A Case-Control Study of Element Levels and Cancer of the Upper Aerodigestive Tract[1]

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Abstract

A population-based case-control study was conducted in western Washington state to investigate possible dietary risk factors for laryngeal, esophageal, and oral cancers. Using results from a food frequency questionnaire, past dietary intakes of iron, zinc, and calcium were estimated for 661 cases and 466 controls. Clippings were also taken from the nails of both halluces to determine concentrations of iron, zinc, calcium, chromium, and cobalt in 507 of the cases and 434 of the controls. After adjustment for smoking, alcohol, and dietary β-carotene and vitamin C intake, individuals who reported dietary intakes of iron and zinc in the upper quartile were less likely to develop cancers of the larynx and esophagus than were individuals with intakes in the lowest quartile [odds ratios (OR), 0.5 for iron and 0.1 for zinc]. However, there were no significant differences in zinc concentrations in nail tissue between subjects with these types of cancer and controls. Esophageal cancer cases had higher nail concentrations of iron and calcium than did controls (OR, 2.9 for high *versus* low quartiles of iron; OR, 2.6 for high *versus* low quartiles of calcium). Individuals who developed esophageal or oral cancer were more likely to have elevated cobalt concentrations in their nail tissue than were individuals without cancer (OR, 9.0 and 1.9 respectively, for high *versus* low quartiles). The results of this study suggest that there may be differences in mineral intake or metabolism between individuals who develop some carcinomas of the upper aerodigestive tract and those who do not.

Introduction

Although there is some evidence to indicate that carotenoids and ascorbic acid may be protective against cancers of the respiratory and digestive tracts (1, 2), little is known regarding prediagnostic mineral levels and the development of upper aerodigestive tract neoplasms. Extremely low levels of iron, as seen in Plummer-Vinson syndrome, have been implicated in the etiology of cancer of upper alimentary tissues, particularly hypopharyngeal cancer (3). In addition, low hematocrit levels have been associated with the risk of oral-pharyngeal cancer (4), and low ferritin levels were reported to increase the risk of gastric cancer, possibly due to achlorhydria (5, 6). In a prospective study using data from the NHANES,[3] individuals with esophageal cancer had very high transferrin saturation and low hemoglobin levels compared to subjects who did not develop cancer (7). However, in a prospective study of individuals in the Kaiser Permanente Medical Care Program, an increased lung cancer risk was observed in women with high iron stores, but no similar association was observed in men, or for other cancer types in either sex (8). Therefore, it is not clear whether iron is directly or inversely related to the development of upper aerodigestive tract or other cancers, nor is it clear whether associations with particular indices of iron status indicate relationships with other underlying disorders of metabolism or with differences in intake.

Investigators in China have observed high rates of esophageal cancer in populations with low zinc levels (9). However, a clinical trial showed no effect of retinol, riboflavin, and zinc supplementation on the prevalence of precancerous esophageal lesions (10). Although there was a significant reduction in the prevalence of micronuclei in esophageal cells, they found no significant difference in the prevalence of micronuclei in buccal mucosa cells after supplementation (11).

The International Agency for Research on Cancer recognizes chromium as a human carcinogen (12). It is carcinogenic to lung tissue after inhalation in occupationally exposed workers (13). There have also been occupational studies of cobalt exposure and cancer risk, but the results of these studies, in the aggregate, are contradictory (14). There has been little research regarding cancer risk from nonoccupational sources of chromium and cobalt such as food and water.

There is some evidence linking low dietary calcium intake and colon cancer (15), although not all studies are supportive of this theory (16, 17). Little is known about calcium intake prior to the development of upper gastrointestinal cancer.

This study was conducted, in part, to investigate further whether iron, zinc, chromium, cobalt, and cal-

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[3] The abbreviations used are: NHANES, National Health and Nutrition Examination Survey; OR, odds ratios.

cium may be involved in the etiology of cancers of the mouth, larynx, and esophagus. Levels of these elements were ascertained by using a food frequency questionnaire and from toenail samples.

