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Micronutrients and laryngeal cancer risk in Italy and Switzerland: a case–control study☆

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Abstract

Objective: To investigate the relation between various micronutrients and laryngeal cancer risk.
Methods: A case–control study was conducted in Italy and Switzerland between 1992 and 2000. Cases were 527 patients with incident cancer of larynx, admitted to the major teaching and general hospitals of the study areas. Controls were 1297 subjects admitted for acute, non-neoplastic diseases to the same network of hospitals. Dietary habits were assessed using a validated food-frequency questionnaire. Odds ratios (OR) and their corresponding 95% confidence intervals (CI) were computed using multiple logistic regression.
Results: Significant inverse relations emerged between laryngeal cancer risk and intake of vitamin C (OR = 0.2, for the highest *versus* the lowest intake quintile; 95% CI: 0.2–0.4), β-carotene (OR = 0.2; 95% CI: 0.2–0.4), α-carotene (OR = 0.3; 95% CI: 0.2–0.5), lutein/zeaxanthin (OR = 0.4; 95% CI: 0.3–0.6), vitamin E (OR = 0.4; 95% CI: 0.3–0.6), β-criptoxanthin (OR = 0.4; 95% CI: 0.2–0.5), folic acid (OR = 0.4; 95% CI: 0.2–0.6), thiamin (OR = 0.4; 95% CI: 0.3–0.6), glutathione (OR = 0.5; 95% CI: 0.4–0.8), reduced glutathione (OR = 0.6; 95% CI: 0.4–0.8), vitamin B6 (OR = 0.6; 95% CI: 0.4–0.9) and potassium (OR = 0.6; 95% CI: 0.4–0.9). Direct associations were found with zinc (OR = 1.5; 95% CI: 1.0–2.2) and vitamin D (OR = 1.8; 95% CI: 1.2–2.6). Combining low intakes of vitamin C, carotene, vitamin E, and folate with heavy smoking and drinking led to ORs between 80 and 170.
Conclusions: This study provides further support that, independently from smoking and alcohol consumption, the intake of several micronutrients, including selected antioxidants, is inversely related to laryngeal cancer risk.

Introduction

Besides smoking and drinking [1–3], some aspects of diet have been linked to laryngeal carcinogenesis [4–7]. A protective effect has been consistently reported for high intakes of fruit and vegetables [8–13]. Information on micronutrients is, however, scanty and inconsistent [12–17].

A case–control study from the USA reported an increased risk of laryngeal cancer for low intake of vitamins A and C [14]. Another North-American study found a significant inverse association with carotene intake, but no association with total vitamin A and retinol [15]. In a study conducted in New York, carotenoids were also found to be inversely related to laryngeal cancer risk, retinol was associated to an increased risk, and no relationship was observed for vitamins C and E [16]. A population-based case–control study conducted in Shanghai, China found a weak, non-significant, decreased risk for the highest intake of both

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carotenoids and vitamin C [12]. In a multicentric investigation from Spain, Italy, Switzerland and France, an inverse association was reported for intake of carotenoids, vitamin C and E, but not for retinol [13].

With reference to minerals, in one study, intake of iron and zinc was associated with a reduced risk of laryngeal cancer, although there was no significant difference between cases and controls in the concentration of iron and zinc in nails [18]. Dietary intake of various minerals (including iron, zinc, sodium, potassium, calcium, and phosphorus) was evaluated in a multicentric study [13], but no significant association was found.

It is therefore unclear whether any of these micronutrients are better predictors of laryngeal cancer risk than the major foods from which they derive.

Most studies on laryngeal cancer, moreover, were relatively small and/or based on simple dietary questionnaires that did not allow for accurate assessment of full dietary pattern, nor of total energy intake. The present case–control study was undertaken to provide further insight in the relationship between intakes of 22 micronutrients and laryngeal cancer in three areas, from northern Italy and Swiss Canton of Vaud, with relatively high incidence rates.

Material and methods

A case–control study on laryngeal cancer was conducted between 1992 and 2000 in two Italian areas (the province of Pordenone, and the greater Milan area, northern Italy), and the Swiss Canton of Vaud [19, 20]. Cases were patients with a first histologically confirmed diagnosis of squamous-cell carcinoma of larynx diagnosed within one year prior to interview (about 91% were interviewed within two months), identified in major teaching and general hospitals in the areas under surveillance. A total of 527 cases (478 men and 49 women; median age 61 years, range 30–79 years) were included in the present analysis (Table 1). Of these, about 74% had carcinomas that originated from the glottis or supraglottis, while 21% had carcinomas of unspecified origin.

