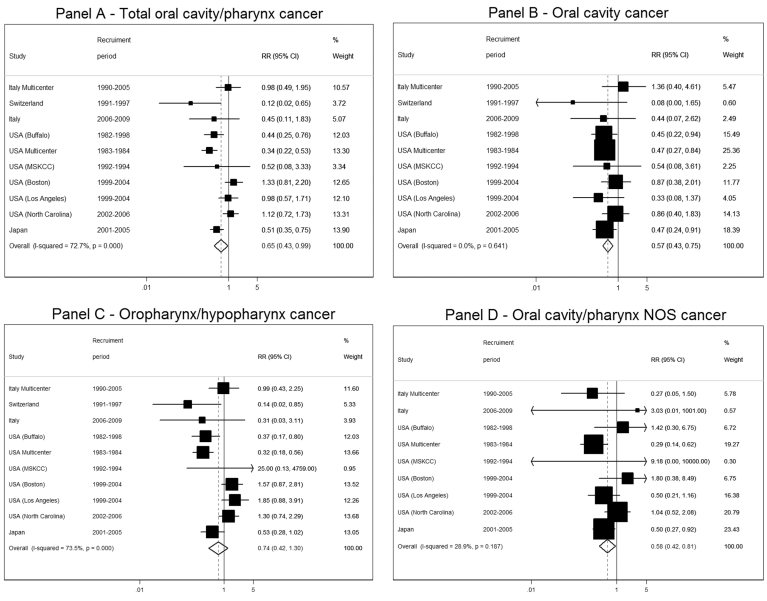

Figure 1.

Table 1

Characteristics of the 10 individual studies on oral cavity/pharynx cancer (OPC) from the International Head and Neck Cancer Epidemiology (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 cut-offs of total folate intake[1]	OPC cases	Controls
					Natural food only	Supplements only	All sources together[2]			
Europe										
Italy Multicenter [29]	Aviano, Milan, Latina	1990–2005	hospital/hospital	>95, >95	Yes	No	No	212.6; 254.6; 291.6; 344.5	801	2,716
Switzerland [33]	Lausanne	1991–1997	hospital/hospital	>95, >95	Yes	No	No	192.1; 238.2; 282.2; 347.2	392	883
Italy [37]	Milan	2006–2009	hospital/hospital	>95, >95	Yes	No	No	198.3; 236.4; 273.8; 322.7	142	755
North America										
USA (Buffalo) [32]	Buffalo	1982–1998	hospital/hospital	~50, ~50	Yes	No	No	267.0; 341.8; 425.5; 539.9	441	1,256
USA Multicenter [28]	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) [35]	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) [30]	Los Angeles	1999–2004	Cancer Registry/Neighborhood	49, 68	Yes	No	No	125.8; 163.2; 207.3; 258.1	338	1,040

Study ID	Study location	Recruitment period	Source (cases/controls)	Participation rate of cases and controls (%)	Sources of folate			Quintile cut-offs of total folate intake[1]	OPC cases	Controls
					Natural food only	Supplements only	All sources together[2]			
USA (North Carolina) [31]	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 [36]	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] Calculation of cut-offs for quintile of the most complete information on folate intake reported in each study were based on distribution of controls.

[2] Two studies reported folate estimates exclusively from natural sources and folate supplementation combined [28, 35], and two studies from natural sources, folate-fortified food products and folate supplementation combined [31, 34].

Table 2

Distribution of oral cavity and pharynx cancer (OPC) cases and controls according to selected variables[1] in the 10 studies included in the International Head and Neck Cancer Epidemiology (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

	OPC cases		Controls	
	n	**%**	**n**	**%**
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)				
Non drinkers	646	13.0	3,303	25.6
>0 – <1	1,143	22.9	4,300	33.4
>=1 – <3	1,051	21.1	3,035	23.5
>=3 – <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				
<25 kg/m^2	2,942	59.4	6,436	48.9
≥25 kg/m^2	2,014	40.6	6,721	51.1
p (χ^2 test)		<0.0001		
Total energy intake (Kcal/die)				
Mean ± SD	1584 ± 1232		1283 ± 939	
p (t-test)		<0.0001		

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

Table 3

Associations between folate intake and risk of oral cavity and pharynx cancer (OPC), overall and stratified by anatomic site. International Head and Neck Cancer Epidemiology (INHANCE) Consortium.

			OPC		Oral cavity		Oropharynx/hypopharynx		NOS[1]	
	Controls (n)	Cases (n)	OR[2] (95% CI)	cases	OR[2] (95% CI)	cases	OR[2] (95% CI)	cases	OR[2] (95% CI)	
Total Folate intake (10 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 for trend			0.04		<0.01		0.28		<0.01	
p for heterogeneity between studies			0.04		0.74		0.06		0.24	
Folate intake from natural sources only (8 studies included[5])										
I Quintile[c]	2,156	781	1(Ref)	241	1(Ref)	410	1(Ref)	130	1	
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 for trend			0.08		<0.01		0.19		0.31	
p 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:[28–37]

[4] Calculation of cut-offs for quintile were based on distribution of controls in each study (study-specific)

[5] Studies included: 29–34, 36, 37

Table 4

Distribution of cases of oral cavity and pharynx cancer (OPC) and controls, and corresponding odds ratio (OR)[1] and 95% confidence intervals (CI), for the highest quintile of total folate intake versus the lowest one in strata of selected covariates. International Head and Neck Cancer Epidemiology (INHANCE) Consortium.

	OPC			
	Cases[2] n:n	Controls[2] n:n	OR (95% CI)	p for heterogeneity between studies
Age (years)				
<55	350:348	810:751	0.69 (0.40–1.20)	<0.01
≥ 55	659:603	1615:1680	0.70 (0.44–1.12)	0.03
P for heterogeneity between strata		0.97		
Gender				
Men	674:769	1637:1820	0.60 (0.37–0.97)	0.03
Women	335:182	788:611	0.80 (0.55–1.16)	0.23
P 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	1010:1020	0.73 (0.58–0.90)	0.22
Asia	113:51	587:600	0.51 (0.35–0.75)	-
P 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	1527:1523	0.71 (0.57–0.87)	0.21
P for heterogeneity between strata		0.28		
Study design				
Hospital based	551:387	1663:1664	0.52 (0.40–0.69)	0.66
Population based	457:564	761:767	0.80 (0.63–1.01)	0.46
P for heterogeneity between strata		0.02		
Body mass index[4]				
<25 kg/m^2	638:542	1222:1156	0.61 (0.48–0.79)	0.59
≥25 kg/m^2	339:394	1186:1262	0.61 (0.33–1.13)	0.03
P 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 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	1266:1300	0.71 (0.35–1.44)	0.08
Heavy drinkers	431:504	489:561	0.59 (0.39–0.90)	<0.01

	OPC			
	Cases[2] n:n	**Controls[2] n:n**	**OR (95% CI)**	**p for heterogeneity between studies**
P 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 cut-offs for quintile were based on 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 2studies from Italy [29, 37] and 1from Switzerland [33]. North America included 6 studies [28, 30–32, 34, 35]. Asia included one study from Japan [36].

[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.

[6] Light drinkers were defined as subjects who drank <3 drinks of alcoholic beverages per day and heavy drinkers ≥3 drinks per day.

Table 5

Odds Ratios[1] and 95% confidence intervals of oral cavity and pharynx cancer (OPC) according to total folate intake and alcohol and tobacco consumption. International Head and Neck Cancer Epidemiology (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 tertiles of intake. Calculation of cut-offs for tertile of total folate intake were based on 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.