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High Intake of Folate from Food Sources Is Associated with Reduced Risk of Esophageal Cancer in an Australian Population[1,2]

Torukiri I. Ibiebele,* Maria Celia Hughes, Nirmala Pandeya, Zhen Zhao, Grant Montgomery, Nick Hayward, Adèle C. Green, David C. Whiteman, and Penelope M. Webb, for the Study of Digestive Health and Australian Cancer Study[3]

Queensland Institute of Medical Research, Genetics and Population Health Division, Herston, Brisbane 4006, Australia

Abstract

Folate plays a key role in DNA synthesis and methylation. Limited evidence suggests high intake may reduce risks of esophageal cancer overall; however, associations with esophageal cancer subtypes and Barrett's esophagus (BE), a precancerous lesion, remain unexplored. We evaluated the relation between intake of folate, B vitamins, and methyl-group donors (methionine, choline, betaine) from foods and supplements, polymorphisms in key folate-metabolizing genes, and risk of BE, esophageal adenocarcinoma (EAC), and esophageal squamous cell carcinoma (ESCC) in 2 population-based case-control studies in Australia. BE patients without (n = 266) or with (n = 101) dysplasia were compared with population controls (n = 577); similarly, EAC (n = 636) or ESCC (n = 245) patients were compared with population controls (n = 1507) using multivariable adjusted logistic regression. Increasing intake of folate from foods was associated with reduced EAC risk (P-trend = 0.01) and mitigated the increased risks of ESCC associated with smoking and alcohol consumption. In contrast, high intake of folic acid from supplements was associated with a significantly elevated risk of BE with dysplasia. High intakes of riboflavin and methionine from food were associated with increased EAC risk, whereas increasing betaine intake was associated with reduced risks of BE without (P-trend = 0.004) or with dysplasia (P-trend = 0.02). Supplemental thiamin, riboflavin, niacin, and vitamin B-12 were associated with increased EAC risk. There were no consistent associations between genetic polymorphisms studied and BE or EAC risk. High intake of folate-containing foods may reduce risk of EAC, but our data raise the possibility that folic acid supplementation may increase risks of BE with dysplasia and EAC. J. Nutr. 141: 274–283, 2011.

Introduction

Cancer of the esophagus is the 5th leading cause of cancer-related death among men and the 7th leading cause of cancer death among women globally (1). There are 2 major histologic types: esophageal adenocarcinoma (EAC)[4] and esophageal squamous cell carcinoma (ESCC). In high-income regions of the world, the incidence of ESCC is declining, whereas that of EAC is increasing rapidly, particularly among white males in Western Europe, North America, and Australia (2–7). ESCC is associated with alcohol consumption and smoking (8–10) and risk factors for EAC include gastroesophageal reflux disease, smoking, and obesity (8,11–15). Long-standing gastroesophageal reflux disease predisposes to Barrett's esophagus (BE), a condition in which metaplastic epithelium resembling the intestine replaces the stratified squamous epithelium that normally lines the distal esophagus (16). BE may progress to dysplasia and invasive adenocarcinoma. The factors that determine progression from BE to cancer are not clear, although micronutrient intake may be important (17).

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[2] Author disclosures: T. I. Ibiebele, M. C. Hughes, N. Pandeya, Z. Zhao, G. Montgomery, N. Hayward, A. C. Green, D. C. Whiteman, and P. M. Webb, no conflicts of interest.

[3] A full list of investigators and contributors to the Study of Digestive Health and Australian Cancer Study is available with the online posting of this paper at jn.nutrition.org.

* To whom correspondence should be addressed. E-mail: Torukiri.Ibiebele@qimr.edu.au.

[4] Abbreviations used: ACS, Australian Cancer Study; BE, Barrett's esophagus; EAC, esophageal adenocarcinoma; EGJAC, esophagogastric junction adenocarcinoma; ESCC, esophageal squamous cell carcinoma; NSAID, nonsteroidal antiinflammatory drug; SDH, Study of Digestive Health; SNP, single nucleotide polymorphism; WCRF, World Cancer Research Fund.

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Folate is a water-soluble B vitamin that occurs naturally in dark green leafy vegetables, dry peas and beans, and citrus fruits. The essential role of folate in preventing neural tube defect has led many countries, including Australia (18,19), to introduce folic acid fortification of certain foods, including ready-to-eat breakfast cereals, fruit juices, and flour. Folate also plays a key role in DNA synthesis and methylation, and adequate intake is essential to maintain DNA integrity, stability and repair (20); thus, folate deficiency may increase cancer risk. The 2007 World Cancer Research Fund (WCRF) review of nutrition and cancer concluded that there was some evidence that foods containing folate might protect against pancreatic and colorectal cancers and might partially mitigate the increased risk of breast cancer associated with increasing alcohol intake (7). However, DNA synthesis is also a key component of cancer development, and antifolates have long been used to treat some types of cancer, including leukemias and breast and lung cancer. Recent studies have suggested that high folate levels might actually increase cancer incidence (21) or promote progression of preneoplastic lesions to cancer (22) and it has been suggested that the introduction of mandatory folate fortification in Canada and the US may explain increases in colorectal cancer rates (23).

Besides folate, other nutrients, including methionine, choline, and betaine, are reported to be involved in one-carbon metabolism (24–26), a network of interrelated biochemical pathways that generate one-carbon groups needed for physiologic processes (27,28). The coenzymes necessary for several of these pathways include riboflavin vitamin B-6 and vitamin B-12 (27). Furthermore, polymorphisms in key folate- and methyl-metabolizing genes have been hypothesized to influence susceptibility to cancer (25,29,30). Other potential modifiers include alcohol, which blocks folate uptake (31), and smoking, which reduces the level of folate in the plasma (32,33).

The role of folate in esophageal carcinogenesis is unclear. Results of epidemiological studies investigating the association between folate intake and EAC (34–37) or ESCC risks (36,38–41) have been mixed. The 2007 WCRF report concluded there was limited evidence that high intake of folate-containing foods was associated with reduced risks of cancer of the esophagus (7), but there was insufficient evidence to consider whether such effects might vary for EAC and ESCC. Furthermore, few studies have considered potential interactions between folate intake, other micronutrients, environmental factors and genotype, or were considered the effect of folate on BE. We report the findings of an investigation into the association between intake of folate, B vitamins, and methyl donors, common polymorphisms in the methylene tetrahydrofolate reductase (MTHFR), methylene synthase (MTR), and methylene synthase reductase (MTRR) genes and other environmental factors, including alcohol and smoking and risk of EAC, its precursor BE, and ESCC.