During the same calendar period, patients admitted for a wide spectrum of acute illnesses to major hos-

Table 1. Distribution of 527 laryngeal cancer cases and 1297 controls according to selected variables in Italy and Switzerland, 1992–2000

	Cases		Controls	
	n	(%)	n	(%)
Gender[a]				
Male	478	90.7	1052	81.1
Female	49	9.3	245	18.9
Age (years)				
<50	59	11.2	133	10.1
50–54	74	14.0	188	14.5
55–59	97	18.4	259	20.0
60–64	113	21.4	266	20.5
65–69	105	19.9	264	20.4
≥70	79	15.0	187	14.4
Education (years)[b]				
<7	289	54.8	652	50.3
7–11	137	26.0	400	30.9
≥12	101	19.2	244	18.8
Smoking status[a,b]				
Never smokers	19	3.6	485	37.3
Ex smokers	159	30.3	460	35.6
Current smokers				
≤25 cigarettes/day	245	46.7	288	22.2
>25 cigarettes/day	102	19.4	63	4.9
Alcohol intake (drinks/day)[a]				
<4	136	25.8	776	59.8
4–7	175	33.2	362	27.9
≥8	216	41.0	159	12.3

[a] $p < 0.05$.

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

pitals of the areas where cases lived were enrolled as controls. None of them had been admitted for malignant tumours or other conditions that may have led to dietary changes. Controls were frequency-matched by gender, age (five year age categories), and study centre. To compensate for the rarity of laryngeal cancer in women, a control-to-case ratio of five was chosen for females as opposed to two for males. A total of 1297 control subjects (1052 men and 245 women; median age 61 years, range 31–79 years) were interviewed (Table 1). They belonged to the following diagnostic categories: traumas, mostly sprains and fractures (27%), other orthopaedic disorders, such as low back or disc disorders (22%), acute diseases requiring surgery (29%), eye diseases (13%), and other illnesses, such as ear, nose, skin and dental conditions (9%).

All interviews were carried out in a hospital setting by nurses introduced to patients by attending clinical staff. Over 95% of cases and controls approached participated. The response rate did not vary across geographical areas.

The questionnaire included information on socio-demographic characteristics, smoking and alcohol drinking, history of selected diseases, body size measures at various ages, and for women, reproductive and menstrual history. A food-frequency questionnaire (FFQ) was used to assess the usual weekly diet during the two years prior to cancer diagnosis or hospital admission (for controls). The FFQ included 78 foods or recipes divided into seven sections: (i) bread, cereals and first courses; (ii) second courses (*e.g.*, meat and other main dishes); (iii) side dishes (*i.e.* vegetables); (iv) fruits; (v) sweets, desserts and soft drinks; (vi) milk, hot beverages and sweeteners; (vii) alcoholic beverages. For vegetables and fruits subject to seasonal variation, consumption in season and the corresponding duration in months were elicited. Intakes less than once a week, but at least once a month, were coded as 0.5 per week. Dietary supplements were not considered, given their infrequent consumption by these populations.

An *ad hoc* developed food composition database was used to compute micronutrient and energy intake [21]. Data on food composition were supplemented with other published data as concerns different types of carotenoids and glutathione [22]. Losses due to cooking were subtracted from the computation of vitamin contents, except for specific carotenoids, since such information was not available. Therefore, the sum of carotenoids (*i.e.* β- and α-carotene, and β-cryptoxanthin), weighted by their vitamin activity, was slightly different from the value of carotene.

Reproducibility and validity of the FFQ were satisfactory, with correlation coefficients between 0.5 and 0.7 for most micronutrients [23, 24].

Statistical analysis

Odds ratios (OR), and corresponding 95% confidence intervals (CI), were obtained by conditional multiple logistic regression models [25]. The models were conditioned for age (five year age categories), gender and area of residence, and included terms for education (<7, 7–11, 12+ years), body mass index (BMI; kg/m^2, quintiles), smoking (never smoker, smoker of <15, 15–24, 25+ cigarettes/day, ex-smokers), alcohol drinking (quintiles plus a dummy variable for ex-drinkers), and non-alcohol energy intake. Adjustment for total energy intake was made using the residual method [26]. When nutrients were entered in the model as quintiles of intake, these were based on the distribution of cases and controls, and when they were entered as continuous variables, the measurement unit was set to one standard deviation (SD) of the distribution of intake among controls. Tests for trend were based on the likelihood-ratio test between the models with and without a linear term for each micronutrient's quintile [25] (interactions were tested by comparing the difference of the −2 log likelihood of the models with and without interaction terms to the χ^2 distribution with degrees of freedom equal to the number of interaction terms).

Results

Table 1 shows the distribution of 527 cases of squamous-cell carcinoma of larynx and 1297 control subjects according to gender, age, education, smoking status, and alcohol intake. By study design, the proportion of women was higher in controls than in cases, while cases and controls had similar age distributions. Education levels were also similar, but cases were more frequently smokers and heavy alcohol drinkers than controls.

