

# Folate intake and squamous-cell carcinoma of the oesophagus in Italian and Swiss men

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Received 20 September 2005; accepted 11 November 2005

**Background:** Dietary folate has been inversely related to the risk of several cancers. However, studies on the role of dietary folate in oesophageal cancer are scanty.

**Patients and methods:** Using data from a multicentric case-control study conducted in Italy and Switzerland between 1992 and 1999, we investigated the association between dietary folate intake and oesophageal squamous-cell carcinoma (OSCC) among 351 men with incident, histologically confirmed OSCC and 875 hospital controls admitted for acute, non-neoplastic conditions, unrelated to alcohol and smoking consumption. Intake of folate and other nutrients was computed from a validated food frequency questionnaire.

**Results:** The multivariate odds ratios (ORs) of OSCC were 0.68 (95% confidence intervals, CI: 0.46–1.00) for the highest versus the lowest tertile of folate intake, and 0.84 (95% CI: 0.72–0.99) for an increment of folate intake equal to a standard deviation (98 µg/day). The inverse relation was somewhat stronger in strata of high methionine, vitamin B6 and alcohol intake, and did not vary substantially according to age and smoking habits.

**Conclusion:** Dietary folate was inversely related to OSCC risk in this population with high alcohol consumption and infrequent use of supplements and multivitamins.

**Key words:** alcohol, case-control study, diet, folate, oesophageal squamous-cell carcinoma

## introduction

In Europe and North America, oesophageal squamous-cell carcinoma (OSCC) is strongly related to tobacco and alcohol consumption [1], but various aspects of diet may also have a role on risk [2]. A high intake of fruit and vegetables has been linked to a lower risk of oesophageal cancer [3], whereas a poor nutritional status and an unbalanced diet have been related to an elevated risk [4].

A deficient supply of folate, a water-soluble B vitamin, has been linked to the risk of cancer at several sites [5]. This may increase the risk of cancer by inducing an imbalance in DNA precursors, leading to modified DNA synthesis and repair [6]. Several epidemiological studies investigated the relationship between dietary folate intake, or serum folate, and risk of various cancers [7], such as oral cavity [8], colorectal [9], breast [10] and ovary [11]. Important interactions between folate, methionine and/or alcohol intake were also reported.

Epidemiological data on the relation between dietary folate intake and OSCC are scanty [12, 13]. In a study from Italy, the odds ratio (OR) for the highest quintile of folate compared to the lowest one was 0.6 [12]. In a case-control study from United

States, comparing the 75th percentile of intake to the 25th, dietary intakes of folate and vitamin B6 were inversely related to OSCC (OR = 0.58, 0.45, respectively) [13]. Moreover, two Chinese studies found that the methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism, which is related to folate metabolism and is involved in risk of various cancers, may influence the risk of SCC of the oesophagus [14, 15].

Thus, we investigated the role of folate and its interaction with alcohol, methionine and vitamin B6, on risk of OSCC in a large, multicentric case-control study conducted in Italy and Switzerland, a population with high alcohol consumption and infrequent use of supplements and multivitamins [12, 16, 17].

## patients and methods

Data were obtained from a case-control study conducted between 1992 and 1999 in Milan, Pordenone, Padua and Udine in the northern Italy, and in the Swiss canton of Vaud, and including 351 male cases of OSCC (median age 60 years) and 875 male controls (median age 60 years) [18]. All cancer cases were incident and histologically confirmed.

Controls were patients hospitalized for a wide spectrum of acute non-neoplastic conditions (23% had non-alcohol-related traumas, mostly fractures and sprains, 27% non-traumatic orthopedic disorders, 32% acute surgical conditions, and 18% miscellaneous other illnesses such as eye, ear or skin diseases).

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Cases and controls were aged <80 years and were identified and questioned by trained interviewers during their hospital stay, in the same network of teaching and general hospitals in the areas under surveillance. The proportion of refusals was less than 5% in both cases and controls.

Data were collected using a structured questionnaire, including information on socio-demographic factors, anthropometric variables, smoking, alcohol and other lifestyle habits, a problem-oriented medical history, physical activity, aspirin use, and history of cancer in relatives. Information on diet referred to the previous 2 years and was based on a reproducible [19] and valid [20, 21] food frequency questionnaire (FFQ) comprising 78 foods, food groups or recipes, and allowing the estimation of total energy intake and of various nutrients. The FFQ was divided into 6 sections: (1) bread, cereals, first courses; (2) second courses (i.e., meat, fish and other main dishes); (3) side dishes (i.e., vegetables, fried/baked potatoes); (4) fruits; (5) sweets, desserts, and soft drinks; (6) milk, hot beverages, and sweeteners. For a few vegetables and fruits, seasonal consumption and the corresponding duration were elicited. At the end of each section, one or two open questions were used to include foods that were not in the questionnaire, but were eaten at least once per week. Energy and nutrient intakes, including folate, methionine and vitamin B6, were computed from the FFQ using an Italian food composition database [22]. A separate section investigated alcohol consumption in detail.