Materials and Methods

Study population. Data for the present study came from 2 studies that were conducted concurrently. The Study of Digestive Health (SDH) was a population-based case-control study of BE (42). In summary, eligible cases were people aged 18−79 y living in the Greater Brisbane area with a new diagnosis of histologically confirmed BE or BE with dysplasia between 2003 and 2006. BE was defined as the presence of specialized intestinal metaplasia or columnar epithelium with goblet cells in a biopsy taken from the esophagus by upper gastrointestinal endoscopy, regardless of the length of involvement (43). Eligible patients were prospectively identified through the 2 major private pathology laboratories and a single public pathology laboratory serving metropolitan Brisbane (population 1.5 million) during the study period. We excluded all those with a previous diagnosis of BE who did not have a first diagnosis of dysplasia during the recruitment period (prevalent cases), those who did not speak English, or those who were too ill to participate. Of 479 eligible cases, 393 (82%) took part in this study. Control participants, sampled from the same geographic regions as cases, were randomly selected from the Australian Electoral Roll (enrolment is compulsory) and broadly matched by age (in 5-y age groups) and sex to the BE case series. Of a total of 1116 potential controls contacted, 646 (58%) agreed to take part.

Australian Cancer Study (ACS) was a population-based case-control study of esophageal cancer, the details of which have been described in full elsewhere (15). Briefly, participants aged 18–79 y with a histologically confirmed primary invasive EAC or esophagogastric junction adenocarcinoma (EGJAC) or ESCC of the esophagus diagnosed between 2002 and 2005, were recruited either through major treatment centers or state-based cancer registries (cancer notification is mandatory in Australia). A total of 1577 patients with esophageal cancer received an invitation to participate in the study, of whom 1102 patients (858 through clinics and 244 through cancer registries) returned a completed questionnaire (70% of all invited; 35% of all living and deceased persons in mainland Australia who had been diagnosed with incident esophageal cancer). Seven case patients were subsequently deemed ineligible on pathology review and were excluded from analysis, leaving 364 EAC cases, 425 EGJAC, and 306 ESCC patients. ACS control participants were randomly selected from the Australian Electoral Roll within strata of age (in 5-y age groups) and state of residence to the distributions of BE and cancer case series. We aimed for similar numbers of male cases and controls in each stratum of age and state; female controls were oversampled for a parallel study of ovarian cancer. Of 3042 potential controls meeting the inclusion criteria, 1680 (55%) gave consent to take part and 1580 returned completed main questionnaires.

Participants provided informed written consent and both studies were approved by the human research ethics committee of the Queensland Institute of Medical Research and all participating institutions.

Data collection. We collected data from all participants via a self-administered questionnaire. Information was collected on education as well as height and weight 1 y previously (1 y before diagnosis for cases). Participants were asked whether, over their whole life, they had ever smoked >100 cigarettes, cigars, or pipes; positive responses elicited further questions about ages started and stopped smoking and typical daily consumption. Pack-years of smoking were derived from duration and intensity of smoking. We assessed the frequency of symptoms of gastroesophageal reflux, defined as the presence of heartburn ('a burning pain behind the breastbone after eating') or acid reflux ('a sour taste from acid or bile rising up into the mouth or throat') 10 y before diagnosis. Participants were also asked to report the frequency with which they consumed different types of alcoholic beverages between ages 20 and 29, 30 and 49, and ≥50 y, as applicable. Total alcohol consumption was summed across all age groups and calculated as the mean number of standard drinks (10 g ethanol) consumed per week between age 20 y and current age. We also obtained information on intake of nonsteroidal antiinflammatory drugs (NSAID).

Dietary assessment. Dietary data were obtained using a 135-item semiquantitative FFQ that asked participants how often they consumed a specified amount of each food item in the previous year (controls) or in the year before their diagnosis (cases). Participants who reported that their diet had changed in the last year or 2 were asked to report their usual diet before the change. The FFQ was based on the instrument developed by Willett et al. (44) but modified and calibrated against weighed food records (45–47) and serum biomarkers (48) and found to be reproducible (49) for use in Australia. The FFQ had 9 possible response options for intake of each item, ranging from never to 4+ times/d. Reported frequencies were converted into intake in grams per day by multiplying the standard serving size of each food item as specified in the FFQ by the frequency of consumption. Dietary intakes of total energy, folic acid added to food, folate, thiamin, riboflavin, niacin (thiamin and niacin were included for completeness), vitamin B-6, vitamin B-12, and methionine were estimated using the 2007 electronic release version of the Australian food composition tables (NUTTAB 2006) (50).

The correlation coefficients of agreement between nutrients estimated using semiquantitative FFQ and nutrients estimated using a mean of 12 d of weighed food records were moderate (51) and are as follows: $r = 0.46$ for thiamin, $r = 0.58$ for riboflavin, $r = 0.42$ for niacin, and $r = 0.45$ for protein (47), while the measure of agreement obtained from administering the same FFQ 1 y apart was $r = 0.58$ for folate (49). The USDA database for choline content of common foods Release2 (2008) was used to estimate intakes of betaine and total choline (calculated as the sum of choline moieties from free choline, phosphocholine, glycerophosphocholine, phosphatidylcholine, and sphingomyelin). To correct intake of micronutrients for total energy intake, we used the regression-residual method described by Willett and Stampfer (52). Participants were asked to report whether they regularly took any multivitamin or vitamin B supplements and, if they did, the brand, type, strength, and number of tablets taken per week were queried. Information regarding ingredients was obtained from the Australian Register of Therapeutic goods database as reported by product sponsors and translated into a common unit for each substance following a process previously reported by Ashton et al. (53)

The nutrition component of the ACS began 6 mo after the main study commenced and no attempt was made to collect dietary data from those who had already taken part. Cases that did not complete an FFQ and those who completed FFQ did not differ significantly in age, gender, education, BMI, smoking status, reflux symptoms, and reported lifetime intake of alcohol. Controls differed only in terms of lifetime alcohol intake and reflux symptoms; controls were less likely to drink alcohol or have reflux symptoms.

Exclusions and final sample size. For nutrient analyses, we excluded participants who did not complete a FFQ (SDH: 60 controls, 12 BE without dysplasia and 6 BE with dysplasia; ACS: 47 controls, 45 EAC, 68 EGJAC, and 39 ESCC). We also excluded those who missed >10% of food items on the FFQ (SDH: 1 control, 0 cases; ACS: 5 controls, 12 EAC, 13 EGJAC, and 10 ESCC) or had implausible total daily energy intake defined as MJ <3.36 or >21.0 for men and <2.94 or >16.8 for women (SDH: 8 controls, 7 BE without dysplasia and 1 BE with dysplasia; ACS: 21 controls, 8 EAC, 7 EGJAC, and 12 ESCC). For SDH this left 577 controls, 266 BE without dysplasia and 101 BE with dysplasia cases for analyses and for ACS there were 1507 controls, 299 EAC, 337 EGJAC, and 245 ESCC cases. For EAC analyses, we included patients with adenocarcinomas arising in both the esophagus and esophagogastric junction, because they share similar histology and incidence patterns (9,54).