Table 2 gives the ORs of laryngeal cancer according to subsequent quintiles of intake of 5 minerals and 17 vitamins, carotenoids and glutathione. ORs for the highest intake quintile of most micronutrients were below unity, with significant inverse trends in risk, except for zinc (OR in highest *versus* lowest quintile of intake = 1.5) and vitamin D (OR = 1.8). With reference to minerals, an inverse association was observed for potassium intake (OR = 0.6 in highest *versus* lowest quintile of intake), no association emerged for other ions, and phosphorus. Most water-soluble vitamins showed a significant protective effect, including thiamin

Table 2. OR and corresponding 95% CI according to intake of selected micronutrients and minerals, among 527 laryngeal cancer cases, and 1297 controls, Italy and Switzerland, 1992–2000

	Mean	(SD)[b]	Quintile, OR[a] (95% CI)					χ^2 for trend	OR[d] continuous
			1[c]	2	3	4	5		
Minerals									
Calcium (g)	1.0	(0.5)	1	0.7 (0.5–1.0)	0.8 (0.6–1.2)	0.9 (0.7–1.4)	0.9 (0.6–1.3)	0.00	1.0 (0.9–1.1)
Potassium (g)	3.7	(1.1)	1	0.9 (0.6–1.3)	1.0 (0.7–1.5)	0.7 (0.5–1.1)	0.6 (0.4–0.9)	6.50[e]	0.9 (0.8–1.0)
Phosphorus (g)	1.6	(0.5)	1	1.0 (0.7–1.5)	1.2 (0.8–1.7)	1.0 (0.7–1.5)	1.1 (0.8–1.6)	0.17	1.0 (0.9–1.1)
Iron (mg)	15.7	(5.3)	1	1.0 (0.6–1.4)	0.9 (0.6–1.4)	0.9 (0.6–1.4)	1.0 (0.6–1.5)	0.04	1.0 (0.9–1.1)
Zinc (mg)	13.0	(4.2)	1	1.1 (0.8–1.6)	1.2 (0.8–1.8)	1.3 (0.9–1.8)	1.5 (1.0–2.2)	3.93[e]	1.1 (1.0–1.2)
Water-soluble vitamins									
Thiamin (mg)	0.8	(0.3)	1	0.8 (0.6–1.2)	0.8 (0.5–1.1)	0.9 (0.6–1.3)	0.4 (0.3–0.6)	9.68[f]	0.9 (0.8–0.9)
Riboflavin (mg)	1.6	(0.6)	1	1.2 (0.8–1.7)	1.0 (0.7–1.5)	1.3 (0.9–1.8)	1.0 (0.7–1.5)	0.01	1.0 (0.9–1.1)
Vitamin C (mg)	112.8	(71.3)	1	0.7 (0.5–0.9)	0.3 (0.2–0.5)	0.3 (0.2–0.5)	0.2 (0.2–0.4)	60.83[f]	0.7 (0.6–0.8)
Vitamin B6 (mg)	1.9	(0.6)	1	0.9 (0.7–1.3)	0.8 (0.6–1.2)	0.9 (0.6–1.3)	0.6 (0.4–0.9)	5.70[e]	0.9 (0.8–1.0)
Folic acid (mcg)	249.3	(83.4)	1	0.7 (0.5–1.0)	0.6 (0.5–0.9)	0.7 (0.5–0.9)	0.4 (0.2–0.6)	18.20[f]	0.8 (0.7–0.9)
Niacin (mg)	17.4	(5.2)	1	1.0 (0.7–1.4)	1.1 (0.8–1.6)	1.5 (1.0–2.1)	1.1 (0.7–1.6)	1.82	1.1 (1.0–1.2)
Fat-soluble vitamins, carotenoids and glutathione									
Retinol (mg)	0.8	(1.0)	1	1.0 (0.7–1.5)	0.8 (0.5–1.2)	1.1 (0.7–1.5)	1.4 (1.0–2.1)	3.60	1.1 (1.0–1.2)
Carotene (mg)	3.7	(2.4)	1	0.9 (0.7–1.3)	0.5 (0.4–0.8)	0.4 (0.3–0.6)	0.3 (0.2–0.4)	57.92[f]	0.7 (0.6–0.8)
α-carotene (mg)	0.8	(0.9)	1	0.7 (0.5–1.1)	0.8 (0.6–1.1)	0.4 (0.2–0.5)	0.3 (0.2–0.5)	43.70[f]	0.7 (0.7–0.8)
β-carotene (mg)	4.1	(2.6)	1	0.9 (0.6–1.3)	0.6 (0.4–0.8)	0.4 (0.3–0.6)	0.2 (0.2–0.4)	58.34[f]	0.7 (0.6–0.8)
β-criptoxanthin (mg)	0.2	(0.3)	1	0.7 (0.5–1.0)	0.7 (0.5–1.0)	0.4 (0.3–0.6)	0.4 (0.2–0.5)	32.29[f]	0.8 (0.7–0.8)
Lutein/zeaxanthin (mg)	4.3	(2.5)	1	0.7 (0.5–1.1)	0.6 (0.4–0.8)	0.5 (0.3–0.7)	0.4 (0.3–0.6)	27.33[f]	0.8 (0.7–0.9)
Lycopene (mg)	6.4	(3.7)	1	0.8 (0.6–1.2)	0.9 (0.6–1.4)	0.7 (0.5–1.1)	0.7 (0.5–1.1)	2.88	0.9 (0.9–1.0)
Vitamin D (mcg)	2.9	(1.3)	1	1.3 (0.9–1.8)	1.5 (1.0–2.2)	1.3 (0.9–1.9)	1.8 (1.2–2.6)	7.81[f]	1.1 (1.0–1.2)
Vitamin E (mg)	14.5	(6.6)	1	0.8 (0.6–1.2)	1.0 (0.7–1.5)	0.7 (0.5–1.1)	0.4 (0.3–0.6)	15.21[f]	0.8 (0.8–0.9)
Glutathione (mg)	62.7	(21.0)	1	0.7 (0.5–1.1)	0.8 (0.5–1.1)	0.6 (0.4–0.9)	0.5 (0.4–0.8)	8.85[f]	0.9 (0.8–1.0)
Reduced glutathione (mg)	46.4	(15.9)	1	0.8 (0.6–1.2)	0.8 (0.5–1.1)	0.8 (0.6–1.2)	0.6 (0.4–0.8)	7.03[f]	0.9 (0.8–1.0)