Materials and Methods

A population-based case-control study was conducted in King, Pierce, and Snohomish counties of Washington state. The methods have been previously described (18). Briefly, individuals from 20 to 74 years of age who were diagnosed with cancers of the larynx, esophagus, or oral cavity (ICD-O 140.0-141.9; 143.0-146.9; 148.0-150.9; 161.0-161.9) from September 1, 1983, through February 28, 1987, were identified through the local Surveillance, Epidemiology and End Results cancer registry. The case group was further restricted to individuals without a history of cancer at any site and to those individuals with cancers of epithelial origin. Diagnosis was based on a positive histological finding or, in a few cases, a positive cytology with follow-up to the attending physician to confirm the diagnosis. People without private telephones were excluded, since individuals without phones were excluded from the control group (see below).

There were 960 eligible cases (241 laryngeal, 203 esophageal, and 516 oral). Subjects were interviewed to obtain information on medical, dental, occupational, smoking, and drinking histories. They were also asked to complete a food frequency questionnaire and to submit toenail clippings from each hallux. A complete dietary questionnaire was obtained from 661 cases (69% of the eligible cases), 100 of which were completed by the subject's next-of-kin, generally the spouse, for subjects who had died or were unable to respond. Of these 661 cases, 175 had laryngeal, 127 had esophageal, and 359 had oral cancer. Nail samples were obtained from 507 individuals (153 laryngeal, 73 esophageal, and 281 oral cancer cases), which is 53% of those eligible. The reasons for nonparticipation were physician refusal (7% of the eligible cases), subject refusal to participate (10%), inability to obtain nails due to death (23%), polish on nails (3%), and miscellany (4%).

The controls were identified using random digit dialing, as described by Waksberg (19), and were frequency-matched by gender and 5-year-age intervals to the oral cancer cases. As with the cases, the control group included only those individuals who had no history of cancer. There were 4012 residential numbers called, of which 3798 households were screened and 214 refused or were unable to be screened because of language or other problems. In the screened households, there were 625 eligible controls, 18% of whom refused to participate in the interview portion of the study. A completed dietary questionnaire was obtained for 75% of the eligible controls (466 subjects). Nail samples were obtained for 69% of the eligible controls (434 subjects); 19 controls with polish on their nails were excluded. The overall random digit dialing response (taking into account individuals who refused to provide a household census when randomly called) was estimated to be 71% for completion of the dietary questionnaire and 66% for nail collection.

A food frequency questionnaire was used to estimate usual dietary habits 10 years prior to the interview. The questionnaire consisted of 125 food items for which the subjects recorded their past consumption according to 9 frequency categories ranging from "never or less than once a year" to "6+ per day." The questionnaire was a modification of a questionnaire in which reliability and validity have been assessed (20). United States Department of Agriculture handbooks were used to determine amounts of iron, calcium, zinc, and ascorbic acid in foods consumed. Amounts of β-carotene in foods were determined by using data from Leveille, Zabik, and Morgan (21). Information regarding iron and zinc supplements was combined with the food frequency data to obtain the total daily intake of each subject. Information regarding supplemental calcium was not collected; therefore, the levels of dietary calcium reflect only intake from foods. The amount of chromium and cobalt in foods could not be determined using United States Department of Agriculture data.

Elements are deposited in the nails during formation of the nail matrix. This generally occurs from 8 months (in younger individuals) to 2 years (in older individuals) prior to clipping (22, 23). For the majority of the subjects, the nails were clipped during the week prior to the interview and were collected at the time of the interview. If the nails were too short to be clipped at that time, the subject was asked to wait until they were long enough (generally 1 week to 2 months) and to mail in the samples. The median length of time from diagnosis to interview was 6.5 months. Thus, the concentrations of elements in these samples were likely to represent prediagnostic levels in the majority of cases.