Single nucleotide polymorphism selection and genotyping. DNA samples for genotyping single nucleotide polymorphisms (SNP) were selected on the basis that they were associated with altered enzyme activity and previously had been associated with cancer risk (29,55–58). Four SNP were selected from 3 key folate and methyl group-metabolizing genes: *MTHFR*, (A1298C, rs1801131, and C677T, rs1801133), *MTRR* (A66G, rs1801394), and *MTR* (A2756G, rs1805087). Genotyping was conducted using the Sequenom iPLEX protocol (Sequenom) as previously described (59). Similar to the dietary data, blood samples for DNA extraction were not available for all participants; we had SNP data for 514 controls, 242 BE without dysplasia and 93 BE with dysplasia cases for SDH; and 1323 controls, 518 EAC and 193 ESCC cases for ACS.

Statistical analyses. We used unconditional multivariable-adjusted logistic regression to estimate OR and 95% CI. Energy-adjusted micronutrients were categorized into 4 equal groups using quartile cutpoints based on the distribution among ACS controls. Quartile 1 served as referent category for all regression analyses. We simultaneously adjusted for the confounding effects of age (y), sex (male, female), BMI 1 y previously (<25, 25.0–29.9, ≥30.0), education (high school only, technical college or diploma, university), frequency of heartburn or acid reflux symptoms in the 10 y before diagnosis (never, less than monthly, less than weekly, more than weekly, daily), pack-years of smoking (0, 1–14.9, 15–29.9, ≥30), average lifetime alcohol intake (never, <1–6, 7–20, ≥21 standard drinks/wk), NSAID use in the past 5 y (never, occasionally, less than weekly, at least weekly), and total energy intake (kJ). To test for linear trends, categorical variables were included in the multivariable model as ordinal variables (with category values taking the median of the range for the group). Chi-squared statistics were used to compare proportions in 2 or more groups.

In separate analyses, tests for multiplicative interactions between SNP in the MTHFR, MTRR, and MTR genes and quartiles of food folate, each of the B-vitamins and each of the methyl donors were performed by including a product term between each SNP and micronutrient in relation to BE, EAC, and ESCC in a multivariable logistic regression model. If interactions were present, we stratified the analysis by high and low levels of intake of the micronutrients. To assess biological (additive) interactions among food folate, alcohol, and smoking, we created new variables that reclassified participants according to their combined levels of exposure to these factors (viz. low/low, low/high, high/low, high/high). Median folate and micronutrient levels were used to define cutpoints for high and low intake, smoking status was categorized as ever/never, and 170 g/wk was used as the cutpoint for alcohol intake, because alcohol intake above this level previously has been associated with increased esophageal cancer risk in this population (60). Intake of supplements (apart from folic acid from fortified foods) was categorized as never use, low intake, and high intake. Risks for each category of combined exposure were estimated relative to the reference (low/low) category.

All analyses were performed using SAS version 9.1 (SAS Institute) and a 2-sided $P < 0.05$ was considered significant.

Results

Overall, participants with cancer were less likely to be 50 y or younger and less likely to have a university degree than BE cases and controls (**Table 1**). BE and EAC cases were more likely to be obese (BMI ≥ 30) and to have more frequent symptoms of daily reflux than ESCC cases and controls. ESCC and EAC cases were more likely to smoke ≥30 pack-years of cigarettes and consume ≥21 standard drinks/wk of alcoholic beverages than BE cases and controls.

Intake of micronutrients from food. Increasing intake of food folate was inversely associated with the risk of EAC (*P*-trend = 0.01) (**Table 2**). We found a borderline increased risk of EAC [OR = 1.32 (95%CI = 0.98–1.80); *P*-trend = 0.07] among participants with high intake of dietary riboflavin relative to low intake, whereas high intake of niacin and vitamin B-6 [OR = 0.53 (95% CI = 0.39–0.74)] was associated with decreased risk. Participants with high intakes of thiamin had decreased ESCC risk (*P*-trend = 0.001). Increasing intake of dietary methionine was associated with increased EAC risk (*P*-trend = 0.04), whereas increasing intake of betaine from food was inversely associated with BE with (*P*-trend = 0.02) or without dysplasia (*P*-trend = 0.004).

Intake of micronutrients from supplements. We found no significant association between folic acid from fortified foods and risks of BE or esophageal cancers (**Table 3**). However, compared with never users, high intake of folic acid from supplement was associated with nonsignificantly increased risk of EAC (*P*-trend = 0.08); additionally, intakes of thiamin (*P*-trend = 0.003), riboflavin (*P*-trend = 0.002), niacin (*P*-trend = 0.008), vitamin B-6 (*P*-trend = 0.03), and vitamin B-12 (*P*-trend = 0.004) from supplements were associated with increased EAC risk. Similarly, high intake of folic acid intake from supplements was associated with increased risk of BE with dysplasia [OR = 2.18 (95% CI = 1.01–4.71)]. Intakes of micronutrients from supplements were not associated with BE without dysplasia or ESCC (Table 3).

