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EPIDEMIOLOGIC STUDIES OF VITAMINS AND CANCER OF THE LUNG,
ESOPHAGUS, AND CERVIX*

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ABSTRACT

Epidemiologic studies of the relationships between vitamins and 3 types of cancer are reviewed. First, the widely reported association between vitamin A and β -carotene and risk of lung cancer is considered. In a large population-based case-control study of lung cancer among white males in New Jersey, increased intake of vegetables, dark green vegetables, dark yellow-orange vegetables, and carotenoids were each associated with reduced risk, but intake of retinol or total vitamin A was not related. The protective effect of vegetables was limited to current and recent cigarette smokers, which suggests that vegetable intake prevents a late-stage event in carcinogenesis. Consumption of dark yellow-orange vegetables was consistently more predictive of reduced risk than either the total carotenoid index or consumption of any other food group, possibly because of the high content of β -carotene in this food group. The results and limitations of other epidemiologic studies of diet and lung cancer are reviewed. Second, the evolving relationship between multiple micronutrient deficiencies and esophageal cancer is discussed. In a death certificate-based case-control study of esophageal cancer in black males in Washington, D.C., several indicators of general nutritional status, including consumption of fresh or frozen meat and fish, dairy products and eggs, and fruit and vegetables, and the number of meals eaten per day, were inversely and independently correlated with the risk of esophageal cancer. Estimates of intake of micronutrients, such as carotenoids, vitamin C, thiamin, and

ABBREVIATIONS: HPLC = high performance liquid chromatography.

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riboflavin, were less strongly associated with reduced risk than were the broad food groups that provide most of each micronutrient. Thus no single micronutrient deficiency was identified. Other studies suggest that generally poor nutrition may partially explain the susceptibility of urban black men to esophageal cancer. Finally, the postulated association between low folacin levels and risk of cervical cancer is examined. Among women who use oral contraceptives, serum and red blood cell folacin levels were reported to be lower among those with cervical dysplasia. In a clinical trial involving oral contraceptive users, cervical dysplasia gradually decreased in the group supplemented with oral folate but remained unchanged in the group given the placebo. Other epidemiologic studies of diet and cervical cancer are discussed.

INTRODUCTION

I was originally asked to report on epidemiologic studies of vitamins and cancer. This is a difficult topic to cover comprehensively, because systematic hypothesis-generating studies have not been conducted for many cancers and many vitamins. Therefore, I have decided to concentrate on 3 evolving relationships for which epidemiologic data exist: (1) the widely discussed association between low levels of vitamin A or β -carotene and the risk of lung cancer, (2) the association between multiple micronutrient deficiencies and the risk of esophageal cancer, and (3) the postulated association between low folacin levels and risk of cervical cancer.

β -CAROTENE, VITAMIN A, AND LUNG CANCER

β -Carotene and vitamin A have been proposed to reduce the risk of cancer in general and of epithelial and lung cancers in particular. β -Carotene is one of many carotenoids that occur in vegetables and fruits. It is the most common of the limited number of carotenoids that can be metabolized to vitamin A by humans.¹ In humans, vitamin A is also obtained directly from the retinol in dairy products, eggs, and liver. Humans can convert β -carotene into retinol but cannot convert retinol into β -carotene.

The β -carotene and vitamin A hypotheses are historically and logically distinct. The vitamin A hypothesis evolved first. Since the mid-1960s, numerous animal experiments have demonstrated that pharmacologic doses of retinol and retinoids can retard or prevent the growth of tumors induced by various agents at a number of different sites.² In the early 1980s, attention turned to β -carotene,³ in part because of its ability to protect lipids, DNA, or both from oxidative degradation.^{4,5} Logically, an association between vitamin A consumption and cancer risk does not provide evidence for an association between β -carotene and cancer, nor does an association between β -carotene consumption and cancer risk guarantee an association between vitamin A and cancer, at least not in a study conducted among individuals on an American or Western European diet. Dietary carotenoids are too small a source of dietary vitamin A. The foods usually available to the U.S. consumer provide only 21% of total vitamin A activity as carotenoids.^{6,7}

In 1980-81, Dr. Tom Mason and I, in conjunction with the New Jersey State Department of Health, conducted a population-based, incident case-control study of lung cancer in white males in 6 high-risk areas of the state.⁸ Interviews were completed for 763 patients and 900 controls; 40% of the interviews had to be conducted with surrogate respondents related to the subjects. To determine whether dietary intake of carotenoids,

Table 1. Smoking-adjusted^a Relative Risks of Lung Cancer for Nutrient and Food Group Intake^b

Micronutrient or Food Group	Level of Consumption			P for Trend
	Upper 25%	Middle 50%	Lower 25%	
Micronutrients				
Retinol	1.0	1.0	0.8	.07
Carotenoids	1.0	1.2	1.3	.10
Vitamin A	1.0	0.9	0.9	.20
Food Groups				
Dairy products	1.0	0.8	1.3	.14
Vegetables and fruit	1.0	1.4	1.3	.04
Fruit	1.0	1.2	1.0	.35
Vegetables	1.0	1.3	1.4	.01
Dark green vegetables	1.0	1.5	1.5	.02
Dark yellow-orange vegetables	1.0	1.5	1.5	.004

^aRelative risks were adjusted over 14 strata: nonsmokers; pipe and cigar smokers only; and cigarette smokers categorized simultaneously by intensity (<25, ≥25 cigarettes/day), duration (<40, >40 years), and time since smoking ceased (0-1, 2-9, ≥10 years).