Nails were stored in plastic specimen bags in a −70° C freezer. The average weight of the samples was 81.6 mg for cases and 66.5 mg for controls. The samples were cleaned in an ultrasonic bath using reagent grade deionized water. They were dried at 75°C in a drying oven for 20 h, weighed, and encapsulated in polyethylene vials for analysis. The nails were analyzed by neutron activation analysis using a General Electric MTR Reactor (24). The samples were irradiated in a flux (1×10^{13} n/cm^2/s) of thermal neutrons for 25 h. The isotopes used were ^{59}Fe, ^{47}Ca, ^{65}Zn, ^{51}Cr, and ^{60}Co. National Bureau of Standards reference materials were used for calibration. The mean percentages of error were: for iron, 14.9%; for calcium, 16.5%; for zinc, 12.8%; for chromium, 14.3%; and for cobalt, 21.6%. The personnel performing the analyses were unaware of the case-control status of the samples.

For the statistical analysis, it was assumed that those at greatest risk would be at the extremes of the distribution. Therefore, to estimate the odds ratios for the elements, the subjects were divided into the lowest 25%, mid-50%, and highest 25% of the distribution when all subjects were combined. Unconditional logistic regression was used to calculate odds ratios as estimates of the relative risk of cancer in individuals with various element levels, adjusted for the effects of potentially confounding variables (25). Since the controls were frequency-matched to the oral cancer cases on age and gender, these two variables were included in the regression models.

Results

Table 1 describes the demographic characteristics and history of alcohol and tobacco use of the subjects. While

Table 1 Distribution of cases and controls by demographic characteristics and use of alcohol and tobacco[a]

	Laryngeal cases		Esophageal cases		Oral cases		Controls	
	n	%	*n*	%	*n*	%	*n*	%
Age (years)								
20-39	9	5	1	1	19	5	25	5
40-59	72	41	38	30	131	36	175	38
60-74	94	54	88	69	209	58	266	57
Sex								
Male	142	81	94	74	238	66	313	67
Female	33	19	33	26	121	34	153	33
Race								
White	167	95	115	91	345	96	445	95
Other	8	5	12	9	14	4	21	5
County of residence								
King	103	59	71	56	219	61	302	65
Pierce	40	23	33	26	93	26	84	18
Snohomish	32	18	23	18	47	13	80	17
Highest level of education attended								
Grade school	21	12	17	13	40	11	25	5
High school	89	51	67	53	176	49	172	37
Technical school/college	65	37	40	31	143	40	269	58
Unknown	0	0	3	2	0	0	0	0
Lifetime alcohol use[b]								
0-3 drink-years	14	8	17	13	48	13	137	29
4-64 drink-years	80	46	37	29	129	36	261	56
65+ drink-years	73	42	54	43	149	42	56	12
Unknown	8	5	19	15	33	9	12	3
Lifetime cigarette use[c]								
0 pack-years	8	5	16	13	38	11	172	37
1-19 pack-years	13	7	10	8	27	8	90	19
20+ pack-years	148	85	92	72	275	77	194	42
Unknown	6	3	9	7	19	5	10	2

[a] The data listed are for the individuals who completed a dietary questionnaire. The proportion of subjects in each category is similar for individuals who submitted nail samples.
[b] Drink-years, number of drinks/day × number of years used.
[c] Pack-years, number of cigarette packs/day × number of years smoked.

the case and controls were similar in race and marital status (data not shown), esophageal cancer cases tended to be older than the controls, and the laryngeal cancer case group had a greater proportion of men than did the control group. Cases were less educated, likely to reside in Pierce County, and heavier consumers of alcohol and tobacco than were controls.

Odds ratios associated with element levels in nail tissue are shown in Table 2. These odds ratios were adjusted for cigarette use, alcohol use, and intakes of energy, β-carotene, and ascorbic acid. Although the consumption of foods containing ascorbic acid and β-carotene did not greatly confound the relationships found in this study, inclusion of these two nutrients in the logistic model did result in odds ratios slightly closer to the null and, therefore, were included. The results in Table 2 show that the risk of esophageal cancer was associated with elevated concentrations of iron and calcium. In addition, individuals who developed esophageal or oral cancer were more likely to have elevated cobalt concentrations in nail tissue than were controls. This was particularly evident in people with esophageal cancer.