TABLE 1 Characteristics of BE and esophageal cancer cases and controls

	SDH				ACS			
	Controls	BE without dysplasia	BE with dysplasia	*P*-value[1]	Controls	EAC	ESCC	*P*-value[1]
		n (%)				*n (%)*		
n	577	266	101		1507	636	245	
Age, y				0.08				<0.0001
<50	110 (19.0)	54 (20.3)	14 (13.9)		268 (17.8)	55 (8.6)	17 (6.8)	
50–59	200 (34.6)	84 (31.6)	24 (23.8)		393 (26.1)	178 (28.0)	59 (24.3)	
60–69	179 (31.0)	84 (31.6)	37 (36.6)		507 (33.6)	220 (34.6)	85 (34.7)	
≥70	89 (15.4)	44 (16.5)	26 (25.7)		339 (22.5)	183 (28.8)	84 (34.3)	
Gender				0.02				<0.0001
Male	369 (64.0)	168 (63.2)	79 (78.2)		996 (66.1)	560 (88.1)	147 (60.0)	
Female	208 (36.0)	98 (36.8)	22 (21.8)		511 (33.9)	79 (12.0)	98 (40.0)	
Educational status				<0.001				<0.0001
High school only	226 (39.3)	130 (48.9)	35 (34.7)		616 (40.9)	266 (41.8)	140 (57.1)	
Technical/diploma	224 (38.8)	101 (38.0)	54 (53.5)		657 (43.6)	315 (49.0)	84 (34.3)	
University	127 (22.0)	35 (13.2)	12 (11.9)		234 (15.5)	55 (8.7)	21 (8.6)	
BMI, kg/m^2				0.02				<0.0001
<25.0,	211 (37.0)	76 (29.1)	27 (27.0)		541 (36.2)	150 (24.1)	130 (55.6)	
25–29.9	235 (41.2)	105 (40.2)	47 (47.0)		642 (42.9)	253 (40.7)	70 (29.9)	
≥30.0	124 (21.9)	80 (30.7)	26 (26.0)		312 (20.9)	219 (35.2)	34 (14.5)	
Heartburn or acid reflux symptoms in previous 10 y				<0.001				<0.0001
Never	282 (49.0)	40 (15.2)	10 (10.0)		649(43.1)	162 (25.6)	111 (45.9)	
<1/mo	156 (27.0)	36 (13.6)	17 (17.0)		460 (30.5)	94 (14.8)	31 (12.8)	
≥1/mo but <1/wk	80 (13.8)	74 (28.0)	25 (25.0)		218 (14.5)	143 (22.6)	32 (13.2)	
Daily	59 (10.2)	114 (43.2)	48 (48.0)		180 (11.9)	235 (37.1)	68 (28.1)	
Pack-years of smoking				<0.001				<0.0001
Never smoked	307 (53.1)	90 (33.8)	23 (22.8)		673 (44.7)	157 (24.7)	56 (23.1)	
>0–14.9	113 (19.6)	63 (23.7)	24 (23.8)		380 (25.2)	127 (20.0)	48 (19.8)	
15–29.9	67 (11.6)	54 (20.3)	27 (26.7)		199 (13.2)	123 (20.8)	49 (20.2)	
≥30.0	90 (15.7)	59 (22.2)	27 (26.7)		255 (16.9)	220 (34.6)	90 (37.0)	
Alcohol intake,[2] standard drinks/wk				0.17				<0.0001
None	65 (11.3)	36 (13.6)	4 (4.0)		161 (10.7)	50 (7.9)	31 (12.7)	
<1–6	221 (38.4)	104 (39.3)	40 (40.0)		573 (38.0)	182 (28.7)	65 (26.6)	
7–20	180 (31.3)	69 (26.0)	32 (32.0)		482 (32.0)	222 (35.0)	51 (20.9)	
≥21	110 (19.1)	56 (21.1)	24 (24.0)		291 (19.3)	181 (28.5)	97 (39.8)	
NSAID use in previous 5 y				0.16				0.23
Never	270 (46.8)	129 (48.5)	49 (48.5)		657 (43.7)	2968 (47.0)	124 (51.5)	
Occasionally	177 (30.7)	70 (26.3)	28 (27.7)		472 (31.4)	173 (27.5)	66 (27.4)	
Less than weekly	62 (10.8)	20 (7.5)	7 (6.9)		145 (9.7)	57 (9.1)	19 (7.9)	
At least weekly	68 (11.8)	47 (17.7)	17 (16.8)		228 (15.2)	114 (16.5)	32 (13.3)	

[1] *P*-value from chi-square test used to compare frequencies or proportions in 2 or more groups.
[2] One standard drink contains 10 g of ethanol.

Folate-metabolizing genes. The genotype frequency distribution for SDH and ACS controls were very similar and there was no deviation from Hardy-Weinberg equilibrium in either control group. The homozygous CC variant of the *MTHFR* A1298C polymorphism was associated with an increased risk of ESCC [OR = 1.79 (95% CI = 1.03–3.13)] compared with AA homozygotes (**Table 4**). Other than this, we found no consistent evidence of increased or decreased risks of BE, EAC, or ESCC associated with any of the polymorphisms studied, notwithstanding a small number of significant associations. For the *MTHFR* C677T polymorphism, we observed a significantly increased risk of BE with dysplasia among CT heterozygotes but a significantly decreased risk among TT homozygotes compared with the CC variant. The *MTRR* A66G and *MTR* A2756G polymorphisms were not associated with risk of BE or esophageal cancer. We investigated the combined effect of the variant alleles compared with the wild type for MTHFR 1298 and 677 genotypes separately and found no significant association between the genotypes and risk of BE or esophageal cancer. In a diplotype analysis, we further investigated the association between the sum of the variant alleles for MTHFR 1298 and 677 genotypes compared with the wild types on risk of BE and the esophageal cancers and found no significant association. There was no indication of a multiplicative interaction between any of the genetic polymorphisms studied and food folate, B vitamins, or one-carbon group donors in relation to risk of BE or esophageal cancer (data not shown).

Alcohol, smoking, and food folate. Consistent with our previous observation (60), we found a strong positive association between alcohol intake ≥ 170 g/wk and risk of ESCC. When participants were cross-classified according to their intakes of both alcohol and folate, we found that the increased risk of ESCC associated with alcohol was restricted to those with

TABLE 2 Associations between food sources of folate, other B-vitamins, methyl group donors, and risk of BE and esophageal cancers in SDH and ACS participants

