REPORTS

Maize and Risk of Cancers of the Oral Cavity, Pharynx, and Esophagus in Northeastern Italy

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The relationship between maize consumption and risk of cancer of the upper digestive tract was investigated in 107 patients with oral cancer, 107 with pharyngeal cancer, 68 with esophageal cancer, and 505 hospital controls who permanently resided in Pordenone Province in the northeastern part of Italy. The analysis was restricted to males. The population of this province has a high incidence of these neoplasms and shows particularly elevated levels of alcohol and tobacco use, in addition to high maize consumption. Highly significant associations with frequent intake of maize emerged for oral cancer, pharyngeal cancer, and esophageal cancer (odds ratios = 3.3, 3.2, and 2.8, respectively). The risk elevation could not be explained in terms of differences in education, occupation, tobacco use, or consumption of fresh fruits and vegetables. The unfavorable effect of maize on risk of cancer of the upper digestive tract, however, was evident only in those individuals who reported heavy drinking (i.e., ≥42 alcoholic drinks/wk). The present findings are likely to be related to the fact that maize can cause deficiencies of various micronutrients (chiefly, niacin and riboflavin) and agree with previous observations from Africa, the People's Republic of China, the United States, and Italy. [J Natl Cancer Inst 82:1407–1411, 1990]

Alcohol and tobacco are two of the best known agents that have been implicated in the etiology of cancers of the upper digestive tract (UDT) (i.e., oral cavity, pharynx, and esophagus) (*1–5*). However, there are areas outside Europe and North America with a very high incidence of UDT cancers, particularly esophageal cancer, in which alcohol and tobacco play a negligible role in the causation of the cancers; therefore, interest has focused on the manner in which poor diets may increase the risk of these cancers (*5–17*).

Some studies (*16,18*) have considered various macronutrient and micronutrient deficiencies or imbalances in the etiology of esophageal cancer. Other studies conducted in various parts of the world (*7,9,11,12,19*), including the northeastern part of Italy (*20*), have suggested that the geographical and temporal distributions, at least of esophageal cancer, could reflect the use of maize as the major staple food as well as the use of maize for brewing beer.

The present case–control study was conducted in Pordenone Province in the Friuli-Venezia Giulia region of the northeastern part of Italy. This area has the highest mortality rates for UDT cancers in Italy (standardized mortality ratios of 266 and 229 for oral and pharyngeal cancers and esophageal cancer, respectively, in males, with the entire male population of Italy having standardized mortality ratios of 100) (*21*). In addition, this area has UDT cancer rates that are among the most elevated in Europe (standardized mortality rates being 23.8 per 100,000 males for cancers of the oral cavity and pharynx and 16.6 per 100,000 males for esophageal cancer) (*21*).

Over 75% of the UDT cancers in northern Italy seem to be attributable to alcohol and tobacco use (*5*). This region of Italy offers also one of the few examples of a highly developed area where maize (i.e., sweet corn or Indian corn) has traditionally been and, to a certain extent, still is the cereal most widely grown and eaten in the form of polenta (*22,23*). In the framework of a multihypothesis case–control surveillance study, we were thus able to explore in this region the influence of a high intake of maize on the risk of developing UDT cancers, allowing for the presence of other risk factors (chiefly, alcohol use).

Patients and Methods

Since June 1985, we have been conducting a case–control study on patients with UDT cancers in the province of Pordenone. Trained interviewers identified and questioned patients who were (*a*) admitted for histologically confirmed UDT cancers (and a wide variety of other conditions) to the Aviano Cancer Center and all other local hospitals, (*b*) below age 75 years, and (*c*) permanent residents in Pordenone Province. Approximately 3% of the patients and 4% of the controls refused to participate. The area studied is not covered by a cancer registry, and it was not possible to estimate the proportion of patients with UDT cancers interviewed. However, the study hospitals comprised

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all the diagnostic and therapeutic facilities available in the province; therefore, the majority of cancer patients would have been referred to one of these hospitals. Furthermore, 90% of the patients were interviewed within 2 months from diagnosis of cancer, thus minimizing losses caused by patient deaths.