[a] Estimates from conditional logistic regression, conditioned on gender, age and centre, and adjusted for education, BMI, alcohol drinking, smoking habits and non-alcohol energy.
[b] Mean and SD among controls, per day.
[c] Reference category.
[d] OR for an increment of intake equal to 1 SD among controls.
[e] $p < 0.05$.
[f] $p < 0.01$.

(OR in highest *versus* lowest quintile of intake = 0.4), vitamin C (OR = 0.2), vitamin B6 (OR = 0.6), and folic acid (OR = 0.4). Significant inverse relations were also found between laryngeal cancer risk and several fat-soluble micronutrients such as α-carotene (OR = 0.3), β-carotene (OR = 0.2), β-cryptoxanthin (OR = 0.4), lutein/zeaxanthin (OR = 0.4), vitamin E (OR = 0.4). Glutathione (OR = 0.5) and reduced glutathione (OR = 0.6) were also inversely associated with cancer risk. The pattern of risk for vitamin B6, retinol, vitamin D, and vitamin E was not linear.

All micronutrients significantly related to laryngeal cancer were simultaneously introduced in the same model as continuous terms in order to allow for mutual confounding (not shown). Associations were weakened on account of co-linearity between several micronutrients. For instance, the Spearman correlation coefficient was about 0.8 for folic acid, thiamin, and vitamin B6, while carotene, vitamin C, vitamin E, and lutein/zeaxanthin showed values between 0.5 and 0.6. The inverse relationship between laryngeal cancer risk and carotene (OR continuous = 0.8; 95% CI: 0.7–0.9), vitamin C (OR continuous = 0.8; 95% CI: 0.7–0.9), potassium (OR continuous = 0.9; 95% CI: 0.8–1.0), and the direct one with vitamin D (OR continuous = 1.2; 95% CI: 1.1–1.3) and zinc (OR continuous = 1.1; 95% CI: 1.0–1.2) remained significant.

Table 3 gives the ORs for the combined effect of tobacco smoking and intake of vitamin C, carotene, vitamin E, and folate. Compared to never/ex-smokers in the highest tertile of consumption of each micronutrient, the ORs for smokers of $\geq$25 cigarettes/day in the lowest tertile of antioxidant consumption were 63.8 for vitamin C, 44.2, for carotene, 57.2 for vitamin E, and 32.3 for folate. A significant departure from effect multiplicativity was found for vitamin E ($p = 0.03$).