	SDH					ACS				
	Controls,	BE no dysplasia		BE with dysplasia		Controls,	EAC		ESCC	
Nutrients[1]	*n*	*n*	OR (95% CI)[2]	*n*	OR (95% CI)[2]	*n*	*n*	OR (95% CI)[2]	*n*	OR (95% CI)[2]
Food folate, *μg/d*										
Q1 [196 (42–230)]	160	70	1.0 (ref)	35	1.0 (ref)	372	191	1.0 (ref)	76	1.0 (ref)
Q2 [255 (230–282)]	146	69	1.22 (0.75–1.98)	27	0.81 (0.38–1.71)	369	182	1.01 (0.76–1.34)	63	1.11 (0.73–1.67)
Q3 [306 (282–336)]	124	61	1.62 (0.97–2.72)	18	0.75 (0.32–1.75)	375	123	0.71 (0.52–0.96)	30	0.45 (0.27–0.73)
Q4 [379 (336–673)]	139	58	1.17 (0.70–1.96)	18	0.76 (0.33–1.76)	374	117	0.72 (0.53–0.98)	56	0.78 (0.51–1.19)
P-trend			0.44		0.50			0.01		0.06
Thiamin, *mg/d*										
Q1 [1.3 (0.4–1.5)]	141	66	1.0 (ref)	31	1.0 (ref)	372	165	1.0 (ref)	67	1.0 (ref)
Q2 [1.6 (1.5–1.8)]	152	71	1.13 (0.69–1.83)	31	1.30 (0.62–2.72)	374	152	1.03 (0.77–1.39)	60	0.86 (0.57–1.30)
Q3 [1.9 (1.8–2.1)]	159	79	1.36 (0.84–2.22)	22	0.95 (0.42–2.14)	372	149	1.16 (0.86–1.56)	41	0.79 (0.52–1.20)
Q4 [2.4 (2.1–5.8)]	117	42	1.12 (0.63–1.96)	15	1.49 (0.61–3.64)	372	147	0.78 (0.57–1.07)	57	0.41 (0.25–0.67)
P-trend			0.53		0.53			0.18		0.001
Riboflavin, *mg/d*										
Q1 [1.6 (0.5–1.8)]	124	56	1.0 (ref)	28	1.0 (ref)	372	150	1.0 (ref)	71	1.0 (ref)
Q2 [2.0 (1.8–2.2)]	133	67	1.21 (0.73–2.02)	30	0.84 (0.38–1.82)	372	156	1.16 (0.86–1.57)	55	0.84 (0.55–1.29)
Q3 [2.5 (2.2–2.7)]	160	65	1.11 (0.67–1.84)	23	0.83 (0.38–1.83)	376	161	1.21 (0.90–1.63)	49	0.81 (0.52–1.26)
Q4 [3.1 (2.7–7.1)]	152	70	0.92 (0.55–1.53)	18	0.62 (0.27–1.42)	372	146	1.32 (0.98–1.80)	50	0.78 (0.50–1.21)
P-trend			0.60		0.27			0.07		0.27
Niacin, *mg/d*										
Q1 [19 (4.3–21)]	120	57	1.0 (ref)	28	1.0 (ref)	372	178	1.0 (ref)	57	1.0 (ref)
Q2 [23 (21–24)]	157	66	0.89 (0.54–1.48)	29	1.29 (0.59–2.80)	374	159	1.06 (0.79–1.41)	67	1.21 (0.79–1.87)
Q3 [26 (24–28)]	133	75	1.19 (0.72–1.97)	21	0.81 (0.35–1.87)	372	133	0.89 (0.66–1.20)	52	1.35 (0.88–2.06)
Q4 [30 (28–50)]	159	60	1.03 (0.61–1.73)	21	0.97 (0.42–2.22)	372	143	0.71 (0.52–0.96)	49	0.69 (0.43–1.12)
P-trend			0.67		0.67			0.02		0.23
Vitamin B-6, *mg/d*										
Q1 [0.93 (0.3–1.1)]	151	78	1.0 (ref)	34	1.0 (ref)	373	197	1.0 (ref)	57	1.0 (ref)
Q2 [1.2 (1.1–1.3)]	148	63	0.86 (0.53–1.39)	23	0.59 (0.27–1.28)	371	153	0.80 (0.60–1.07)	69	1.04 (0.67–1.61)
Q3 [1.4 (1.3–1.5)]	144	59	0.97 (0.60–1.59)	26	0.93 (0.43–2.05)	375	117	0.83 (0.62–1.11)	47	1.02 (0.66–1.58)
Q4 [1.7 (1.5–3.0)]	126	58	1.11 (0.67–1.83)	16	0.65 (0.27–1.55)	371	146	0.53 (0.39–0.74)	52	0.66 (0.42–1.05)
P-trend			0.62		0.50			0.002		0.08
Vitamin B-12, *μg/d*										
Q1 [0.8 (0.0–1.1)]	136	63	1.0 (ref)	25	1.0 (ref)	370	149	1.0 (ref)	63	1.0 (ref)
Q2 [1.3 (1.1–1.5)]	138	61	1.19 (0.72–1.98)	18	0.67 (0.28–1.59)	372	151	0.81 (0.59–1.09)	50	0.79 (0.51–1.23)
Q3 [1.8 (1.5–2.1)]	142	54	0.85 (0.50–1.42)	26	1.08 (0.48–2.47)	375	158	0.87 (0.64–1.17)	47	0.78 (0.50–1.22)
Q4 [2.5 (2.1, 7.8)]	153	80	1.41 (0.86–2.32)	30	1.66 (0.75–3.70)	373	155	0.96 (0.71–1.30)	65	0.89 (0.58–1.32)
P-trend			0.26		0.10			0.96		0.74
Methionine, *mg/d*										
Q1 [938 (256–1069)]	113	53	1.0 (ref)	30	1.0 (ref)	370	144	1.0 (ref)	65	1.0 (ref)
Q2 [1174 (1069–1259)]	135	61	1.10 (0.65–1.87)	17	0.53 (0.22–1.27)	374	146	1.15 (0.85–1.56)	51	0.76 (0.49–1.17)
Q3 [1361 (1259–1482)]	151	64	0.93 (0.55–1.56)	24	0.73 (0.33–1.62)	371	168	1.18 (0.87–1.60)	58	1.02 (0.71–1.67)
Q4 [1667 (1482–3068)]	170	80	0.91 (0.55–1.52)	28	0.87 (0.39–1.93)	375	155	1.39 (1.02–1.89)	51	0.84 (0.51–1.32)
P-trend			0.57		0.98			0.04		0.74
Betaine, *mg/d*										
Q1 [85 (36–100)]	218	119	1.0 (ref)	53	1.0 (ref)	373	175	1.0 (ref)	49	1.0 (ref)
Q2 [112 (100–124)]	151	68	0.66 (0.42–1.02)	22	0.54 (0.28–1.03)	370	146	0.88 (0.65–1.19)	49	0.95 (0.60–1.52)
Q3 [138 (124–154)]	104	48	0.73 (0.45–1.20)	14	0.54 (0.26–1.12)	372	142	0.75 (0.56–1.03)	42	0.72 (0.44–1.18)
Q4 [185 (154, 725)]	96	23	0.40 (0.22–0.74)	10	0.41 (0.18–0.96)	375	150	0.79 (0.58–1.07)	85	1.30 (0.83–1.99)
P-trend			0.004		0.02			0.12		0.21
Choline, *mg/d*										
Q1 [380 (124–435)]	122	54	1.0 (ref)	26	1.0 (ref)	373	107	1.0 (ref)	37	1.0 (ref)
Q2 [510 (435–618)]	150	63	0.62 (0.37–1.06)	21	0.54 (0.23–1.25)	370	165	1.34 (0.97–1.85)	63	1.67 (1.03–2.69)
Q3 [754 (618–1003)]	150	68	0.78 (0.46–1.32)	22	0.72 (0.31–1.70)	370	169	1.25 (0.91–1.73)	61	1.48 (0.91–2.40)
Q4 (1171 (1003–2984)]	147	73	0.69 (0.41–1.16)	30	0.59 (0.26–1.34)	373	172	1.25 (0.91–1.72)	64	1.39 (0.88–2.30)
P-trend			0.45		0.44			0.48		

[1] Values are [medians (range)]. Nutrients were categorized into quartiles based on intake in controls and corrected for energy intake using the residual method described by Willett and Stampfer (52).

[2] Multivariable adjustment for age, gender, education, BMI 1 y previously, frequency of heartburn or acid reflux 10 y prior to diagnosis, lifetime alcohol intake, pack-years of smoking, NSAID use, and total energy intake.