^bSee ref. 8.

performed retinol, or total vitamin A influenced the risk of lung cancer, subjects were asked about their usual frequency of consumption, several years earlier, of 44 food items that provide 83% of the vitamin A in the American diet⁹ and about their use of vitamin supplements. Table 1 presents the smoking-adjusted relative risks of lung cancer for decreasing intakes of retinol, carotenoids, vitamin A, and several related food groups. Among the micronutrients, only carotenoids showed an increase in risk with decreased intake, with subjects in the lowest quartile of consumption having 1.3 times the risk of those in the highest quartile. No increase in risk with decreased intake of retinol or total vitamin A could be detected. Certain food groups, specifically vegetables, dark green vegetables, dark yellow-orange vegetables, and vegetables and fruit, all of which were partially correlated with each other and with carotenoids, also showed an increase in risk with decreased intake. Moreover, the inverse associations with these food groups were stronger than that with carotenoids.

The increase in risk of lung cancer associated with decreased vegetable intake was seen in both cigarette smokers and in pipe and cigar smokers who reported no cigarette use. There was no evidence for a vegetable-related increase in risk among the nonsmokers, although the number of cases was small (Table 2). When the cigarette smokers were divided into current smokers, including those who quit smoking within 1 year of diagnosis, and smokers who quit 2-5, 6-9, or 10 or more years prior to diagnosis, a vegetable-related increase in risk was seen only among current and recent smokers. Analogously, a vegetable-related increase in risk of lung cancer was noted only among cigarette smokers of moderate or long duration and not among those who had smoked for less than 30 years. These 2 findings are related, because current and recent smokers in any age group have generally smoked for many years.

Table 2. Relative Risks of Lung Cancer for Vegetable Intake by Cigarette Smoking and Years Since Quitting^a

Cigarette Smoking	No. of Cases, Controls	Level of Vegetable Consumption			P for Trend
		Upper 25%	Middle 50%	Lower 25%	
Non-cigarette Smokers					
Nonsmokers	13, 140	1.0	1.2	0.3	.20
Pipes, cigars only	21, 81	1.0	1.5	1.8	.22
Cigarette Smokers ^b					
Years since quitting					
0-1	465, 303	1.0	1.3	1.8	.004
2-5	59, 51	1.0	2.2	1.9	.19
6-9	49, 38	1.0	0.8	0.5	.25
10+	134, 255	1.0	1.4	1.2	.30

^aSee ref. 8.

^bRelative risks were adjusted over 4 strata among the cigarette smokers: low intensity (<25 cigarettes/day) and low duration (<40 years), low intensity/high duration, high intensity/low duration, and high intensity/high duration.

The concentration of the vegetable-related reduction in risk among current and recent smokers and smokers of moderate and long duration suggests that vegetable intake may prevent a late-stage event in lung cell carcinogenesis, an event involving promotion rather than initiation. It is noteworthy that in a number of animal models, retinoids block the promotion or progression of carcinogenesis when administered after initiation.¹⁰

Table 3 shows the smoking-adjusted relative risks of lung cancer among current and recent cigarette smokers for decreasing intake of the micronutrients of interest and the related food groups. Results were similar for all study subjects, but for current and recent cigarette smokers, the associations were stronger, and dose-response relationships were apparent. Among the micronutrients, only carotenoids showed an increase in risk of lung cancer with decreased intake. No increase in risk with decreased intake of retinol or total vitamin A could be seen. As in the study population in general (Table 1), vegetables, dark green vegetables, dark yellow-orange vegetables, and vegetables and fruit showed increases in risk with decreased intake; in general, the inverse associations with these food groups were stronger than the association observed with carotenoids. Subjects in the lowest quartile of vegetable consumption had 1.7 times the risk of lung cancer of those in the highest quartile. For dark green vegetables, the corresponding relative risk was 1.8; for dark yellow-orange vegetables, it was 2.2.

One explanation for the stronger inverse associations with food groups than with the carotenoid index is simply that the protective agent in vegetables may be something other than carotenoids; it could be vitamin C,¹¹ indoles,¹² plant phenols,⁵ or trace minerals,¹³ all of which have been proposed to reduce the risk of cancer. Another possible explanation stems from the nature of carotenoid determinations for foodstuffs. The

Table 3. Smoking-adjusted^a Relative Risks of Lung Cancer for Nutrient and Food Group Intake Among Current and Recent Cigarette Smokers^{b,c,d}

Micronutrient or Food Group	Level of Consumption			P for Trend
	Upper 25%	Middle 50%	Lower 25%	
Micronutrients				
Retinol	1.0	1.1	1.0	.48
Carotenoids	1.0	1.5	1.7	.02
Vitamin A	1.0	1.2	1.2	.26
Food Groups				
Dairy products	1.0	0.8	0.9	.26
Vegetables and fruit	1.0	1.7	1.8	.005
Fruit	1.0	1.4	1.2	.28
Vegetables	1.0	1.3	1.7	.004
Dark green vegetables	1.0	1.4	1.8	.002
Dark yellow-orange vegetables	1.0	1.6	2.2	<.001

^aRelative risks were adjusted over 4 strata: low intensity (<25 cigarettes/day) and low duration (<40 years), low intensity/high duration, high intensity/low duration, and high intensity/high duration.

^bSee ref. 8.

^cCurrent cigarette smokers are current smokers and those who quit within 1 year of diagnosis. Recent smokers are those who quit 2-5 years prior to diagnosis.

^dIncluded for analysis are 524 cases and 354 controls.

assay method most often utilized detects not only β -carotene but also a number of other carotenoids, some with vitamin A activity and some without, and measures each carotenoid with a different efficiency unrelated to its potential efficacy in cancer prevention.¹ Therefore, if the protective agent were β -carotene itself or some other chemically unique carotenoid, the estimate of total carotenoid intake might still be no more reliable a measure of exposure than food group consumption.