We reported earlier (18) that individuals in the lowest quartile of zinc levels in nail tissue had an elevated risk of oral cancer compared to individuals with higher zinc concentrations, when adjusted for smoking and drinking. To investigate whether there was a trend in risk with zinc concentrations, three levels of zinc were used (lowest 25%, mid-50%, and upper 25%). Increasing levels of nail zinc did not correspond with decreasing risk (Table 2).

In general, subjects with high iron, calcium, and zinc intake, as estimated from the food frequency questionnaire, were less likely to develop upper aerodigestive tract cancer than were persons with lower intake (Table 3). The strongest associations were seen between dietary zinc and laryngeal and esophageal cancers. Individuals who reported diets high in zinc were one-tenth as likely to have cancer at these two sites as individuals with low levels. Further adjustment for education did not materially change the point estimates of the relative risks shown in the table. These associations were not modified by smoking status (nonsmokers *versus* people who smoked 100 or more cigarettes in their lifetimes) or drinking status. Dietary zinc was inversely associated with the risks of laryngeal and esophageal cancers even in those with a lifetime alcohol intake of 0-3 drink-years (OR, 0.00 and 0.22, respectively, for the upper 25% of the distribution compared to the lowest 25%). For dietary iron, the corresponding OR were 0.46 and 0.69.

Table 2 Odds ratios for element levels in nail tissue by site of cancer

	Units (ppm)	Larynx			Esophagus			Oral cavity		
		No.	OR[a]	95% CI[b]	No.	OR[a]	95% CI	No.	OR[a]	95% CI
Iron	<48	144	1.0		120	1.0		182	1.0	
	48-116	203	1.6	0.9-2.8	167	1.7	0.7-3.9	225	0.9	0.6-1.5
	>116	91	1.0	0.5-2.2	86	2.9	1.1-7.5	119	1.1	0.7-2.0
Calcium	<1045	146	1.0		126	1.0		168	1.0	
	1046-1989	160	1.5	0.8-2.7	129	1.4	0.5-3.6	193	1.3	0.8-2.2
	>1989	75	1.6	0.8-3.4	61	2.6	1.0-7.1	84	1.2	0.7-2.3
Zinc	<98	126	1.0		96	1.0		156	1.0	
	98-148	283	0.8	0.4-1.3	250	1.3	0.6-2.9	326	0.6	0.4-0.9
	>148	133	0.8	0.4-1.5	117	1.7	0.7-4.1	165	0.9	0.5-1.4
Chromium	<1.1	122	1.0		107	1.0		164	1.0	
	1.1-3.7	287	1.3	0.7-2.3	244	1.1	0.5-2.4	328	0.8	0.5-1.3
	>3.7	130	1.6	0.8-3.1	109	1.7	0.7-3.9	151	1.0	0.6-1.7
Cobalt	<0.05	114	1.0		92	1.0		135	1.0	
	0.05-0.17	168	2.0	1.0-3.8	127	2.4	0.8-7.2	190	1.5	0.9-2.6
	>0.17	62	1.0	0.4-2.6	66	9.0[c]	2.7-30.0	92	1.9	1.0-3.6

[a] All odds ratios were adjusted for age, sex, pack-years of cigarette use, drink-years of alcohol, energy intake (kcal/day), [beta]-carotene intake (mg/day), and ascorbic acid intake (mg/day).
[b] CI, confidence interval.
[c] Test for trend, $P < 0.001$.

In these data, zinc intake was strongly associated with energy intake ($r = 0.7$; $P < 0.001$). With such high correlation, we were unable to determine whether zinc, *per se*, or caloric intake was associated with the risk of disease, since inclusion of both energy and zinc in the logistic model yielded unstable odds ratios. Therefore, the results for zinc are presented without adjusting for energy intake (Table 3).