The present analysis was restricted to males, who represented 89% of the interviewed individuals. The analysis was based on 107 patients with cancer of the tongue and oral cavity [International Classification of Diseases, 9th revision (ICD) = 140, 141, 143, 144, and 145; median age = 60 yr]; 107 patients with cancer of the pharynx, the junction between the hypopharynx and the larynx included (ICD = 146, 148, and 161.1; median age = 59 yr); 68 patients with cancer of the esophagus (ICD = 150; median age = 62 yr); and 505 hospital controls (median age = 58 yr) interviewed before November 1989 (table 1). Of these controls, 25% were admitted for traumatic conditions, 22% for orthopedic conditions, 19% for infections or acute surgical conditions, 14% for skin diseases, and 20% for other illnesses (e.g., disorders of the ear, nose, or teeth). Excluded from the comparison group were patients whose diagnosis causing the current admission was of a malignant disorder, of a disease related to alcohol and/or tobacco consumption, or of a condition that might have resulted in diet modifications.

The questionnaire concerned sociodemographic indicators and personal habits such as smoking and frequency of consumption per week of alcoholic beverages (i.e., wine, beer, and hard liquors, separately) and 40 selected indicator foods, including major sources of proteins, fats, fibers, methylxanthines, and vitamins A and C in the Italian diet. Questions on dietary and drinking habits referred to the year before the onset of the symptoms of the disease that led to hospital admission.

The case–control studies were analyzed by standard statistical methods (*24–27*).

Table 1. Distribution of 282 patients with UDT cancers and 505 controls according to sociodemographic factors and smoking and drinking habits: Pordenone, Italy, 1985–1989

	Patients by site of cancer							
	Oral cavity		Pharynx		Esophagus		Controls	
	No.*	%	No.†	%	No.‡	%	No.§	%
Age (yr)								
≤54	36	33.6	37	34.6	17	25.0	186	36.8
55-64	38	35.5	44	41.1	28	41.2	192	38.0
≥65	33	30.8	26	24.3	23	33.8	127	25.1
Education (yr)‖								
≤4	27	25.5	23	21.5	19	27.9	99	19.6
5-6	57	53.8	58	54.2	40	58.8	249	49.3
≥7	22	20.8	26	24.3	9	13.2	157	31.1
Occupation								
Clerical-professional worker	24	22.4	25	23.4	13	19.1	143	28.3
Industrial worker	50	46.7	61	57.0	40	58.8	287	56.8
Farmer	33	30.8	21	19.6	15	22.1	75	14.9
Smoking habits‖								
Never smokers	4	3.8	1	0.9	3	4.4	96	19.0
Exsmokers	5	4.8	10	9.3	13	19.1	112	22.2
Pipe-cigar smokers	3	2.9	0	0.0	1	1.5	2	0.4
Cigarette smokers								
<15/day	22	21.0	22	20.6	13	19.1	104	20.6
15-24/day	44	41.9	48	44.9	26	38.2	134	26.6
≥25/day	27	25.7	26	24.3	12	17.6	56	11.1
Alcoholic drinks/wk‖·¶								
≤41	16	15.0	17	16.0	10	14.7	208	41.3
42-76	50	46.7	38	35.8	28	41.2	183	36.3
≥77	41	38.3	51	48.1	30	44.1	113	22.4

*Total = 107.
†Total = 107.
‡Total = 68.
§Total = 505.
‖Sum does not add up correctly because of missing values.
¶One drink corresponded to 150 mL of wine, 330 mL of beer, and 30 mL of hard liquor.

Results

Table 1 gives the distribution of patients with UDT cancer and of controls according to sociodemographic factors and smoking and drinking habits. Table 2 shows this distribution according to consumption of fresh fruits, vegetables, and maize. Highly significant associations with frequent intake of maize emerged for all three tumor sites; age-adjusted odds ratios were (for highest vs. lowest frequency of consumption) 3.3 [95% confidence interval (CI) = 2.0–5.4], 3.2 (95% CI = 2.0–5.3), and 2.8 (95% CI = 1.5–5.1) for cancers of the oral cavity, pharynx, and esophagus, respectively (table 3). Maize was further included in a multiple logistic regression equation, including terms for age, education, occupation, tobacco use, and frequency of consumption of fresh fruits and vegetables. Although being a farmer turned out to be an important confounder of the association of maize with the development of UDT cancers, all the trends in risk remained significant (table 3).