Although both vegetables and fruit contain carotenoids, only vegetables displayed protective activity in this study. Dark yellow-orange and dark green vegetables, rich in β -carotene, seemed to account for most of the protection conferred by vegetable consumption, with dark yellow-orange vegetables being most strongly associated with reduced risk. Recent HPLC analysis of the various carotenoids in foods has demonstrated that dark yellow-orange vegetables contain more α - and β -carotene but less of other carotenoids than are contained in dark green vegetables.¹⁴ Other HPLC studies have suggested that certain fruit may contain much less β -carotene than was previously assumed on the basis of their total carotenoid content.^{15,16} Thus the relative strength of the associations with the food groups in this study suggests that β -carotene may be the protective agent.

The risk of squamous cell lung cancer, 49% of the cases in this study, was significantly increased among subjects with a low level of vegetable consumption, but the risk of lung adenocarcinoma, 16% of the cases, was unrelated to vegetable intake. However, this apparent histologic speci-

ficity is partially explained by the tendency of the vegetable-related reduction in lung cancer risk to be concentrated among current and recent smokers. Because squamous cell lung carcinoma is more strongly associated with cigarette smoking than is lung adenocarcinoma, more of the squamous cell patients are current and recent smokers. Among the relatively few current and recent cigarette smokers with adenocarcinoma, vegetable intake did seem to be protective. In fact, for most lung cancer cell types, current and recent cigarette smokers experienced a reduction in risk with increased vegetable intake, although the strongest reduction in risk was definitely seen with squamous cell carcinoma.

Four other case-control and 3 cohort studies also examined relationships between diet and risk of lung cancer (Table 4). Kvale et al.²³ and Bjelke¹⁷ analyzed the same cohort at different times. Only one of the studies, Hinds et al.,²⁴ used an interview that included most of the foods containing carotenoids and vitamin A; the other investigators drafted their interviews before these 2 hypotheses were well defined. MacLennan et al.¹⁸ and Hirayama¹⁹ did not form weighted micronutrient indices and only analyzed food group consumption. To separately evaluate the relationship of carotenoids and total vitamin A to the risk of lung cancer, indices of both carotenoid and preformed retinol intake must be formed and tested. In only 1 analysis, by Shekelle and associates,²² was this done. However, the original dietary interviews for the cohort were lost, and a series of approximations and assumptions were used to analyze the derived data.

Table 4. Epidemiologic Studies on Dietary Carotenoids and Vitamin A and Risk of Lung Cancer

Study Design	Carotenoids	Vitamin A	Reference
Cohort Norwegian men 36 cases	Not assessed	Inverse association, RR ^a ~2.6	Bjelke, 1975 (17)
Case-control hospital controls Singapore Chinese men and women 233 cases	Inverse association with green vegetables, men and women combined, RR~2.2	Possible inverse association. Not assessed directly, but green vegetables may be a major source of vitamin A in Chinese diet	MacLennan et al., 1977 (18)
Cohort Japanese men and women 807 cases	Inverse association with green-yellow vegetables in both men and women	Possible inverse association. Not assessed directly, but green- yellow vegetables provide 44% of vitamin A in Japanese diet	Hirayama, 1979 (19)
Case-control hospital controls Upstate New York white men 292 cases	Not assessed	Inverse association, RR~1.7	Mettlin et al., 1979 (20)
Case-control hospital controls London men and women 100 cases	Probably no association. Not assessed directly, but green vegetables, carrots unrelated to risk	Inverse association in men, RR~2.5 Direct association in women, RR~0.3	Gregor et al., 1980 (21)
Cohort Chicago men 33 cases	Inverse association, RR~7	No association	Shekelle et al., 1981 (22)
Cohort Norwegian men and women 168 cases	Not assessed	Inverse association in men, RR~1.6 Inverse association in women	Kvale et al., 1983 (23)
Case-control population controls Hawaiian multiethnic men and women 364 cases	Inverse association in men, RR~2.2 Direct association in women, RR~0.6	Inverse association in men, RR~1.8 Direct association in women, RR~0.7 Did not assess whether these associations with vitamin A only reflect underlying associations with carotenoids	Hinds et al., 1984 (24)

^aAbbreviation used: RR = relative risk.

The results of the 7 studies are not consistent. Three of the studies, Mettlin et al.,²⁰ Gregor and colleagues,²¹ and Kvale's group,²³ showed a protective effect of vitamin A and included evidence to suggest that protection by carotenoids alone was not responsible for the appearance of protection by vitamin A. These studies did not directly assess carotenoids. Two studies, MacLennan et al.¹⁸ and Hirayama,¹⁹ noted a protective effect of carotenoid-containing vegetables but did not directly assess vitamin A. In another study, Shekelle and co-workers,²² found that carotenoids protected against lung cancer but that vitamin A did not. Finally, in the last study, Hinds et al.,²⁴ observed protection by both carotenoids and vitamin A but did not determine whether the apparent effect of vitamin A was due to carotenoids alone.

Four of these studies examined the effects in men and women separately. Kvale et al.²³ and Hirayama¹⁹ obtained similar results in both sexes, but Hinds and associates²⁴ and Gregor's group²¹ found that increased intake was protective in men but deleterious in women.

In addition to these studies of dietary vitamin A, 4 cohort studies have examined serum vitamin A levels in blood collected prior to diagnosis of cancer. Stahelin and colleagues²⁵ and Willett et al.²⁶ failed to find any association of serum vitamin A with the subsequent incidence of cancer in general or of lung cancer in particular. Peleg's group²⁷ observed an inverse relationship between serum vitamin A and the incidence of lung cancer, though not at a level of statistical significance, but they found no association between serum vitamin A and cancer in general. Wald and colleagues²⁸ found that serum vitamin A was inversely related to incidence of all cancer, including lung cancer. However, this research is difficult to relate to studies of dietary vitamin A and cancer, because in adequately nourished populations, serum vitamin A levels are maintained within a narrow range by liver stores and do not reflect recent dietary intake of vitamin A.²⁹⁻³¹

On the other hand, the level of serum carotenoids is believed to reflect recent intake of carotenoids.^{30,31} The only published cohort study to assay carotenoids in stored sera found no relationship to subsequent incidence of cancer in general or of lung cancer in particular.²⁶ Total carotenoids were measured spectrophotometrically in this study. However, in 3 other cohort studies, β -carotene itself was isolated and measured in the sera using HPLC.³²⁻³⁴ Preliminary reports indicate that in all 3 serum β -carotene was reduced among those who ultimately developed lung cancer. Thus the studies of serum levels of micronutrients and subsequent incidence of lung cancer are consistent with the suggestion in our case-control study that dietary β -carotene may be the protective agent.