The difference in dietary zinc levels for laryngeal and esophageal cases and controls was principally due to the zinc obtained from foods rather than from supplements (Table 3). The foods high in zinc from the food frequency questionnaire were "clams, oysters or mussels," "beef as a main dish," "beef as a sandwich or mixed dish," "liver," "chicken, turkey or other poultry," "pork," "lamb," "bran breakfast cereal," and "wheatgerm." Laryngeal cancer cases were significantly less likely to consume bivalves (clams, oysters, and mussels) and poultry at least once a week than were controls (Table 4). Esophageal cancer cases were significantly less likely to consume bran breakfast cereal at least once a week than were controls, and oral cancer cases were significantly less likely to consume poultry at least once a week than were individuals without oral cancer. None of the estimates of relative risk in Table 4 are as low as the odds ratio of 0.1 for both esophageal and laryngeal cancers in relation to estimated dietary zinc intake (Table 3).

Since the nails were collected after diagnosis, there was some concern that the elements deposited in the nail matrix reflected levels after disease onset. To investigate this, we stratified the cases by stage at diagnosis (*in situ*/localized versus regional/distant) and by time from diagnosis to interview (<7 months *versus* ≥7 months). There were no statistically significant differences (at the 0.05 level) in the odds ratios by stage or by time from diagnosis to interview. However there were

Table 3 Odds ratios for dietary intake of elements by site of cancer

	mg/day	Larynx			Esophagus			Oral cavity		
		No.	OR[a]	95% CI[b]	No.	OR[a]	95% CI	No.	OR[a]	95% CI
Zinc[c]	<13.87	146	1.0		116	1.0		136	1.0	
	13.87-26.75	287	0.3	0.2-0.5	262	0.5	0.3-0.9	351	1.0	0.6-1.5
	>26.75	142	0.1[d]	0.0-0.2	140	0.1[d]	0.0-0.3	216	0.9	0.5-1.5
Zinc supplements[e]	No	547	1.0		492	1.0		670	1.0	
	Yes	28	0.7	0.2-2.0	26	0.8	0.3-2.7	33	0.8	0.3-1.8
Iron[c]	<14.26	141	1.0		120	1.0		164	1.0	
	14.26-31.02	298	0.4	0.2-0.7	275	0.5	0.2-0.9	377	0.7	0.5-1.2
	>31.02	147	0.5	0.2-1.0	134	0.5	0.2-1.2	182	0.7	0.4-1.3
Iron supplements[e]	No	391	1.0		345	1.0		479	1.0	
	Yes	195	0.9	0.5-1.4	184	1.1	0.6-1.8	244	1.0	0.7-1.4
Calcium[f]	<571	143	1.0		128	1.0		176	1.0	
	571-1419	296	0.7	0.4-1.2	272	0.8	0.4-1.5	368	0.8	0.5-1.2
	>1419	148	0.7	0.3-1.6	130	0.6	0.3-1.5	181	0.8	0.4-1.4

[a] Odds ratios were adjusted for age, sex, pack-years of cigarette use, drink-years of alcohol, energy intake (kcal/day), [beta]-carotene intake (mg/day), and ascorbic acid intake (mg/day), except for zinc. Odds ratios for zinc were adjusted for age, sex, pack-years, drink-years, [beta]-carotene, and ascorbic acid.
[b] CI, confidence interval.
[c] Includes intake from foods and supplements.
[d] Test for trend, $P < 0.001$.
[e] Individuals who used supplements were compared to individuals who did not (reference group).
[f] Intake from foods only (no supplement information).

Table 4 Odds ratios for subjects who consumed specific zinc-containing foods once per week or more compared to subjects who consumed these foods less than once per week (reference category)

Food category[a]	Larynx		Esophagus		Oral cavity	
	OR[b]	95% CI[c]	OR[b]	95% CI	OR[b]	95% CI
Clams, oysters, mussels	0.2	0.1-0.8	0.7	0.2-2.0	0.9	0.5-2.0
Beef as a main dish	0.8	0.5-1.4	0.8	0.4-1.4	1.1	0.7-1.7
Beef as a sandwich	0.8	0.5-1.2	1.0	0.6-1.7	0.9	0.6-1.2
Poultry	0.5	0.3-0.8	0.8	0.5-1.4	0.7	0.5-1.0
Pork excluding bacon and ham	1.1	0.7-1.7	1.2	0.8-2.5	0.9	0.6-1.2
Bran breakfast cereal	0.9	0.5-1.4	0.5	0.3-1.0	1.1	0.8-1.7
Wheatgerm	1.2	0.4-5.0	1.2	0.3-5.0	1.4	0.6-3.3