The odds ratios were heterogeneous for maize consumption across levels of alcohol intake, which led to the investigation of the specific effects of maize intake on consumers of alcohol (table 4). Consistently for each tumor (not shown) and overall, we found an elevated odds ratio for highest versus lowest frequency of maize intake in heavy drinkers (i.e., ≥42 drinks/wk); this finding contrasted with the apparent lack of effect in light drinkers and abstainers from alcohol (table 4). Conversely, the pattern of risk for maize was similar across various strata of sociodemographic factors, tobacco use, and intake of fresh fruits and vegetables, thus suggesting no appreciable modification of effect (data not shown).

An attempt was made to explore maize intake 10 years before diagnosis or (among controls) before interview. Only 4% of the patients reported a lower consumption of maize in the past compared with the present, whereas 30% of the patients with oral and pharyngeal cancers, 50% of the patients with esophageal cancer, and 33% of the control subjects reported substantially higher intake in the past.

Discussion

Our results from the northeastern part of Italy not only are consistent with previous

Table 2. Distribution of 282 patients with UDT cancers and 505 controls according to consumption of various indicator foods: Pordenone, Italy, 1985-1989

Food	Patients by site of cancer						Controls	
	Oral cavity		Pharynx		Esophagus			
	No.*	%	No.†	%	No.‡	%	No.§	%
Fresh fruits (servings/wk)								
≤4	40	37.4	43	40.2	30	44.1	153	30.3
5-12	30	28.0	25	23.4	18	26.5	144	28.5
≥13	37	34.6	39	36.4	20	29.4	208	41.2
Vegetables (servings/wk)								
<7	24	22.4	22	20.6	18	26.5	71	14.1
7-13	30	28.0	21	19.6	22	32.4	134	26.5
≥14	53	49.5	64	59.8	28	41.2	300	59.4
Maize‖ (servings/wk)								
Never-occasionally	54	50.5	53	49.5	37	54.4	351	69.6
1-2	20	18.7	21	19.6	12	17.6	84	16.7
≥3	33	30.8	33	30.8	19	27.9	69	13.7

*Total = 107.
†Total = 107.
‡Total = 68.
§Total = 505.
‖Sum does not add up correctly because of missing values.

Table 3. Odds ratios for UDT cancers associated with frequency of maize consumption: Pordenone, Italy, 1985-1989

Cancer site	Method of statistical analysis*	Odds ratio: frequency of consumption of maize (servings/wk)†			χ^2_1 trend
		Never-occasionally‡	1-2	≥3	
Oral cavity	MH	1	1.6 (0.9-2.8)	3.3 (2.0-5.4)	21.09§
	MLR	1	1.5 (0.8-2.8)	2.5 (1.4-4.4)	9.21§
Pharynx	MH	1	1.7 (1.0-2.9)	3.2 (2.0-5.3)	21.13§
	MLR	1	1.5 (0.8-2.8)	2.5 (1.4-4.4)	10.34§
Esophagus	MH	1	1.4 (0.7-2.8)	2.8 (1.5-5.1)	10.11§
	MLR	1	1.2 (0.6-2.6)	2.1 (1.1-4.0)	4.42‖
All sites	MH	1	1.6 (1.1-2.4)	3.2 (2.2-4.6)	36.82§
	MLR	1	1.4 (0.9-2.2)	2.3 (1.5-3.5)	15.60§

*MH = Mantel-Haenszel estimates adjusted for age in decades; MLR = derived from multiple logistic regression equations, including terms for age, education, occupation, tobacco use, and frequency of consumption of fresh fruits, vegetables, and maize.
†Nos. in parentheses in columns = 95% CIs.
‡Reference category.
§$P < .01$.
‖$P = .04$.

Table 4. Odds ratios* for all UDT cancers associated with maize and alcohol consumption: Pordenone, Italy, 1985-1989

Alcoholic drinks/wk	Odds ratio: frequency of consumption of maize (servings/wk)			χ^2_1 trend
	Never-occasionally†	1-2	≥3	
≤41	1	1.02	0.72	0.26
42-76	1	1.29	2.52	5.95‡
≥77	1	2.04	2.90	10.00§

*Derived from multiple logistic regression equation, including terms for age, tobacco use, and frequency of consumption of maize.
†Reference category.
‡$P = .02$.
§$P < .01$.

findings, but also offer an unprecedented opportunity to investigate in depth the joint effect of high exposure to dietary maize and use of alcohol and tobacco. Also new was the finding of the consistency in the effect of high maize consumption on the development of cancers of the oral cavity, pharynx, and esophagus, at least in the presence of a high prevalence of smoking and alcohol consumption.