MICRONUTRIENT DEFICIENCIES AND ESOPHAGEAL CANCER

Esophageal cancer is another cancer that is related to diet. Heavy alcohol use and tobacco use are major risk factors for this cancer,³⁵ but poor nutrition is also suspected to be a cause for several reasons. First, in Iran,³⁶ China,³⁷ the Soviet Union,³⁸ the Caribbean,^{39,40} and southern Africa,⁴¹ esophageal cancer is endemic in regions with limited diets and impoverished agriculture. Second, within the U.S., mortality rates for esophageal cancer are inversely related to county socioeconomic indices and are higher among blacks than whites.⁴² Third, until recently, esophageal cancer was usually common in women from the rural, northern areas of Sweden and adjacent countries.^{35,43} Many of these women also had the Plummer-Vinson syndrome, which is associated with anemia and

various micronutrient deficiencies. Finally, esophageal cancer has been reported as a sequel^{44,45} of celiac disease, a malabsorption disorder of the small intestine.

Several years ago, Dr. Bill Blot, Linda Brown, Linda Pottern, and I conducted a case-control study of esophageal cancer among black male residents of Washington, D.C., the U.S. metropolitan area with the highest esophageal cancer mortality rate for nonwhite males.⁴⁶ The next of kin of 120 esophageal cancer patients who died during 1975-77 and of 250 black males who died of other causes were interviewed. The major risk factor was alcoholic beverage consumption, with a risk of 6.4 for drinkers relative to nondrinkers. Diet was assessed by asking about the usual adult frequency of consumption of 31 food items. Table 5 presents the alcohol-adjusted relative risks of esophageal cancer by consumption of food groups and micronutrients. Risk increased in a dose-response manner with decreasing intake of dairy products and eggs, fruit and vegetables, and fresh or frozen meat and fish but was largely unrelated to intake of carbohydrates or precooked or processed meat and fish. For those micronutrients for which indices of intake could be formed, specifically

Table 5. Alcohol-Adjusted^a Relative Risks of Esophageal Cancer for Food Groups and Micronutrients^b

Nutrition Index	Level of Consumption		
	Upper 33%	Middle 33%	Lower 33%
Food Groups			
Meat, fish, eggs, and cheese	1.0	1.7	1.3
Meat and fish	1.0	1.3	1.2
Dairy products and eggs	1.0	1.7	1.9† ^c
Fruit and vegetables	1.0	1.7	2.0†
Vegetables	1.0	1.5	1.6*
Green vegetables	1.0	1.0	1.3
Yellow vegetables	1.0	1.0	1.7
Fruit	1.0	2.4	2.0†
Carbohydrates	1.0	1.1	1.2
Bread	1.0	1.1	1.1
Fresh or frozen meat and fish	1.0	1.6	2.2†
Precooked or processed meat and fish	1.0	0.9	0.9
Nitrite-containing foods	1.0	1.1	1.0
Micronutrients			
Vitamin A	1.0	1.5	1.5
Carotenoids	1.0	1.3	1.3
Vitamin C	1.0	1.2	1.8†
Thiamin	1.0	1.2	1.2
Riboflavin	1.0	1.0	1.7†

^aRelative risks were adjusted over 5 strata: 0, 1.0-5.9, 6.0-14.9, 15.0-29.9, and 30.0-80.6 fl. oz. of hard liquor or its equivalent/day.

^bSee ref. 45.

^cStatistical significance of trend is indicated by *, $P < 0.10$ or †, $P < 0.05$.

vitamin A, carotenoids, vitamin C, thiamin, and riboflavin, risk increased with decreasing intake. However, each micronutrient index was less strongly associated with risk than were the broad food groups that provide most of the micronutrient. For example, risk was elevated among those with a low intake of vitamin C or carotenoids but was even more elevated with low consumption of fruit and vegetables. In addition, the number of meals eaten per day and weight adjusted for height, which are general measures of nutritional status, were better predictors of reduced risk than were the individual micronutrient indices. Thus we found no direct evidence that a single nutritional deficiency increases risk of esophageal cancer. Instead, generally poor nutrition, probably involving deficiencies of several micronutrients, was proposed as the major dietary predictor of risk.

When fresh or frozen meat and fish consumption, dairy product and egg consumption, or fruit and vegetable consumption was used as an indicator of nutritional status, the least nourished third of the study population had twice the risk of the most nourished third. When these 3 food group consumption measures were combined into a single overall index of general nutritional status, the risk of esophageal cancer decreased steadily with improving patterns of food consumption (Table 6). The relative risk between extremes (those who consumed low quantities of all 3 food groups compared to those who consumed high quantities of all 3) reached 14.

Table 6. Relative Risks of Esophageal Cancer for Overall Food Consumption Patterns^a

Food Consumption Pattern ^b	No. of Cases, Controls	Relative Risk (95% confidence interval)	Relative Risk Adjusted for Alcohol ^c
HHH	2, 20	1.0	1.0
HHM HMM	24, 65	3.7 (0.8-17.0)	3.8
HHL MMM HML HLL	32, 68	4.7 (1.0-21.4)	4.5
MML MLL	36, 46	7.8 (1.7-35.7)	6.7
LLL	11, 8	13.8 (2.5-27.4)	15.0

^aSee ref. 45.