TABLE 3 Associations between supplemental sources of folic acid, other B vitamins, methyl group donors, and risk of BE and esophageal cancers in SDH and ACS participants

	SDH					ACS				
	Controls,	BE no dysplasia		BE with dysplasia		Controls,	EAC		ESCC	
Supplements[1]	*n*	*n*	OR (95% CI)[2]	*n*	OR (95% CI)[2]	*n*	*n*	OR (95% CI)[2]	*n*	OR (95% CI)[2]
Folic acid from fortified foods,[5] μg/d										
Q1 [6.8 (0–22)]	132	57	1.0 (ref)	32	1.0 (ref)	374	136	1.0 (ref)	67	1.0 (ref)
Q2 [39 (22–59)]	145	77	1.10 (0.68–1.79)	23	0.61 (0.27–1.34)	373	180	1.53 (1.13–2.07)	64	1.15 (0.76–1.74)
Q3 [80 (59–109)]	165	71	1.13 (0.69–1.85)	23	0.16 (0.28–1.33)	372	170	1.27 (0.94–1.73)	51	0.89 (0.57–1.37)
Q4 [149 (109–672)]	127	53	1.20 (0.71–2.03)	20	0.93 (0.41–2.12)	371	127	1.18 (0.86–1.62)	42	0.77 (0.49–1.21)
P−trend			0.51		0.97			0.79		0.14
Folic acid from supplements[1]										
Never use	438	202	1.0 (ref)	72	1.0 (ref)	1200	506	1.0 (ref)	190	1.0 (ref)
Low intake[3]	81	32	0.81 (0.47–1.37)	15	1.37 (0.64–2.94)	165	66	1.30 (0.92–1.83)	23	0.86 (0.51–1.45)
High intake[4]	58	32	1.07 (0.62–1.87)	14	2.18 (1.01–4.71)	142	64	1.29 (0.90–1.85)	32	1.39 (0.85–1.26)
P−trend			0.94		0.04			0.08		0.34
Thiamin from supplements[1]										
Never use	436	207	1.0 (ref)	74	1.0 (ref)	1214	506	1.0 (ref)	193	1.0 (ref)
Low intake[3]	71	27	0.68 (0.38–1.19)	12	1.15 (0.52–2.51)	144	58	1.12 (0.78–1.61)	22	0.91 (0.53–1.57)
High intake[4]	70	32	0.88 (0.51–1.52)	15	1.74 (0.82–3.70)	149	72	1.73 (1.22–2.46)	30	1.22 (0.75–1.98)
P-trend			0.39		0.16			0.003		0.55
Riboflavin from supplements[1]										
Never use	425	206	1.0 (ref)	72	1.0 (ref)	1189	491	1.0 (ref)	198	1.0 (ref)
Low intake[3]	96	31	0.59 (0.35–1.00)	16	1.18 (0.58–2.40)	168	70	1.19 (0.85–1.67)	19	0.62 (0.35–1.11)
High intake[4]	56	29	0.93 (0.52–1.66)	13	1.94 (0.86–4.37)	150	75	1.72 (1.22–2.44)	28	1.08 (0.66–1.77)
P−trend			0.33		0.13			0.002		0.77
Niacin from supplements[1]										
Never use	426	206	1.0 (ref)	73	1.0 (ref)	1188	491	1.0 (ref)	198	1.0 (ref)
Low intake[3]	86	32	0.70 (0.41–1.18)	13	1.05 (0.49–2.24)	162	75	1.34 (0.96–1.87)	24	0.79 (0.46–1.34)
High intake[4]	65	28	0.77 (0.43–1.36)	15	1.95 (0.91–1.19)	157	70	1.50 (1.06–2.14)	23	0.89 (0.53–1.50)
P−trend			0.19		0.12			0.008		0.48
Vitamin B-6 from supplements[1]										
Never use	416	205	1.0 (ref)	72	1.0 (ref)	1162	490	1.0 (ref)	193	1.0 (ref)
Low intake[3]	131	50	0.70 (0.45–1.10)	24	1.34 (0.73–2.45)	168	75	1.26 (0.90–1.75)	31	1.08 (0.67–1.74)
High intake[4]	30	11	0.71 (0.29–1.74)	5	2.33 (0.68–7.96)	177	71	1.38 (0.98–1.94)	21	0.74 (0.43–1.27)
P−trend			0.12		0.13			0.03		0.39
Vitamin B-12 from supplements[1]										
Never use	440	211	1.0 (ref)	77	1.0 (ref)	1218	501	1.0 (ref)	199	1.0 (ref)
Low intake[3]	77	31	0.75 (0.44–1.28)	14	1.51 (0.72–3.14)	163	79	1.33 (0.96–1.85)	27	0.92 (0.56–1.52)
High intake[4]	60	24	0.74 (0.41–1.34)	10	1.28 (0.54–3.02)	126	56	1.63 (1.11–2.41)	19	0.84 (0.47–1.50)
P−trend			0.21		0.36			0.004		0.52

[1] Supplements were categorized into never use, low intake, and high intake [except folic acid from fortified foods; value was median, (range)].
[2] Multivariable adjustment for age, gender, education, BMI 1 y previously, frequency of heartburn or acid reflux 10 y prior to diagnosis, lifetime alcohol intake, pack-years of smoking, and NSAID use. No energy adjustment.
[3] Low intake = <median for folic acid supplement = 180 μg/d; thiamin = 9.2 mg/d; riboflavin = 10.0 mg/d; niacin = 25.0 mg/d; vitamin B-6 = 5.8mg/d; vitamin B-12 = 10.0 μg/d; choline = 10.25 mg/d based on intakes in controls.
[4] High intake = ≥median for folic acid supplement = 180 μg/d; thiamin = 9.2 mg/d; riboflavin = 10.0 mg/d; niacin = 25.0 mg/d; vitamin B-6 = 5.8mg/d; vitamin B-12 = 10.0 μg/d; choline = 10.25mg/d based on intakes in controls.
[5] Values are [medians (range)].

low food folate intake [OR = 2.67 (95% CI = 1.65–4.35)] (Table 5). Risks of EAC were significantly lower among participants with high food folate intake regardless of their level of alcohol consumption.

Overall, smoking was associated with increased risk of BE and esophageal cancer. The risk of ESCC associated with smoking appeared to be partially mitigated by high food folate intake such that, compared with nonsmokers with low folate intake, the RR of ESCC among smokers with high folate intake [OR = 2.96 (95% CI = 1.57–5.58)] was one-half that of smokers with low folate intake [OR = 5.90 (95% CI = 3.21–10.9)]. There was little evidence of such effect modification for BE or EAC.