**data analysis**

OR, and their corresponding 95% confidence intervals (CI), were computed using conditional multiple logistic regression. All regression models were matched on age and study centre, and included terms for education (<7, 7 to <12, ≥12 years), body mass index (in quintiles), alcohol (in quintiles: <9, 9–<24, 24–<37, 37–<61 and ≥61 glasses per week, plus a term for ex-drinkers) and smoking habits (never smokers and ex-smokers since ≥20 years, current smokers and ex-smokers since <20 years of <15, 15 to <25, ≥25 cigarettes per day). Allowance was also made for non-alcohol energy intake, using the residual method [23].

**results**

Table 1 shows the relationship between dietary folate, methionine, vitamin B6 and risk of OSCC among 351 male cases

and 875 controls. Oesophageal cancer was inversely associated with dietary folate intake (OR = 0.68 for the highest versus the lowest tertile of intake, 95% CI: 0.46–1.00). The OR for an increase of folate intake equal to a standard deviation was 0.84 (95% CI: 0.72–0.99). For methionine, the OR for the highest tertile of intake was 1.11 (95% CI: 0.76–1.62). The corresponding value for vitamin B6 was 0.88 (95% CI: 0.60–1.31).

Table 2 presents the OR of OSCC in men according to dietary folate in strata of age, methionine and vitamin B6. The inverse relation between folate and OSCC was similar in <60 (OR = 0.77) and ≥60 years old (OR = 0.58), while it was apparently stronger in men with higher intake of methionine (OR = 0.45) and vitamin B6 (OR = 0.48). However, no significant heterogeneity was observed across strata of any of these covariates.

The continuous OR of OSCC according to folate intake, in strata of alcohol drinking and smoking habits are reported in Table 3. The protection of folate was stronger in heavy drinkers (OR = 0.74) than in moderate drinkers (OR = 0.96). The OR were 0.68 for non-smokers, 0.81 for smokers of <15, 0.74 for 15–24 and 1.07 for ≥25 cigarettes/day.

Table 4 considers the combined effect of folate with alcohol intake and smoking habits. The reference category were subjects in the lowest tertile of folate intake and with, respectively, alcohol intake above the median or current smokers or ex-smokers since <20 years. Subjects with low alcohol/low folate intake had an OR of 0.19, those with high alcohol/high folate had an OR of 0.81, and those with low alcohol/high folate had an OR of 0.10. Non-smokers with low folate intake had an OR of 0.36, current smokers with high folate had an OR of 0.63 and non-smokers with high folate had an OR of 0.31.

**discussion**

Folate influences both methylation of DNA and available nucleotide pool for DNA replication and repair [24]. A low

**Table 1.** Relation between dietary folate, methionine, vitamin B6 intake and risk of oesophageal SCC among 351 male cases and 875 male controls in Italy and Switzerland, 1992–1999

	Mean	SD	Tertile <sup>a</sup> of intake			$\chi^2_{\text{trend}}$	Continuous OR <sup>b</sup>
			1 (low)	2	3 (high)		
<b>Folate (µg/day)</b>							
Cases:Controls	276.3	98.0	131:274	130:287	90:314		
OR <sup>c</sup> (95% CI)			1 <sup>d</sup>	0.97 (0.68–1.41)	0.68 (0.46–1.00)	3.73 (P = 0.053)	0.84 (0.72–0.99)
<b>Methionine (mg/day)</b>							
Cases:Controls	2273.3	730.2	124:281	106:311	121:283		
OR <sup>c</sup> (95% CI)			1 <sup>d</sup>	0.76 (0.52–1.10)	1.11 (0.76–1.62)	0.30 (P = 0.584)	1.03 (0.89–1.20)
<b>Vitamin B6 (mg/day)</b>							
Cases:Controls	2.1	0.6	117:287	126:291	108:297		
OR <sup>c</sup> (95% CI)			1 <sup>d</sup>	1.02 (0.71–1.48)	0.88 (0.60–1.31)	0.35 (P = 0.554)	0.92 (0.78–1.08)

<sup>a</sup>Energy adjusted using the residual method. Cut-off points, for absolute intake of folate: 228.1, 305.1 µg/day; methionine: 1862.56, 2479 mg/day; vitamin B6: 1.722, 2.249 mg/day.