^bAbbreviations indicate concurrent level of consumption of fresh or frozen meat and fish, fruit and vegetables, and dairy products and eggs, each ranked as in the upper (H), middle (M), or lower (L) tertile of consumption. For example, HML indicates high consumption of 1 of the 3 food groups, moderate consumption of a second, and low consumption of a third.

^cRelative risks were adjusted over 2 strata: 0-5.9 and 6.0-80.0 fl. oz. of hard liquor or its equivalent/day.

Table 7. Relative Risks of Esophageal Cancer by Nutritional Status and Alcohol Consumption^a

Alcohol Consumption, in Hard Liquor Equivalents	Nutritional Status ^b		
	High	Moderate	Low
0-5.9 fl. oz./day	1.0 (6, 43) ^c	1.7 (6, 25)	3.0 (8, 19)
6.0-80.0 fl. oz./day	2.7 (13, 34)	4.1 (21, 37)	8.0 (29, 26)

^aSee ref. 45.

^bNutritional status indicates concurrent level of consumption of fresh or frozen meat and fish, fruit and vegetables, and dairy products and eggs. High, moderate, and low nutritional status are defined as food consumption patterns HHH, HHM, and HMM; patterns HHL, MMM, HML, and HLL; and patterns MML, MLL, and LLL, respectively.

^cNumbers in parentheses are the numbers of cases and controls.

Using this overall measure of food consumption patterns as the nutrition index, we examined the interaction of nutritional status and alcohol intake. The risks for poor nutrition and alcohol intake remained distinct (Table 7). The elevated risk associated with poor nutrition could be detected across each level of alcohol consumption, even when alcohol consumption was divided into more strata than shown. It was not possible to determine whether poor nutrition was a risk factor among those unexposed to alcohol because only 5 patients did not drink. In this study, the association of esophageal cancer with poor nutrition appeared to be not only independent of any association with alcohol consumption but also of associations with smoking and socioeconomic status.

There are no generally accepted animal models for the role of alcohol in carcinogenesis.⁴⁷ Because poor nutrition was a risk factor for esophageal cancer in our study, we proposed that alcohol consumption might increase risk by reducing appetite and limiting nutrient intake. Beer, wine, and hard liquor provide almost none of the daily requirements for micronutrients and protein and therefore can be considered empty calories. When alcoholic beverage consumption for the study subjects was converted to intake of empty calories and related to the risk of esophageal cancer, the relative risk rose from 1.0 to 4.1 to 6.4 as the percentage of the estimated caloric intake of the average adult male⁷ that was being supplied by alcoholic beverages rose from less than 0.03% to 0.03-20% to 21-80% (Table 8).

Other case-control and cohort studies have demonstrated that broad dietary inadequacies are associated with esophageal cancer. In Iran, a history of low intake of vegetables and fruit was identified as a risk factor⁴⁸; in Japan, low intake of meat and fruit increased risk.²¹ In New York City, low intake of milk, vegetables, and fruit⁴⁹ and in upstate New York, low intake of vegetables and fruit⁵⁰ have been associated with increased risk of esophageal cancer. In addition, multiple nutrient deficiencies have been found in subpopulations at high risk of esophageal cancer: deficiencies of vitamin A, riboflavin, calcium, and protein in Puerto Rico;³⁹ deficiencies of vitamins A and C, riboflavin, and protein in Iran;³⁶ deficiencies of vitamin C, nicotinic acid, riboflavin, and calcium in South Africa;⁵¹ and deficiencies of vitamin C, riboflavin,

Table 8. Relative Risk of Esophageal Cancer for Consumption of Empty Calories in Alcoholic Beverages^a

Beer, Wine, and Hard Liquor Consumed Weekly, kcal	Caloric Needs Filled by Alcoholic Beverages, ^b %	No. of Cases, Controls	Relative Risk for Empty Calories (95% confidence interval)
<500	<.03	5, 55	1.0
500-3,360	.03-20	16, 43	4.1 (1.4, 12.1)
3,361-6,720	21-40	18, 31	6.4 (2.1, 18.8)
6,721-13,440	41-80	28, 49	6.3 (2.3, 17.6)
>13,440	>80	23, 35	7.2 (2.5, 20.8)

^aSee ref. 45.

^bDaily caloric need of each individual is assumed to be 2,400 kcal, based on the National Academy of Sciences' recommendation for U.S. males, 51-75 years of age.

calcium, and protein in China.³⁷ In addition to generally poor nutrition, other aspects of diet have been proposed as risk factors for esophageal cancer, though at present the evidence for them is more anecdotal than scientific. Among the hypotheses are molybdenum and zinc deficiencies,³⁵ silica fragments in staple grains,⁵² toxins in fermented and possibly moldy foods,^{37,53} and thermal irritation from drinking hot liquids.³⁵

FOLACIN DEFICIENCY AND CERVICAL CANCER

Folacin deficiency has recently been suggested as a risk factor for cervical cancer. In 1973, Whitehead et al.⁵⁴ observed megaloblastic features in cervical epithelial cells from a group of women who were using steroid hormones as oral contraceptives. Megaloblasts are the large, multinucleated, immature red blood cells noted in women with folacin deficiency. Although these megaloblastic changes in the cervical epithelium were not associated with systemic folacin deficiency, they were reversed with oral folate supplementation. The authors postulated that a localized folacin deficiency existed in the cervix as a result of oral contraceptive use.