[a] Odds ratios for liver, lamb, and ham are not listed, since few subjects consumed these foods once per week or more.
[b] Odds ratios were adjusted for age, gender, pack-years of cigarettes, drink-years of alcohol, energy intake (kcal/day), [beta]-carotene intake (mg/day) and ascorbic acid intake (mg/day).
[c] CI, confidence interval.

some differences, although not statistically significant, which are worth mentioning. The association between high iron concentrations in nail tissue and esophageal cancer was more evident in individuals with *in situ* or localized tumors (OR, 9.4 for high *versus* low categories; 95% confidence interval, 1.6–55) than with regional or distant tumors (OR, 1.8; 95% confidence interval, 0.5–6.4), suggesting that the observed association was not a result of the influence of the disease on nail levels. However, as previously reported (18), an association between low zinc concentrations in nail tissue and cancer of the oral cavity was evident in those individuals who were interviewed 7 months or more after diagnosis but not in subjects interviewed less than 7 months after diagnosis, although the difference in these odds ratios was not statistically significant. This may indicate that the low zinc levels in the nails of oral cancer patients may be a result of the disease.

There were no significant linear associations between dietary intake of the elements and amount in the nail tissue when expressed as continuous variables. The correlation coefficient between dietary and nail zinc was 0.05; for dietary and nail iron it was −0.03; for dietary and nail calcium it was −0.05.

Discussion

The results of this study suggest that individuals with cancers of the upper aerodigestive tract have altered element levels. Low dietary intake of iron and zinc was associated with laryngeal and esophageal cancer. Esophageal cancer cases had elevated nail tissue levels of iron and calcium compared to controls. In addition, esophageal and oral cancer cases had higher cobalt concentrations in nail tissue than did controls.

Zinc. The strongest association observed in this study was an apparent protective effect of consumption of foods high in zinc. A decreasing trend in risk with the amount consumed was evident for both laryngeal and esophageal cancer. The food frequency questionnaire used in this study is a fairly reliable and valid instrument for assessing the intake of common minerals (26, 27). A recent validation study of an expanded version of this questionnaire yielded correlation coefficients from 0.50 to 0.71 comparing dietary zinc from the questionnaire and from dietary records (27). Some investigators have shown that dietary information can be recalled from the past fairly well, although there is some bias towards current intake (28). However, there is a possibility of recall bias, particularly since the site of these cancers was the mouth and throat. Similar to people who develop lung cancer, the cases in this study tended to be thinner than the controls, and loss of appetite as a result of the disease could have resulted in underreporting of overall food (energy) consumption by the cases. Since zinc intake and energy were correlated, this could explain the low zinc levels in some cases. Furthermore, the results in Table 4 do not show a uniform pattern of a protective effect from eating zinc-rich foods, although there are some significant associations. This heterogeneity of results may indicate that it may be some other determinant of risk, perhaps energy intake. However, if this were true, it is not clear why the dietary recall bias would be more evident in subjects with laryngeal and esophageal cancer than in subjects with oral cancer (Table 3).

This difference in zinc levels between cases and controls was reflected only in foods consumed and not in nail tissue levels. It is important to note that nail concentrations of minerals do not necessarily correspond to dietary intake. The principle reason for this is widely varying differences in absorption and metabolism of these minerals. Absorption of zinc varies with the type of zinc compound ingested and the presence of other factors such as phytate or fiber, iron, calcium, glucose, lactose, and alcohol (29). Since the case group contained proportionately more heavy drinkers, the results were presented after adjustment for alcohol intake. Furthermore, dietary zinc was inversely associated with the risks of laryngeal and esophageal cancers even in those with a lifetime alcohol intake of 0–3 drink-years. Therefore, alcohol consumption does not appear to account for the relationship between dietary zinc and cancer in this study.