Several case–control studies have examined the effect of diet on the etiology of UDT cancers, but the information on the role of maize is scanty. Most investigations, including the present one, have found that diets high in raw vegetables and fresh fruits confer some protection against oral and pharyngeal cancers (*28–33*) as well as against esophageal cancer and precursor lesions (*31,34–40*).

Where it is common, maize emerges consistently as an important risk factor, at least for esophageal cancer. Van Rensburg et al. (*41*) found a more than fivefold elevated risk of developing esophageal cancer among Zulu men who daily purchased maize meals. Segal et al. (*42*) observed in South Africa a 25-fold enhanced risk of developing esophageal cancer among heavy drinkers of traditional beer made from maize. In Linxian, People's Republic of China, Li et al. (*40*) detected a rather strong trend of increasing risk of esophageal cancer with increasing consumption of maize and wheat, but not millet and sweet potatoes. Wahrendorf et al. (*39*) found that in Henan, People's Republic of China, frequent consumption of maize enhanced cancer risk, even after these investigators allowed for several other risk factors. Finally, Rossi et al. (*20*) reported a 4.5-fold increased risk of developing esophageal cancer among individuals who ate two or more slices of polenta per day in an investigation conducted in the Veneto region of Italy. This region borders Pordenone Province and is economically and historically similar to it.

By far, however, the strongest evidence for the role of maize comes from surveys in Africa. Maize is an introduced crop in Africa, and its spread as a staple food and an ingredient in traditional beer seems to coincide (after one allows for a latent period) with the rise in the frequency of esophageal cancer (*8,10,12,13*). Maize is easier to grow and more resistant to fungus and attacks by birds than other grains. However, particularly if it is refined,

maize is less nutritious than other grains and can cause deficiencies of several micronutrients (chiefly, riboflavin and niacin) (*43*).

Riboflavin deficiency has been suggested as one of the deficiencies that cause Plummer-Vinson syndrome, a long-recognized precancerous lesion of the UDT (*6*). Maize not only is low in niacin and tryptophan (its precursor) but also contains large quantities of leucine, which is capable of interfering with oxidation–reduction reactions. Like the Plummer-Vinson syndrome, pellagra, a life-threatening disease caused by niacin deficiency, can result in widespread inflammation of the mucous surfaces, dysphagia, and esophageal lesions (*44*).

The elevation in risk resulting from frequent consumption of maize in the present study could not be explained in terms of the confounding effect of age, education, occupation, tobacco use, or consumption of fresh fruits and vegetables. However, the difference in the effect of maize according to level of alcohol consumption is highly significant. In other words, the unfavorable impact of high intake of maize found in the present study seems to be relevant only in heavy drinkers. Long-term, high alcohol intake is seen rather frequently in the male population of Pordenone Province. It is often accompanied by deficiencies of niacin and riboflavin in the diet and, perhaps most importantly, by increases in micronutrient requirements caused by high levels of ethanol oxidation (*45*). This observation may explain the extraordinarily increased risk of esophageal cancer observed in Africa (*8*), in various parts of the United States (*29,34,46*), and in northeastern Italy (*20*) among individuals on diets low in nutrients who consumed large quantities of maize and alcoholic beverages.

Alcohol may aggravate the nutritional deficiency induced by maize-rich diets. Alternatively, mucosal lesions caused by niacin and/or riboflavin deficiency may enhance the topical action of alcohol, possibly favoring the penetration of carcinogenic compounds (*47*). While we can only speculate on such mechanisms, the present data suggest that maize retains a role in the etiology of UDT cancers even in developed areas of the world, where maize is still a major staple food and/or where the occurrence of alcohol-related diseases is high.