The morphologic changes in cervical epithelium that are associated with folacin deficiency are known to be deceptively similar to the changes observed in cervical dysplasia.⁵⁵ Furthermore, Butterworth and colleagues⁵⁶ noted that plasma and red blood cell folacin levels were lower in oral contraceptive users than in nonusers and that oral contraceptive users with cervical dysplasia had the lowest values of all (Table 9). Therefore, they designed a clinical trial of oral folic acid supplementation in 47 women with mild or moderate cervical dysplasia. Subjects were given either 25 times the Recommended Dietary Allowance of folic acid or a vitamin C placebo every day for 3 months. All had been on oral contraceptives for at least 6 months and continued their use for the 3 months of the trial. During the trial, cervical smears were cytologically reviewed each month and a cervical biopsy was taken at the end of the trial. Results are shown in Fig. 1. Treatment with folate led to a gradual improvement in cytology scores, and the scores of the women taking the placebo were essentially unchanged. In addition, at the end of the study, the biopsy scores of the women on folate were significantly

Table 9. Blood Folacin Levels in Oral Contraceptive Users With and Without Cervical Dysplasia and in Nonusers Without Dysplasia^a

Subjects	No. of Subjects	Plasma Folacin, ng/ml	Red Blood Cell Folacin, ng/ml
Oral contraceptive users with cervical dysplasia	34	3.6±0.37 ^b	161±13
Oral contraceptive users without cervical dysplasia	20	5.22±0.69	189±16
Non-contraceptive users without cervical dysplasia	20	7.14±0.98	269±21

^aSee ref. 55.

^bValues are expressed as mean ± standard error of the mean.

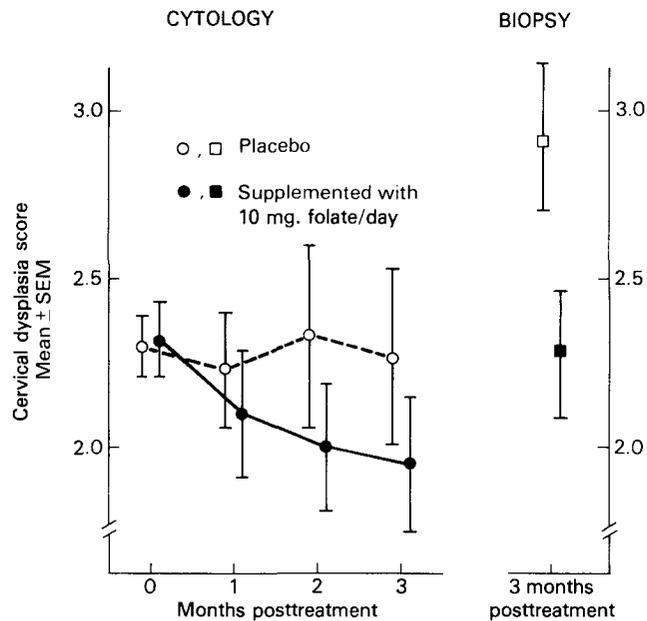


Fig. 1. Changes in cervical dysplasia in a clinical trial of oral folic acid supplementation in 47 oral contraceptive users with mild or moderate dysplasia. Cytology smears and biopsies were arbitrarily graded from 1 to 5 with 1 as negative, 2 as mild dysplasia, 3 as moderate dysplasia, 4 as severe dysplasia, and 5 as carcinoma in situ.

better than those of the women given the placebo. During the trial, the megaloblastic appearance of the cervical epithelium decreased in severity among the women on folate but increased in severity among the women given the placebo.

This clinical trial looks only at cervical dysplasia and its reversal and does not prove that low folacin levels are a risk factor for invasive cervical cancer. Nor does the study, which included only women on oral contraceptives, indicate whether women with cervical dysplasia who are not using oral contraceptives would respond similarly to folate supplementation. However, its findings are provocative.

Another suggestion that cervical cancer may be related to diet stems from its consistent association with socioeconomic status. Both within the U.S. and in other countries, cervical cancer rates are elevated in regions of low socioeconomic status.⁵⁷ In a recent case-control study within the U.S., the increased risk with low socioeconomic status was not explained by accepted risk factors, such as age at first intercourse, the number of sexual partners, smoking, or time since the last Pap smear.⁵⁸ In addition to folacin, other dietary factors have been proposed to increase the risk of cervical cancer. In 2 case-control studies, one in Italy with 191 cases of invasive cervical cancer⁵⁹ and the other in upstate New York with 513 cases of cervical cancer,⁶⁰ intake of foods high in carotenoids was associated with reduced risk. In a case-control study in New York City involving 87 patients with cervical dysplasia, dietary vitamin C was associated with reduced risk.⁶¹

Because of these indications that diet may be related to cervical cancer, Dr. Louise Brinton and I carried out a multicenter, population-based case-control study of cervical cancer within the U.S. Usual adult dietary patterns were assessed in an interview, and blood was collected, 6 months after completion of any treatment for cancer, for the measurement of retinol, β -carotene, total carotenoids, vitamin E, and folacin in serum and red blood cell folacin. Approximately 480 patients with invasive cervical cancer, 293 patients with in situ cervical cancer, and 799 controls were successfully interviewed. Analysis is now in progress.

CONCLUSION

It is crucial that epidemiologists and laboratory experimentalists continue to test each other's hypotheses about diet and cancer. However, the epidemiologist must be rigorous in interpreting results and in comparing them to the findings of other studies. For example, the studies on dietary carotenoids and vitamin A and risk of lung cancer are often said to agree even though some support a carotenoid hypothesis, some support a vitamin A hypothesis, and most did not separately test both hypotheses. Most of these studies did not evaluate whether the frequency of consumption of vegetables and fruit was a better predictor of reduced risk of lung cancer than an estimate of carotenoid intake. A vegetable and fruit association does not necessarily implicate β -carotene, because β -carotene is not the only putative protective agent in vegetables and fruit. Similarly, in discussions of esophageal cancer, emphasis is often placed on the deficiency of a specific nutrient, such as vitamin A or vitamin C, even though several major studies suggest that poor nutrition in general, involving the deficiency of several nutrients, is the risk factor. Epidemiologists delight in consistency among epidemiologic studies and between epidemiologic and laboratory findings and use such consistency as evidence for causality. Yet vague consistency with a hypothesis is not enough. If our epidemiologic findings are to be believed, we must be demanding in our study designs and analyses and objective in the interpretation of our results.