Besides differences in absorption and metabolism, the lack of agreement for nail and diet indices in this study may also be due to differences in the time when the levels were measured (8 months to 2 years prior to interview for nail tissue; for dietary intake, approximately 10 years prior to the interview).

There are possible mechanisms by which zinc could influence the risk of cancer. There is considerable evidence to support the theory that oxygen radicals and lipid peroxidation promote carcinogenesis (30). This theory provides a unifying mechanism for the action of many possible etiological agents. Oxygen radicals are known to cause cellular damage and DNA mutations which may

result in carcinogenesis. Zinc protects against peroxidation and free-radical formation and is a constituent of the enzyme superoxide dismutase which eliminates superoxides (31, 32). In addition, zinc deficiency is known to result in epidermal hyperplasia and lesions of the mucosal membrane (33). Esophageal mucosa in rats has been found to be highly sensitive to dietary zinc content and a zinc-deficient diet resulted in hyperplasia of the esophageal epithelium (34). Dietary zinc deficiency increases the incidence of and shortens the lag time for induction of esophageal tumors in rats by methylbenzylnitrosamine (35). In addition, zinc is known for its role in maintaining immunocompetence and for stabilizing the structure of DNA during transcription (33).

Iron. In this study, individuals with esophageal cancer had higher concentrations of iron in nail tissue than did controls. This was more evident in individuals with *in situ* or localized tumors than with regional or distant tumors, suggesting that the elevated iron levels may not be a result of the disease. Here again, the dietary analysis yielded a different picture. Although the OR were not statistically significant, they do suggest that esophageal cancer cases had diets low in iron. Stevens *et al.* (7), using data from NHANES, found that esophageal cancer patients had low total iron-binding capacity and high transferrin saturation, indicating high iron stores; yet they also had low hemoglobin levels. Similar to our results, the iron intake of the NHANES subjects with esophageal cancer tended to be lower than the iron intake of individuals without cancer.

Differences in absorption and metabolism of various iron-containing compounds may be one explanation for these consistent but paradoxical findings. The amount of heme iron absorbed varies by the amount of iron stores and is affected very little by meal composition (36). Nonheme iron accounts for most of the dietary iron absorbed but is greatly affected by concurrent ingestion of ascorbic acid, calcium, animal proteins, phytates, and other complexing agents and by the presence of intrinsic factor, gastric acidity, and the chemical form of iron consumed such as ferrous lactate or ferrous citrate (29, 37). Some investigators have found an association between low serum ferritin levels and the risk of gastric cancer, possibly because achlorhydria reduces iron absorption in the gastric mucosa (5, 6).

Another explanation for these findings may be that measurements of dietary intake of iron do not reflect body stores unless iron deficiency exists. It may be difficult to interpret correlations between levels of nail iron and diet since little is known regarding the incorporation of iron in the nail matrix and its possible relationship to body stores. In this study, there was no linear correlation between dietary and nail iron levels. Sobolewski *et al.* (38) found a relationship between levels of nail iron and iron deficiency anemia, as measured by hemoglobin, mean corpuscular volume, serum iron, total iron binding capacity, and bone marrow iron. However, other researchers have not found such an association when using hemoglobin or iron content of liver tissue (39–41).

Within the past decade, the hypothesis that an excess amount of iron may contribute to cancer risk in humans has led to extensive dialogue (42, 43). However, there are few observational studies in humans which specifically address this issue. From animal studies, it has been found that iron-catalyzed oxidation may occur when iron is decompartmentalized, resulting in highly reactive and damaging free radicals (42, 43). The presence of iron may increase oxidative stress to a cell and thereby the risk of transformation. Furthermore, since iron is necessary for cellular metabolism, iron availability may influence the chances that a cancer cell will survive. Iancu (44) calls ionic iron "an ultimate carcinogen and toxicant." In this data set, only individuals with esophageal cancer had higher nail levels of iron than did controls. At this point, it is unclear how mechanisms of action may differ for esophageal cancer cases and individuals with cancer at other sites.