Table 3. OR[a] and corresponding 95% CI according to the combined effect of tobacco smoking and selected micronutrients intake among 527 laryngeal cancer cases and 1297 controls, Italy and Switzerland, 1992–2000

Tertiles of intake	Tobacco smoking		
	Never/ex-smokers	<25 cigarettes/day	≥25 cigarettes/day
Vitamin C			
3 (highest)	1[b]	8.3 (3.8–18.3)	19.6 (7.3–52.7)
2	1.0 (0.3–3.1)	10.3 (4.7–22.6)	29.4 (11.0–78.9)
1 (lowest)	1.8 (0.6–5.2)	22.9 (10.5–50.0)	63.8 (24.9–163.3)
Carotene			
3 (highest)	1[b]	6.7 (3.1–14.7)	9.6 (3.0–30.5)
2	1.1 (0.4–3.2)	13.5 (6.2–29.1)	59.7 (22.1–161.3)
1 (lowest)	1.5 (0.5–4.6)	20.6 (9.5–44.6)	44.2 (18.1–107.9)
Vitamin E			
3 (highest)	1[b]	14.3 (5.6–36.7)	19.2 (5.9–62.3)
2	1.7 (0.5–5.7)	20.2 (7.9–51.6)	130.0 (40.8–414.2)
1 (lowest)	3.2 (1.0–10.2)	24.0 (9.4–61.2)	57.2 (20.1–162.2)
Folic acid			
3 (highest)	1[b]	7.2 (3.4–15.2)	24.8 (9.6–63.9)
2	0.8 (0.3–2.3)	10.3 (4.8–21.7)	26.8 (10.4–69.2)
1 (lowest)	1.0 (0.3–3.1)	13.3 (6.3–28.0)	32.3 (13.0–80.7)

[a] Estimates from conditional logistic regression, conditioned on gender, age, and centre, and adjusted for education, BMI, alcohol drinking and non-alcohol energy.
[b] Reference category.

Table 4. OR[a] and corresponding 95% CI according to the combined effect of alcohol drinking and selected micronutrients intake among 527 laryngeal cancer cases and 1297 controls, Italy and Switzerland, 1992–2000

Tertiles of intake	Alcohol drinking (drinks/day)		
	<4	4–<8	≥8
Vitamin C			
3 (highest)	1[b]	3.6 (2.0–6.2)	9.2 (4.8–17.5)
2	1.7 (1.0–2.7)	4.0 (2.4–6.8)	10.9 (6.1–19.4)
1 (lowest)	4.7 (2.8–7.6)	8.9 (5.5–14.6)	22.1 (13.3–36.9)
Carotene			
3 (highest)	1[b]	3.2 (1.8–5.6)	5.0 (2.5–9.9)
2	2.8 (1.7–4.6)	6.0 (3.6–10.0)	13.4 (7.5–24.2)
1 (lowest)	3.2 (1.9–5.3)	8.7 (5.2–14.6)	28.8 (17.1–48.7)
Vitamin E			
3 (highest)	1[b]	3.2 (1.9–5.5)	6.6 (3.4–12.8)
2	2.0 (1.3–3.2)	4.3 (2.7–7.0)	14.4 (8.0–25.8)
1 (lowest)	2.4 (1.4–3.9)	6.0 (3.7–9.7)	14.8 (9.2–23.8)
Folic acid			
3 (highest)	1[b]	2.9 (1.8–4.8)	6.1 (3.2–11.6)
2	1.4 (0.9–2.2)	3.7 (2.3–5.9)	9.3 (5.4–16.0)
1 (lowest)	1.8 (1.1–3.0)	4.6 (2.8–7.3)	13.5 (8.4–21.7)

[a] Estimates from conditional logistic regression, conditioned on gender, age, and centre, and adjusted for education, BMI, smoking habit and non-alcohol energy.
[b] Reference category.

Table 4 shows the combined effect of the same four micronutrients with alcohol consumption (<4, 4–<8, ≥8 drinks/day). Compared to subjects who drank <4 drinks/day and reported a high intake of each micronutrient, those who drank ≥8 drinks/day and with a low micronutrient intake had ORs of 22.1 for vitamin C,

Table 5. OR[a] and corresponding 95% CI according to the combination of tobacco smoking and alcohol drinking and selected antioxidants (vitamin C, carotene, and vitamin E) intake among 527 laryngeal cancer cases and 1297 controls, Italy and Switzerland, 1992–2000