REFERENCES

1. K. L. Simpson and C. O. Chichester, Metabolism and nutritional significance of the carotenoids, Annu. Rev. Nutr. 1:351 (1981).
2. M. B. Sporn, N. M. Dunlop, D. L. Newton, et al., Prevention of chemical carcinogenesis by vitamin A and its synthetic analogs (retinoids), Fed. Proc. 35:1332 (1976).
3. R. Peto, R. Doll, J. D. Buckley, et al., Can dietary β -carotene materially reduce human cancer rates? Nature 290:201 (1981).
4. N. I. Krinsky and S. M. Dencke, The interaction of oxygen and oxyradicals with carotenoids, J. Natl. Cancer Inst. 69:205 (1982).
5. B. N. Ames, Dietary carcinogens and anticarcinogens, Science 221:1256 (1983).
6. U. S. Department of Agriculture, "Food and Nutrient Intakes of Individuals in One Day in the United States, Spring, 1977. Nationwide Food Consumption Survey 1977-1978. Preliminary Report No. 2," U.S.D.A., Washington, D.C. (1980).
7. National Academy of Sciences, "Recommended Dietary Allowances, 9th rev. ed.," N.A.S., Washington, D.C. (1980).
8. R. G. Ziegler, T. J. Mason, A. Stenhagen, et al., Carotenoid intake, vegetables, and the risk of lung cancer among white men in New Jersey, Am. J. Epidemiol., in press (1986).
9. G. Block, C. M. Dresser, A. M. Hartman, et al., Nutrient sources in the American diet: Quantitative data from the NHANES II survey. I. Vitamins and minerals, Am. J. Epidemiol. 122:13 (1985).
10. M. B. Sporn and D. L. Newton, Chemoprevention of cancer with retinoids, Fed. Proc. 38:2528 (1979).
11. E. Cameron, L. Pauling, and B. Leibovitz, Ascorbic acid and cancer: A review, Cancer Res. 39:663 (1979).
12. L. W. Wattenberg and W. D. Loub, Inhibition of polycyclic aromatic hydrocarbon-induced neoplasia by naturally occurring indoles, Cancer Res. 38:1410 (1978).
13. W. C. Willett, B. F. Polk, J. S. Morris, et al., Prediagnostic serum selenium and risk of cancer, Lancet 2:130 (1983).
14. G. R. Beecher and F. Khachik, Evaluation of vitamin A and carotenoid data in food composition tables, J. Natl. Cancer Inst. 73:1397 (1984).
15. I. Stewart, Provitamin A and carotenoid content of citrus juices, J. Agric. Food Chem. 5:1132 (1977).
16. M. Zakaria, K. Simpson, P. R. Brown, et al., Use of reversed-phase high-performance liquid chromatographic analysis for the determination of provitamin A carotenes in tomatoes, J. Chrom. 176:109 (1979).
17. E. Bjelke, Dietary vitamin A and human lung cancer, Int. J. Cancer 15:561 (1975).
18. R. MacLennan, J. Da Costa, N. E. Day, et al., Risk factors for lung cancer in Singapore Chinese, a population with high female incidence rates, Int. J. Cancer 20:854 (1977).
19. T. Hirayama, Diet and cancer, Nutr. Cancer 1:67 (1979).
20. C. Mettlin, S. Graham, and M. Swanson, Vitamin A and lung cancer, J. Natl. Cancer Inst. 62:1435 (1979).
21. A. Gregor, P. N. Lee, F. J. C. Roe, et al., Comparison of dietary histories in lung cancer cases and controls with special reference to vitamin A, Nutr. Cancer 2:93 (1980).
22. R. B. Shekelle, S. Liu, W. J. Raynor, Jr., et al., Dietary vitamin A and risk of cancer in the Western Electric study, Lancet 2:1185 (1981).
23. G. Kvale, E. Bjelke, and J. J. Gart, Dietary habits and lung cancer risk, Int. J. Cancer 31:397 (1983).
24. M. W. Hinds, L. N. Kolonel, J. N. Hankin, et al., Dietary vitamin A, carotene, vitamin C, and risk of lung cancer in Hawaii, Am. J. Epidemiol. 119:227 (1984).