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Clinical Pharmacokinetics of High-Dose Leucovorin Calcium After Intravenous and Oral Administration

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The clinical formulation of leucovorin calcium (leucovorin, LV) is a mixture of stereoisomers [(6R,S)-5-formyltetrahydrofolate], which have been shown to differ significantly in plasma clearance and route of elimination after intravenous administration; the (6S) isomer is rapidly converted to 5-CH_3 tetrahydrofolate (5-CH_3 THF), and the (6R) isomer is slowly eliminated by renal excretion. The relative importance of (6S) LV and 5-CH_3 THF in expanding reduced folate pools in tumor cells is unknown, but it is known that high concentrations of (6R) LV can support growth of folate-depleted cells and thus have the potential to interfere with the biological activity of the (6S) isomer. To examine the pharmacokinetics of the LV isomers and metabolites, we administered 1,000 mg of LV to five normal subjects as a 2-hour intravenous infusion and in divided oral 100-mg doses given over 24 hours. Plasma and urine samples were analyzed by reverse phase followed by chiral high-performance liquid chromatography. Following intravenous administration, peak plasma concentrations of (6R) LV, (6S) LV, and 5-CH_3 THF were 148 ± 32, 59.1 ± 22, and 17.8 ± 17 μM, respectively. During oral administration of LV, virtually no (6S) LV appeared in the plasma. Steady-state plasma concentrations of (6R) LV and 5-CH_3 THF were approximately 1.5 ± 0.23 and 2.8 ± 0.41 μM, respectively. Intravenous administration of LV resulted in an area under the curve (AUC) for (6R) LV that was more than four times that of the biologically active (6S) folates, whereas oral administration produced an AUC for (6S) reduced folates [(6S) LV and 5-CH_3 THF] that was approximately twice that of (6R) LV. After administration of high doses of LV intravenously, conversion of (6S) LV to 5-CH_3 THF was saturable, as indicated by the prolonged (6S) LV half-life of 58 minutes and the slow (6S) LV clearance of 119.2 ± 38 mL/min, compared with previously reported data for administration of low doses. This study illustrates that intravenous administration of LV produces equivalent AUCs of (6S) LV and 5-CH_3 THF but a substantially higher AUC for (6R) LV. Oral administration over 24 hours results in an AUC of 5-CH_3 THF equivalent to that obtained after intravenous dosing in the presence of only small amounts of (6R) LV. The optimal route of LV administration will ultimately be determined by ongoing studies of the cellular pharmacology of LV that will determine if high concentrations of (6R) LV interfere with the biological activity of the (6S) reduced folates. [J Natl Cancer Inst 82:1411–1415, 1990]

Leucovorin calcium ([6R,S]-5-formyltetrahydrofolate, LV) is a reduced folate that has been used for many years to prevent toxic effects after intermediate- and high-dose methotrexate therapy. Recent experimental and clinical data indicate that leucovorin also plays an important role in modulating the cytotoxicity of the pyrimidine antimetabolite fluorouracil (5-FU). Exposure of tumor cells to LV in vitro results in expanded intracellular pools of 5-10·CH_2-tetrahydrofolate and stabilization of the ternary complex formed by this folate cofactor, thymidylate synthase, and 5-fluorodeoxyuridylate. The results are sustained inhibition of thymidylate synthesis and enhanced cytotoxicity (*1–6*). A number of clinical trials comparing the combination of 5-FU and LV to 5-FU alone have clearly demonstrated improved response rates and survival for patients with advanced colorectal cancer receiving the two-drug combination (*7–10*).

Despite the clinical success of treatment with 5-FU plus LV, the optimal dose, route, and schedule of LV administration have not yet been clearly defined. Preclinical studies suggest that (6S) LV concentrations in the range of 1–10 μM are necessary to optimally enhance the cytotoxicity of 5-FU in vitro (*2,4,5*). However, these studies have generally not considered the unique pharmacologic characteristics of LV that become apparent in vivo due to drug metabolism and the existence of LV stereoisomers.

The clinical formulation of LV is a mixture of stereoisomers around the C6 carbon of the pteridine ring, with most of the biological activity confined to the (6S) isomer. The individual isomers differ significantly in their plasma clearance and route of elimination after intravenous administration, with the (6S) isomer being rapidly converted to 5-CH_3-tetrahydrofolate (5-CH_3 THF) and the (6R) isomer being slowly eliminated by renal excretion (*11–13*). The relative importance of (6S) LV and 5-CH_3 THF in expanding reduced folate pools in human tumors is unknown, and the potential for high concentrations of (6R) LV to interfere with the biological activity of the (6S) isomer must be considered, in view of the fact that (6R) LV can gain entry into cells and, in high concen-

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