Tertiles of intake	Never/ex-smokers + light drinkers	Intermediate smokers and drinkers	≥25 cigarettes/day + heavy drinkers
Vitamin C			
3 (highest)	1[b]	10.5 (4.7—23.7)	49.4 (16.5–147.8)
2	1.0 (0.3–3.3)	13.9 (6.2–31.1)	60.0 (20.3–177.4)
1 (lowest)	1.8 (0.5–6.3)	38.1 (17.1–84.9)	126.8 (46.3–347.0)
Carotene			
3 (highest)	1[b]	7.1 (3.3–15.4)	18.9 (5.4–66.1)
2	0.9 (0.3–2.8)	16.2 (7.6–34.6)	87.9 (29.8–259.8)
1 (lowest)	0.9 (0.2–3.5)	29.4 (13.8–62.8)	90.4 (35.9–227.4)
Vitamin E			
3 (highest)	1[b]	21.2 (7.2–62.1)	30.0 (7.6–117.9)
2	1.5 (0.4–5.8)	35.5 (12.1–103.8)	274.0 (73.5–1021.2)
1 (lowest)	3.4 (1.0–12.1)	49.2 (16.9–143.1)	169.6 (52.7–545.7)
Folic acid			
3 (highest)	1[b]	11.0 (4.8–25.3)	55.6 (18.7–165.1)
2	0.8 (0.3–2.5)	16.2 (7.1–37.0)	62.7 (22.1–178.1)
1 (lowest)	0.7 (0.2–2.7)	25.3 (11.2–57.3)	80.7 (30.7–212.1)

[a] Estimates from conditional-logistic regression, conditioned on gender, age, and centre, and adjusted for education, BMI, and non-alcohol energy.

[b] Reference category.

28.8 for carotene, 14.8 for vitamin E, and 13.5 for folate. No significant departure from effect multiplicativity was found.

Table 5 gives the combination of vitamin C, carotene, vitamin E, and folic acid with tobacco and alcohol together. Compared to non- or ex-smokers, with low alcohol intake and in the highest tertile of micronutrient intake, the ORs for smokers of ≥25 cigarettes/day, with high alcohol intake and lowest micronutrient intake were 126.8 for vitamin C, 90.4 for carotene, 169.6 for vitamin E, and 80.7 for folic acid.

Discussion

The present study suggests that squamous-cell carcinoma of larynx is inversely related to consumption of several micronutrients, including various carotenoids, vitamin C, vitamin E, folic acid, thiamin, vitamin B6, and glutathione. A few of these associations may be a chance finding due to the performance of multiple tests. However, inverse associations were previously reported for carotene [14–16], vitamin C [13, 14, 27] and vitamin E [13] as well as for fruits and vegetables [8, 9, 11, 12, 28].

The inverse relation with vitamin C is of specific interest. Vitamin C inhibits endogenous formation of carcinogenic n-nitroso compounds and exogenous tobacco-specific nitrosamines and aromatic amines [29]. The increased oxidative stress in smokers but also in heavy drinkers may substantially increase the requirements of vitamin C [30], and this may explain the substantially elevated risks observed in this study in heavy alcohol and tobacco consumers reporting low vitamin C intake. Alcohol intake, tobacco, and several (antioxidant) micronutrients appear to have a synergistic effect on laryngeal cancer risk.

Among various carotenoids considered, an inverse association emerged with α-carotene, β-carotene, β-cryptoxanthyn and lutein/zeaxanthin, confirming previous results on carotenoids from United States [15, 16] and from a study based on pre-diagnostic serum [31]. Disentangling the separate effect of various carotenoids is, however, not possible from either a statistical (*i.e.* co-linearity), or a biological viewpoint.

Although vitamin E derived in part from fruits and vegetables, its main dietary source in our study was olive oil used in vegetable seasoning [32], which has been shown to exert a favourable effect also against other cancer sites, including breast, colon-rectum, and mouth [5].

The issue of folate is interesting because of its possible interaction with alcohol as described for colorectal [33] and breast cancer [34]. Folate is important for DNA methylation and repair, and abnormalities in DNA methylation may contribute to loss of normal control of protooncogene expression. Folate and methionine are involved in production of S-adenosylmethionine, the primary methyl donor in the body. The metabolism of ethanol leads to the generation of acetaldehyde and free radicals that may increase folate requirements. Low folate ingestion has been associated with a moderately increased breast-cancer risk in women with high alcohol intake in Italy [35]. To our knowledge, this is the first study reporting a similar pattern of risk for laryngeal cancer. The combined effects of alcohol and folate were more than additive, and consistent with a multiplicative model.

An increased risk of borderline statistical significance associated with the highest quintile of retinol intake, as opposite to carotene, is noteworthy. The likely effect of carotenoids may thus be due to their antioxidants effect, rather than being pre-cursors of vitamin A. Retinol and carotene may also be considered markers of two more general eating patterns, one, indicated by retinol intake, rich in fat of animal origin, the other rich in plant foods, from which β-carotene and carotenoids derive. Interestingly, the only two micronutrients directly associated to laryngeal cancer risk (zinc and vitamin D) derive, as retinol, from foods of animal origin.