Calcium. There are few data to indicate that calcium intake may be etiologically related to upper alimentary tract cancer, although there is some speculation that a deficiency may be involved with colon cancer (45). In our study, there were no significant differences in calcium intake between cases and controls. However, esophageal cancer cases had elevated levels of calcium in nail tissue. Skal'nyi *et al.* (46) found that alcoholics have increased levels of calcium in keratinous tissue. Even after adjustment for alcoholic intake, however, calcium levels in nail tissue were still elevated in esophageal cancer cases in this data set.

Serum calcium levels are known to rise due to bone resorption in some patients with head and neck tumors, particularly squamous cell carcinomas (47, 48). Some tumors have been found to secrete a protein similar in structure to parathyroid hormone which results in hypercalcemia (49). If the element concentrations in nails reflect levels after disease onset, the elevated values may be explained. However, there was no significant difference in odds ratios for calcium by stage at which the tumors were diagnosed.

Chromium. There is ample evidence from animal studies and from occupational studies in humans that certain hexavalent chromium compounds are carcinogenic (13). Most of the evidence for chromium comes from workers in metal industries, where the quantities of chromium were much greater than exposure in the general population. In this study, there were no differences in chromium concentrations in cases and controls, although the odds ratios for laryngeal and esophageal cancer were slightly elevated in persons with high nail levels. Unfortunately, the concentrations of chromium obtained from the nail tissue indicate total chromium content, not specific chromium compounds. These results need to be replicated, since there are few studies of human cancer risk and low dose exposure to chromium.

Cobalt. Associations were found between elevated nail levels of cobalt and cancers of the esophagus and oral cavity, which were evident in both individuals with *in situ*/localized tumors and individuals with regional/distant tumors. It seems unlikely that cases would consume higher amounts of vitamin B_{12} (cobalamin) since the cases tended to eat fewer animal products than did controls. Furthermore, there was no difference between cases and controls with respect to the intake of vitamin B_{12} supplements. It is possible that some cobalt may be present in beer, although we found no significant association between beer consumption and cobalt levels in nail tissue. Jensen *et al.* (14) concluded that there was sufficient evidence that cobalt and both soluble and insoluble cobalt compounds are carcinogens in animal experi-

ments. There have been a few studies of cobalt exposure in the hard-metal industries, in cobalt production, and in medical use, but the results are not consistent. Sarcomas are occasionally reported at injection sites in animals, but the use of cobalt alloys for medical use has not been shown to be carcinogenic (14). Collecchi *et al.* (50) found that mean plasma cobalt levels were higher in patients with laryngeal carcinoma than in healthy control subjects and that cobalt levels in tumor tissue were higher than in adjacent nonmalignant tissue.

The mechanisms by which cobalt may affect cellular constituents are not clear. Cobalt compounds bind irreversibly to nucleic acids in the cell nucleus and adversely affect cell division, although in animal studies they are only weak mutagens (51). It is unknown why nail levels of cobalt were elevated in esophageal and oral cancer patients in our study. While it is possible that the source of the elevated cobalt may be dietary, it is also possible that there may be occupational exposure in some subjects.

Summary. There were a number of significant differences between mineral levels of cases and controls, both in diet and in nail tissue. Individuals with laryngeal and esophageal cancer consumed foods lower in zinc and iron than did individuals without cancer. Subjects with esophageal cancer had elevated levels of iron, calcium, and cobalt in their nail tissue, while oral cancer cases had elevated levels of cobalt. Some of the discrepancies that were observed between the results of the dietary information and nail tissue could be due to differences in absorption or metabolism of these elements. While there are limitations of this study, its size was such that site-specific associations could be investigated and information regarding other potential confounding or modifying factors could be considered. Neither tobacco nor alcohol use could account for the differences in element levels found between cases and controls.

It is clear that no one measurement provides a sufficient overall assessment of mineral status, with the possible exception of serum ferritin for iron (52). Because of this, it may be necessary to measure many dietary and biochemical indices in one study. This may help to elucidate possible pathological processes involved in either cancer initiation or promotion. While much attention has been devoted to vitamin intake and carcinogenesis, this study suggests that it may be worthwhile to investigate other nutrients as well. Most notably, the results for dietary zinc warrant further investigation.

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