Discussion

In these paired studies of BE and esophageal cancer, we found a decreased risk of EAC, and to a lesser extent ESCC, associated with higher intakes of food folate. This finding is consistent with nonsignificant (37,41,61) and significant (35,36,40) inverse associations found between food folate and esophageal cancers in epidemiological studies, with folate's role in mediating transfer of one-carbon group for DNA synthesis and methylation hypothesized as the basis for the decreased cancer risk (20,62). Inadequate folate intake has been hypothesized to influence cancer risk by inducing misincorporation of uracil into

TABLE 4 Associations between polymorphisms in *MTHFR* (A1298C, C677T), *MTRR* (A66G), and *MTR* (A2756G) and the risk of BE and esophageal cancers in SDH and ACS participants

			SDH		ACS	
SNP	Genotype	Frequency in ACS controls	BE without dysplasia, 510 Controls, 236 cases	BE with dysplasia, 510 Controls, 91 cases	EAC 1303 Controls, 501 cases	ESCC 1303 Controls, 177 cases
		%	OR (95%CI)[2]			
MTHFR A1298C, rs1801131	AA	(46.8)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
	AC	(43.9)	1.33 (0.91–1.94)	1.45 (0.84–2.50)	1.19 (0.93–1.52)	0.98 (0.68–1.43)
	CC	(9.3)	0.82 (0.40–1.69)	0.51 (0.15–1.77)	1.22 (0.80–1.84)	1.79 (1.03–3.13)
P-value[1]			0.22	0.16	0.35	0.07
MTHFR C677T, rs1801133	CC	(45.6)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
	CT	(42.6)	1.15 (0.77–1.70)	1.90 (1.08–3.36)	1.06 (0.83–1.35)	0.85 (0.58–1.23)
	TT	(11.8)	0.94 (0.52–1.71)	0.28 (0.08–0.97)	0.70 (0.47–1.05)	0.96 (0.56–1.66)
P-value[1]			0.72	0.003	0.61	0.61
MTRR A66G, rs1801394	GG	(31.2)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
	GA	(50.7)	1.10 (0.71–1.69)	0.61 (0.32–1.14)	0.93 (0.72–1.20)	0.94 (0.63–1.40)
	AA	(18.1)	1.16 (0.68–1.98)	1.28 (0.64–2.57)	0.96 (0.69–1.35)	1.46 (0.90–2.37)
P-value[1]			0.84	0.17	0.84	0.13
MTR A2756G, rs1805087	AA	(63.9)	1.0 (ref)	1.0 (ref)	1.0 (ref)	1.0 (ref)
	AG	(32.7)	0.84 (0.56–1.26)	1.42 (0.82–2.45)	0.89 (0.69–1.15)	1.08 (0.74–1.57)
	GG	(3.4)	1.81 (0.74–4.40)	1.57 (0.30–8.31)	1.28 (0.68–2.39)	1.02 (0.41–2.97)
P-value[1]			0.24	0.43	0.48	0.91

[1] Type 3 analysis of effect, Wald chi-square.
[2] Multivariable adjustment for age, gender, education, BMI 1 y previously, frequency of heartburn or acid reflux 10 y prior to diagnosis, lifetime alcohol intake, pack-years of smoking, NSAID use, and total energy intake.

DNA, which could lead to chromosomal breaks and mutations and by causing aberrant DNA methylation, resulting in altered expression of tumor suppressor genes (25).

Our data showed that the association between food folate and EAC was largely independent of alcohol and smoking status, but high folate intake appeared to eliminate the increased

TABLE 5 Associations between alcohol intake, smoking, combined levels of food folate, alcohol, and smoking and risk of BE and esophageal cancer in SDH and ACS participants

	SDH					ACS				
	Controls,	BE without dysplasia		BE with dysplasia		Controls,	EAC		ESCC	
	n	*n*	OR (95% CI)[3]	*n*	OR (95% CI)[3]	*n*	*n*	OR (95% CI)[3]	*n*	OR (95% CI)[3]
Alcohol										
Low alcohol intake, <170 g/wk	408	181	1.0 (ref)	69	1.0 (ref)	1046	446	1.0 (ref)	116	1.0 (ref)
High alcohol intake, ≥170 g/wk	98	43	0.96 (0.57–1.60)	26	1.65 (0.86–3.17)	284	138	1.01 (0.75–1.36)	84	1.81 (1.20–2.73)
P-value[2]			0.86		0.13			0.94		0.0043
Low alcohol,[1] low food folate	208	91	1.0 (ref)	36	1.0 (ref)	469	239	1.0 (ref)	55	1.0 (ref)
Low alcohol,[2] high food folate	200	90	1.15 (0.77–1.71)	33	0.88 (0.48–1.62)	577	207	0.75 (0.59–0.95)	61	0.87 (0.60–1.28)
High alcohol,[1] low food folate	62	25	0.78 (0.41–1.48)	16	1.41 (0.63–3.15)	155	98	1.08 (0.75–1.54)	69	2.67 (1.65–4.35)
High alcohol,[2] high food folate	36	18	1.50 (0.72–3.12)	10	1.88 (0.71–5.01)	129	40	0.64 (0.41–0.99)	15	0.68 (0.35–1.34)
Smoking										
Never smoked	254	65	1.0 (ref)	21	1.0 (ref)	541	124	1.0 (ref)	32	1.0 (ref)
Ever smoked	252	159	2.53 (1.47–4.30)	74	2.89 (1.34–6.23)	789	460	2.53 (1.88–3.41)	168	4.29 (2.70–6.80)
P-value			0.0006		0.007			<0.001		<0.001
Never smoked,[1] low food folate	130	31	1.0 (ref)	11	1.0 (ref)	242	78	1.0 (ref)	15	1.0 (ref)
Never smoked,[2] high food folate	124	34	1.00 (0.57–1.75)	10	0.73 (0.28–1.90)	299	46	0.65 (0.44–0.95)	17	1.02 (0.55–1.90)
Ever smoked,[1] low food folate	140	85	2.11 (1.12–3.97)	41	2.47 (1.00–6.08)	382	259	2.33 (1.62–3.33)	109	5.90 (3.21–10.9)
Ever smoked,[2] high food folate	112	74	3.08 (1.62–5.86)	33	2.61 (1.04–6.34)	407	201	1.74 (1.20–2.54)	59	2.96 (1.57–5.58)

[1] Low food folate < 275 μg/d.
[2] High food folate ≥ 275μg/d.
[3] Multivariable adjustment for age, gender, education, BMI 1 y previously, frequency of heartburn or acid reflux 10 y prior to diagnosis, lifetime alcohol intake, pack-years of smoking, NSAID use, and total energy intake.

risk of ESCC associated with alcohol consumption and to partially mitigate the risk associated with smoking. This is in part consistent with results of a smaller multicenter case-control study conducted in Italy and Switzerland where a stronger protective effect of food folate among heavy drinkers than smokers was found (40). These findings add further evidence that, as suggested by the 2007 WCRF report, consumption of foods rich in folate is associated with decreased risk of esophageal cancers (7,25).