<sup>b</sup>The measurement unit was set at 1 standard deviation (SD) of the combined distribution of cases and controls.

<sup>c</sup>OR, odds ratio; CI, confidence interval. Estimates from conditional logistic regression conditioned on age and center, and adjusted for education, body mass index, tobacco smoking and alcohol drinking.

<sup>d</sup>Reference category.

**Table 2.** Relation between dietary folate intake and risk of oesophageal SCC among 351 male cases and 875 male controls in strata of selected covariates. Italy and Switzerland, 1992–1999

Stratum	Tertile <sup>a</sup> of folate intake			$\chi^2_{\text{trend}}$	Continuous OR <sup>b</sup>
	1	2	3		
<b>Age</b>					
<b>&lt;60 years</b>					
Cases:Controls	60:132	58:147	47:150		
OR <sup>c</sup> (95% CI)	1 <sup>d</sup>	1.02 (0.57–1.81)	0.77 (0.43–1.39)	0.76	0.86 (0.67–1.10)
<b>≥60 years</b>					
Cases:Controls	71:142	72:140	43:164		
OR <sup>c</sup> (95% CI)	1 <sup>d</sup>	0.99 (0.61–1.62)	0.58 (0.34–1.00)	3.57	0.82 (0.67–1.02)
<b>Methionine intake</b>					
<b>&lt; Median Value</b>					
Cases:Controls	67:147	57:142	49:151		
OR <sup>c</sup> (95% CI)	1 <sup>d</sup>	1.11 (0.64–1.92)	1.04 (0.59–1.86)	0.03	0.95 (0.76–1.20)
<b>≥ Median Value</b>					
Cases:Controls	64:127	73:145	41:163		
OR <sup>c</sup> (95% CI)	1 <sup>d</sup>	0.87 (0.52–1.46)	0.45 (0.25–0.78)	7.69 <sup>f</sup>	0.75 (0.59–0.94)
<b>Vitamin B6 intake</b>					
<b>&lt; Median Value</b>					
Cases:Controls	95:208	65:144	25:76		
OR <sup>c</sup> (95% CI)	1 <sup>d</sup>	1.21 (0.73–1.98)	0.93 (0.49–1.78)	0.004	0.96 (0.75–1.22)
<b>≥ Median Value</b>					
Cases:Controls	36:66	65:143	65:238		
OR <sup>c</sup> (95% CI)	1 <sup>d</sup>	0.70 (0.38–1.29)	0.48 (0.26–0.88)	5.93 <sup>e</sup>	0.75 (0.58–0.97)

<sup>a</sup>Energy adjusted using the residual method. Cut-off points for absolute intake of folate: 228.1, 305.1  $\mu\text{g}/\text{day}$ ; median value of methionine: 2140 mg/day; vitamin B6: 1.986 mg/day.

<sup>b</sup>The measurement unit was set at 1 standard deviation of the combined distribution of cases and controls.

<sup>c</sup>OR, odds ratio; CI, confidence interval. Estimates from conditional logistic regression conditioned on age and center, and adjusted for education, body mass index, tobacco smoking and alcohol drinking.

<sup>d</sup>Reference category.

<sup>e</sup> $P < 0.05$ .

<sup>f</sup> $P < 0.01$ .

dietary folate intake has been related to suboptimal cellular DNA repair capacity [25]. Two major mechanisms have been proposed to link low folate status to increased cancer risk. First, folate deficiency may lead to decreased levels of S-adenosylmethionine (SAM), and cause DNA hypomethylation and proto-oncogene activation. Second, folate deficiency induces uracil misincorporation in DNA synthesis, leading to chromosome breaks in humans, which could contribute to increase cancer risk [6, 26].

Vitamin B6 is a coenzyme of folate in biological reactions for DNA synthesis and methylation. As folate, vitamin B6 deficiency could be associated to chromosome breakage [27]. Methionine is involved together with folate in the production of SAM, the primary methyl donor in the body. If methionine levels are low, more folate is used as methyltetrahydrofolate to form methionine. This may lower the level of methylene-tetrahydrofolate, which is necessary for DNA synthesis [26]. In this study we did not find associations between methionine and vitamin B6 and oesophageal cancer risk, but folate intake had a somewhat stronger protective effect in strata of high intake of both methionine and vitamin B6, as was found in a prior study on oral cancer [8].