In contrast, strong inverse relations with laryngeal cancer risk tend to be shared by most micronutrients of plant origin. It is therefore difficult to understand which micronutrient(s) are protective against laryngeal cancer [4, 6, 13]. When we simultaneously accounted for the effect of several micronutrients, an inverse relation persisted for carotene and vitamin C. An additional difficulty in the interpretation of results is given by the co-linearity of micronutrients derived from fruits and vegetables [36].

As in all case–control studies, recall and selection biases are of potential concern [25]. However, awareness about any particular dietary hypothesis in laryngeal cancer aetiology was still limited in the Italian and Swiss public, as the issue had not received media attention. It is also possible that dietary habits of hospital controls may differ from those of the general population, but great attention was paid in excluding all diagnoses that might have been associated with or have determined special dietary habits among controls. Moreover, the comparability of dietary history between cases and controls should have been improved by interviewing all subjects in the same hospital setting. Adjustment for total energy intake should have reduced potential bias due to differential over- or under-reporting of food intakes.

The major strength of this study is related to its large dataset, which allowed to obtain reasonably precise risk estimates and to identify significant associations for several micronutrients. Other strengths are due to the consistency of the present findings when major categories of controls were separately used, to the nearly complete participation of identified cases and controls, to the assessment of a broad range of nutrients, and to the reliance on a validated FFQ [23, 24]. Finally, supplementation or food fortification with vitamins or minerals is rare in the Italian and Swiss populations [37], and, therefore, unlikely to distort or attenuate the association with food-derived micronutrients.

In conclusion, the findings of the present study support the hypothesis that, independently of smoking and drinking, various micronutrients such as vitamin C, carotenoids, vitamin E, and folate are inversely related to laryngeal cancer. Whether this observation reflects a specific effect of some of these micronutrients, or a general favourable influence pattern of a diet rich in vegetable oils, fruits, and vegetables and poor in foods of animal origin remains, however, outside the scope of observational epidemiological studies.

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References

1. Franceschi S, Talamini R, Barra S, *et al.* (1990) Smoking and drinking in relation to cancers of the oral cavity, pharynx, larynx, and esophagus in northern Italy. *Cancer Res* **50**: 6502–6507.
2. Tavani A, Negri E, Franceschi S, Barbone F, La Vecchia C (1994) Attributable risk for laryngeal cancer in Northern Italy. *Cancer Epidemiol Biomarkers Prev* **3**: 121–125.
3. Doll R, Peto J (1985) Effects on health of exposure to asbestos. London: Her Majesty's stationery Office.
4. Block G, Patterson B, Subar A (1992) Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. *Nutr Cancer* **18**: 1–29.
5. World Cancer Research Fund in association with the American Institute for Cancer Research (1997) *Food, Nutrition and the Prevention of Cancer: A Global Perspective*. Washington DC: World Cancer Research Fund.
6. Riboli E, Kaaks R, Estève J (1996) Nutrition and laryngeal cancer. *Cancer Causes Control* **7**: 147–156.
7. Kjærheim K, Gaard M, Andersen A (1998) The role of alcohol, tobacco, and dietary factors in upper aerogastric tract cancers: a prospective study of 10,900 Norwegian men. *Cancer Causes Control* **9**: 99–108.
8. De Stefani E, Correa P, Oreggia F, *et al.* (1987) Risk factors for laryngeal cancer. *Cancer* **60**: 3087–3091.
9. Notani PN, Jayant K (1987) Role of diet in upper aerodigestive tract cancers. *Nutr Cancer* **10**: 103–113.
10. Zatonski W, Becher H, Lissowska J, Wahrendorf J (1991) Tobacco, alcohol, and diet in the etiology of laryngeal cancer:

a population-based case–control study. *Cancer Causes Control* **2**: 3–10.