Our observation of elevated risks of precancerous BE with dysplasia and EAC in relation to high intake of folic acid supplement is interesting and may be analogous to the finding of Cole et al. (22). In that study, administration of high doses of folic acid appeared to accelerate the progression of preexisting colorectal cancer precursors and increase the risk of developing advanced adenomas, supporting the notion that folic acid might increase cancer risk once initial lesions are present (63).

Like dietary folate, riboflavin, vitamin B-6, vitamin B-12, and methyl donors have also been considered potential cancer-preventive agents because of the role they play in one-carbon metabolism. Methionine, choline, and betaine are interchangeable sources of one-carbon moieties; riboflavin is a precursor for flavin adenine dinucleotide, the cofactor for critical folate-dependent enzyme MTHFR; vitamin B-6 and vitamin B-12 are coenzymes of serine hydroxymethyl transferase and methionine synthase, respectively, both of which are involved in folate metabolism, while methionine is required for the synthesis of *S*-adenosyl methionine, the universal methyl donor required for DNA methylation for all biological processes (26). Results of studies examining the association between these micronutrients and esophageal cancers have been mixed. In our study, we found that high intake of vitamin B-6 from food sources was significantly associated with decreased risks of EAC; this is consistent with the findings of a case-control study from the US (36,37), with others also reporting nonsignificant inverse associations (35,40). We found a borderline positive association between high intake of riboflavin and EAC risk; this is consistent in part with findings of a case-control study from the US (36) that reported a nonsignificantly increased risk for EAC but was inconsistent with inverse findings reported by Chen et al. (35) and Brown et al. (34). Studies that have investigated the association among riboflavin, methionine, and risk of esophageal cancers are very few; thus, we are uncertain as to the reason why high intakes of riboflavin and methionine might increase the risk of EAC. Results from epidemiologic studies investigating the association between riboflavin and risk of cancers have been inconsistent. For instance, whereas inverse associations have been reported for plasma riboflavin and gastric (64) or colorectal adenomas (65,66), a null association was reported between riboflavin and risk of breast cancer in a prospective study (67). It has been suggested that effects of folate deficiency on DNA methylation are gene and site specific, highly complex, and seem to depend on cell type, target organ, stage of transformation, and degree and duration of folate depletion (68). We do not claim a causal association, because the observed association may reflect residual confounding or a chance finding. Thus, our observation must await replication from other studies before considering further investigation. The inverse association we observed between betaine from food and risk of BE with and without dysplasia may be analogous to a weak inverse association found between betaine intake and colorectal adenoma risk (24). Neither methionine nor choline from food was associated with decreased BE or esophageal cancer risk; rather, we found an elevated risk of EAC with increasing methionine intake. Importantly, our findings showed no beneficial effect for folic acid or vitamin B supplementation in the prevention of BE or esophageal cancers. In contrast, we found 2-fold increased risk of BE with dysplasia, a nonsignificantly increased risk of EAC (*P*-trend = 0.08) with high intake of folic acid supplement, and elevated EAC risk with high intakes of B vitamin supplement. It is quite possible that increased risks with intake of vitamins from supplement could represent increasing intake of supplements among those diagnosed with BE or esophageal cancer. However, all cases were asked to report their diet 1 y prior to their diagnosis to minimize this possibility.

Our finding of a significantly increased risk of ESCC among carriers of the CC compared with AA variant of *MTHFR* at nucleotide 1298 is consistent with an earlier Chinese study (57). A case-cohort study conducted in China had only 3 ESCC cases and no controls with *MTHFR* 1298 CC genotype, making it difficult to compare our results to that study (55); however, the same study found a nonsignificant increased risk of gastric cardia adenocarcinoma among carriers of the *MTHFR* 1298 CC genotypes compared with the AA variant (55). We found no association between the *MTHFR* 677 TT genotype and ESCC risk but did find higher risks of BE with dysplasia among those carrying the CT variant of *MTHFR* 677 genotype and a decreased risk among those carrying the TT variant compared with the CC variant. The majority of previous studies on genetic variations in relation to one-carbon metabolism and esophageal cancers have been conducted in Asian populations. Published results from predominantly Caucasian populations from the European Prospective Investigation into Cancer and Nutrition studies also showed no associations between polymorphisms in MTHFR, MTRR, and MTR genes and other gastrointestinal cancers, including colorectal (65) and gastric (64) cancers. Nevertheless, we do not discount the possibility that our sample size might have been too small to detect modest associations between the genetic variants and risk of BE or esophageal cancer.

Our study has strengths and limitations. The sample size was larger than most previous investigations and cases were recruited soon after diagnosis across the country. Moreover, by conducting 2 case-control studies in parallel, we were able to examine both cancer and precancer. Finally, ours is one of very few studies in the field of esophageal neoplasia to capture information regarding environmental and dietary factors, phenotype, and medical history and to collect DNA samples. Taken together, these design features provided a rare opportunity to test these topical hypotheses. Limitations include the possibilities of selection bias, recall bias with respect to reporting of dietary intake, and also the possibility that cases might have changed their diet with the onset of disease. Whereas the participation fractions, especially among controls, were less than ideal, we are reassured that mean daily intakes of total energy, thiamin, riboflavin, and folate in our ACS control population (9270 kJ, 1.8 mg, 2.3 mg, and 279 μg, respectively) were very similar to those reported for adults aged 19 y and over in the Australian population (9240 kJ, 1.6 mg, 2.1 mg, and 269 μg, respectively) (69). We also reanalyzed our data excluding those who reported changing their usual eating habits in the year prior to recruitment and observed no material difference in the estimates obtained. All dietary assessment tools (FFQ) are prone to measurement error; however, any nondifferential measurement error is likely to have attenuated the associations that we have observed. Objective measures of biomarkers such as plasma folate or B vitamins would overcome some of these limitations. However, in a case-control study, it is likely that nutrient levels in samples collected after diagnosis would be

affected by changes in diet arising from the disease process and treatment and thus would not be representative of the usual premorbid diet in cases.

In conclusion, our findings provide some support for the hypothesis that increasing intake of folate-rich foods may reduce overall risks of EAC and ESCC; the effects on the latter appear to be greatest among smokers and drinkers. There remains the possibility that high folic acid intake may increase risk of BE progressing to dysplasia and EAC, although such a finding needs to be tested in other studies, ideally using a prospective design. We found no evidence that genetic polymorphisms in folate-metabolizing genes play a major role in esophageal carcinogenesis.

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