25. H. B. Stahelin, E. Buess, F. Rosel, et al., Vitamin A, cardiovascular risk factors, and mortality. Lancet 1:394 (1982).
26. W. C. Willett, B. F. Polk, B. A. Underwood, et al., Relation of serum vitamins A and E and carotenoids to the risk of cancer, N. Engl. J. Med. 310:430 (1984).
27. I. Peleg, S. Heyden, M. Knowles, et al., Serum retinol and risk of subsequent cancer: Extension of the Evans County, Georgia, study, J. Natl. Cancer Inst. 73:1455 (1984).
28. N. Wald, M. Idle, J. Boreham, et al., Low serum vitamin A and subsequent risk of cancer, Lancet 2:813 (1980).
29. W. N. Pearson, Biochemical appraisal of nutritional status in man, Am. J. Clin. Nutr. 11:462 (1962).
30. W. C. Willett, M. J. Stampfer, B. A. Underwood, et al., Vitamins A, E, and carotene: Effects of supplementation on their plasma levels, Am. J. Clin. Nutr. 38:559 (1983).
31. W. C. Willett, M. J. Stampfer, B. A. Underwood, et al., Validation of a dietary questionnaire with plasma carotenoid and tocopherol levels, Am. J. Clin. Nutr. 38:631 (1983).
32. H. B. Stahelin, F. Rosel, E. Buess, et al., Cancer, vitamins, and plasma lipids: Prospective Basel study, J. Natl. Cancer Inst. 73:1463 (1984).
33. L. Heilbrun, A. Nomura, G. Stemmerman, et al., Serum vitamins and the risk of cancer (abstract), Am. J. Epidemiol. 120:491 (1984).
34. M. Menkes and G. W. Comstock, Vitamins A and E and lung cancer (abstract), Am. J. Epidemiol. 120:490 (1984).
35. N. E. Day and N. Munoz, Esophagus, in: "Cancer Epidemiology and Prevention," D. Schottenfeld and J. F. Fraumeni, Jr., eds., W. B. Saunders, Philadelphia (1982).
36. Joint Iran-International Agency for Research on Cancer Study Group, Esophageal cancer studies in the Caspian Littoral of Iran: Results of population studies--A prodrome, J. Natl. Cancer Inst. 59:1127 (1977).
37. D. S. Yang, Research on esophageal cancer in China: A review, Cancer Res. 40:2633 (1980).
38. N. I. Kolycheva, Epidemiology of esophageal cancer in the USSR, in: "Cancer Epidemiology in the USA and the USSR, DHHS Publication No. 80-2044," D. L. Levin, ed., DHHS, Washington, D.C. (1980).
39. I. Martinez, Factors associated with cancer of the esophagus, mouth, and pharynx in Puerto Rico, J. Natl. Cancer Inst. 42:1069 (1969).
40. S. C. Freni, Long-term trends in the incidence rates of upper digestive tract cancer in the Netherlands Antilles, Cancer 53:1618 (1984).
41. E. Rose, Esophageal cancer in the Transkei: 1955-69, J. Natl. Cancer Inst. 51:7 (1973).
42. J. F. Fraumeni, Jr., and W. J. Blot, Geographic variation in esophageal cancer mortality in the United States, J. Chronic Dis. 30:759 (1977).
43. L. G. Larsson, A. Sandstrom, and P. Westling, Relationship of Plummer-Vinson disease to cancer of the upper alimentary tract in Sweden, Cancer Res. 35:3308 (1975).
44. O. D. Harris, W. T. Cooke, H. Thompson, et al., Malignancy in adult coeliac disease and idiopathic steatorrhea, Am. J. Med. 42:899 (1967).
45. G. K. Holmes, P. L. Stokes, T. M. Sorahan, et al., Coeliac disease, gluten-free diet, and malignancy, Gut 17:612 (1976).
46. L. M. Potters, L. E. Morris, W. J. Blot, et al., Esophageal cancer among black men in Washington, D.C. I. Alcohol, tobacco, and other risk factors, J. Natl. Cancer Inst. 67:777 (1981).
47. D. Schottenfeld, Alcohol as a co-factor in the etiology of cancer, Cancer 43:1962 (1979).

48. P. J. Cook-Mozaffari, F. Azordegan, N. E. Day, et al., Oesophageal cancer studies in the Caspian Littoral of Iran: Results of a case-control study, Br. J. Cancer 39:293 (1979).
49. E. L. Wynder and I. J. Bross, A study of etiological factors in cancer of the esophagus, Cancer 14:389 (1961).
50. C. Mettlin, S. Graham, R. Priore, et al., Diet and cancer of the esophagus, Am. J. Epidemiol. 112:422 (1980).
51. G. Groenewald, M. L. Langenhoven, M. J. C. Beyers, et al., Nutrient intakes among rural Transkeians at risk for oesophageal cancer, S. Afr. Med. J. 60:964 (1981).
52. C. H. O'Neill, G. M. Hodges, P. N. Riddle, et al., A fine fibrous silica contaminant of flour in the high oesophageal cancer area of north-east Iran, Int. J. Cancer 26:617 (1980).
53. S. H. Lu, A. M. Camus, L. Tomatis, et al., Mutagenicity of extracts of pickled vegetables collected in Linhsien County, a high-incidence area for esophageal cancer in northern China, J. Natl. Cancer Inst. 66:33 (1981).
54. N. Whitehead, F. Reyner, and J. Lindenbaum, Megaloblastic changes in the cervical epithelium: Association with oral contraceptive therapy and reversal with folic acid, J.A.M.A. 226:1421 (1973).
55. L. G. Koss, "Diagnostic Cytology and Its Histopathologic Bases, 3d ed.," J. P. Lippincott, Philadelphia (1979).
56. C. E. Butterworth, Jr., K. D. Hatch, H. Gore, et al., Improvement in cervical dysplasia associated with folic acid therapy in users of oral contraceptives, Am. J. Clin. Nutr. 35:73 (1982).
57. D. W. Cramer, Uterine cervix, in: "Cancer Epidemiology and Prevention," D. Schottenfeld and J. F. Fraumeni, Jr., eds., W. B. Saunders, Philadelphia (1982).
58. L. A. Brinton, Current epidemiologic studies: Emerging hypotheses, in: "Banbury Report--Aetiology of Genital Cancer: Virological and Epidemiological Aspects," R. Peto and H. Zur Hausen, eds., Cold Spring Harbor, Cold Spring Harbor Laboratory (1986).
59. C. La Vecchia, S. Francheschi, A. Decarli, et al., Dietary vitamin A and the risk of invasive cervical cancer, Int. J. Cancer 34:319 (1984).
60. J. R. Marshall, S. Graham, T. Byers, et al., Diet and smoking in the epidemiology of cancer of the cervix, J. Natl. Cancer Inst. 70:847 (1983).
61. S. Wassertheil-Smoller, S. L. Romney, J. Wylie-Rosett, et al., Dietary vitamin C and uterine cervical dysplasia, Am. J. Epidemiol. 114:714 (1981).