11. La Vecchia C, Negri E, D'Avanzo B, Franceschi S, Decarli A, Boyle P (1990) Dietary indicators of laryngeal cancer risk. *Cancer Res* **50**: 4497–4500.
12. Zheng W, Blot WJ, Shu X-O, *et al*. (1992) Diet and other risk factors for laryngeal cancer in Shanghai, China. *Am J Epidemiol* **136**: 178–191.
13. Estève J, Riboli E, Péquignot G, *et al*. (1996) Diet and cancers of the larynx and hypopharynx: the IARC multi-center study in southwestern Europe. *Cancer Causes Control* **7**: 240–252.
14. Graham S, Mettlin C, Marshall J, Priore R, Rzepka T, Shedd D (1981) Dietary factors in the epidemiology of cancer of the larynx. *Am J Epidemiol* **113**: 675–680.
15. Mackerras D, Buffler PA, Randall E, Nichanan MZ, Pickle LW, Mason TJ (1988) Carotene intake and the risk of laryngeal cancer in coastal Texas. *Am J Epidemiol* **128**: 980–988.
16. Freudenheim JL, Graham S, Byers TE, *et al*. (1992) Diet, smoking, and alcohol in cancer of the larynx: a case–control study. *Nutr Cancer* **17**: 33–45.
17. Austin DF, Reynolds P (1996) In: Schottenfeld, Fraumeni Jr. eds. *Cancer Epidemiology and Prevention, 2nd Edn*. Oxford: Oxford University Press.
18. Rogers MAM, Thomas DB, Davis S, Vaughan TL, Nevissi AE (1993) A case–control study of element levels and cancer of the upper aerodigestive tract. *Cancer Epidemiol Biomarkers Prev* **2**: 305–312.
19. Talamini R, Bosetti C, La Vecchia C, *et al*. (1992) Combined effect of tobacco and alcohol on laryngeal cancer risk: a case–control study. *Cancer Causes Control* **13**: 957–964.
20. Bosetti C, La Vecchia C, Talamini R, *et al*. (2002) Food groups and laryngeal cancer risk: a case control study from Italy and Switzerland. *Int J Cancer* **100**: 355–360.
21. Salvini S, Parpinel M, Gnagnarella P, *et al*. (1998) Banca dati di composizione degli alimenti per studi epidemiologici in Italia. Milano: Istituto Europeo di Oncologia.
22. Jones DP, Coates RJ, Flagg E, *et al*. (1992) Glutathione in foods listed in the National Cancer Institute's Health Habits and History Food Frequency Questionnaire. *Nutr Cancer* **17**: 57–75.
23. Franceschi S, Barbone F, Negri E, *et al*. (1995) Reproducibility of an Italian food frequency questionnaire for cancer studies. Results for specific nutrients. *Epidemiol* **5**: 69–75.
24. Decarli A, Franceschi S, Ferraroni M, *et al*. (1996) Validation of a food frequency questionnaire to assess dietary intakes in cancer studies in Italy. Results for specific nutrients. *Ann Epidemiol* **6**: 110–118.
25. Breslow NE, Day NE (1980) *Statistical Methods in Cancer Research*. Vol. 1. *The Analysis of Case–Control Studies*. IARC Scientific Publications No. 32. Lyon: International Agency for Research on Cancer.
26. Willett WC, Stampfer MJ (1986) Total energy intake: implications for epidemiologic analysis. *Am J Epidemiol* **124**: 17–27.
27. De Stefani E, Ronco A, Mendilaharsu M, Deneo-Pellegrini H (1999) Diet and risk of cancer of the upper aerodigestive tract – II. Nutrients. *Oral Oncol* **35**: 22–26.
28. De Stefani E, Boffetta P, Oreggia F, *et al*. (2000) Plant foods and risk of laryngeal cancer: a case–control study in Uruguay. *Int J Cancer* **87**: 129–132.
29. IARC (1986) *IARC Monographs on the Evaluation of Carcinogenic Risks to Human. Tobacco Smoking*. Vol. 38. Lyon: International Agency for Research on Cancer.
30. Rogers MAM, Vaughan TL, Davis S, Thomas DB (1995) Consumption of nitrate, nitrite and nitrosodimethylamine and the risk of upper aerodigestive tract cancer. *Cancer Epidemiol Biomarkers Prev* **4**: 29–36.
31. Nomura AMY, Ziegler RG, Stemmermann GN, Chyou PH, Craft NE (1997) Serum micronutrients and upper aerodigestive tract cancer. *Cancer Epidemiol Biomarker Prev* **6**: 407–412.
32. Favero A, Salvini S, Russo A, *et al*. (1997) Sources of macro- and micronutrients in Italian women: results from a food-frequency questionnaire for cancer studies. *Eur J Cancer Prev* **6**: 277–287.
33. Giovannucci E, Rimm EB, Acherio A, Stampfer MJ, Colditz GA, Willett WC (1995) Alcohol, low-methionine–low-folate diets, and risk of colon cancer in men. *J Natl Cancer Inst* **7**: 265–273.
34. Rohan TE, Jain MG, Howe, GR, Miller AB (2000) Dietary folate consumption and breast cancer risk. *J Natl Cancer Inst* **92**: 266–269.
35. Negri E, La Vecchia C, Franceschi S (2000) Dietary folate consumption and breast cancer risk. *J Natl Cancer Inst* **92**: 1270–1271.
36. Negri E, La Vecchia C, Franceschi S (2002) Relation between vegetable, fruit and micronutrient intake. Implications for odds ratios in a case–control study. *Eur J Cancer* **56**: 166–170.
37. La Vecchia C, Tavani A (1998) Fruit and vegetables, and human cancer. *Eur J Cancer Prev* **7**: 3–8.