Alcohol consumption and tobacco smoking are two well-recognized and strong-risk factors for OSCC in industrialized

countries. High intake of alcohol can lead to decreased folate absorption in the body, and thus increase folate requirements [28]. In our data, the protection conferred by folate appeared somewhat stronger in heavy drinkers. Also cigarette smoking may increase folate requirements by interfering with folate utilization and/or metabolism [29], but in our study there was no evidence that folate intake was more protective for oesophageal cancer in non-smokers than in current smokers.

Aspirin may exert an anti-folate activity [30]. In this male population, aspirin was regularly used by only 13 cases and 27 controls. Adjustment for aspirin use and exclusion of regular users, did not substantially change the results. Use of supplements and multivitamins is still uncommon in Italy and Switzerland [16, 17] and it is therefore unlikely that they may have materially distorted any of the results.

We tried to minimize bias of hospital-based case-control studies by excluding all control patients with diagnoses linked to long-term changes in diet or admitted for chronic conditions. Folate is found in a wide variety of foods, many of which are not commonly related to cancer risk in the general population. The main contribution to folate intake in this population derives from bread (~16.7%) [31]. Also, hospital controls should reduce recall bias and improve comparability of information of cases and controls [32, 33]. Finally, strengths of this

**Table 3.** Relationship between dietary folate intake and risk of oesophageal SCC in strata of alcohol and smoking habits. Italy and Switzerland, 1992–1999

	Cases:Controls <sup>c</sup>	Continuous OR <sup>d</sup>	95% CI
Tertile of alcohol <sup>a</sup> (glasses per week)			
<18	21:384	0.96	(0.70–1.31)
18– <44	102:308	0.80	(0.60–1.07)
≥44	228:183	0.74	(0.58–0.95)
Smoking habits <sup>b</sup> (cigarettes per day)			
Non-smokers	31:268	0.68	(0.40–1.20)
1– <15	62:204	0.81	(0.56–1.17)
15– <25	155:244	0.74	(0.58–0.96)
≥25	103:157	1.07	(0.78–1.45)

<sup>a</sup>Estimates from conditional logistic regression conditioned on age and center, and adjusted for education, body mass index, tobacco smoking and alcohol drinking. Energy adjusted using the residual method.

<sup>b</sup>The same model was applied, excluding adjustment for tobacco smoking.

<sup>c</sup>The sum does not add up to the total because of some missing values.

<sup>d</sup>The measurement unit was set at 1 standard deviation (98 µg/day of folate) of the combined distribution of cases and controls.

OR = odds ratio; CI = confidence interval.

**Table 4.** Odds ratios<sup>a</sup> and 95% confidence intervals of oesophageal SCC according to combined intake of folate with alcohol and smoking habits. Italy and Switzerland, 1992–1999

	Tertile of folate intake <sup>b</sup>		
	Low	Intermediate	High
Alcohol consumption <sup>c</sup>			
≥ Median	1 <sup>d</sup>	0.70 (0.44–1.10)	0.81 (0.49–1.33)
< Median	0.19 (0.11–0.31)	0.25 (0.15–0.41)	0.10 (0.06–0.17)
Smoking habits			
Current smokers	1 <sup>d</sup>	0.95 (0.65–1.39)	0.63 (0.42–0.96)
Non-smokers	0.36 (0.16–0.81)	0.32 (0.15–0.66)	0.31 (0.15–0.66)

<sup>a</sup>Estimates from conditional logistic regression conditioned on age and center, and adjusted for education, body mass index, alcohol drinking and tobacco smoking. Energy adjusted using the residual method.

<sup>b</sup>Cut-off points for absolute intake of folate: 228.1, 301.5 µg/day.

<sup>c</sup>Cut-off point (median value) for alcohol was 29.5 drinks per week.

<sup>d</sup>Reference category.

investigation are its large size, the use of a validated and reproducible FFQ [19–21], allowing to adjust for total energy intake and several micro- and macro-nutrients, and the low percentage of refusals of the subjects contacted.

Our study supports a favorable role of folate in the aetiology of OSCC in men. Given the findings obtained in similar populations for oral and pharyngeal [8], prostate [34] and colorectal cancer [9], these data confirm the favorable effect of folate in the process of carcinogenesis. In our analysis, this inverse association was confirmed after adjustment for major known risk factors of OSCC (i.e. smoking and alcohol drinking) and for energy intake, and was consistent across age strata.

## acknowledgements

This work was conducted with the contribution of the Italian Association for Cancer Research, the Italian and Swiss Leagues against Cancer, the Swiss Research against Cancer/Oncosuisse (KFS 700 and OCS-1633), and the Italian Ministry of Education (COFIN 2003). The authors thank Mrs. C. Pasche and F. Lucchini for Swiss data collection and validation, and Mrs. I. Garimoldi for editorial assistance.

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