Postdiagnostic intake of one-carbon nutrients and alcohol in relation to colorectal cancer survival[1–3]

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ABSTRACT

Background: Observational data have suggested that intakes of nutrients involved in one-carbon metabolism are inversely associated with risk of colorectal carcinoma and adenomas. In contrast, results from some preclinical studies and cardiovascular and chemoprevention trials have raised concerns that high folate intake may promote carcinogenesis by facilitating the progression of established neoplasia.

Objective: We tested the hypothesis that higher total folate intake (including food folate and folic acid from fortified foods and supplements) or other one-carbon nutrient intakes might be associated with poorer survival after a diagnosis of colorectal cancer.

Design: We used rectal and colon cancer cases within the following 2 US prospective cohort studies: the Nurses' Health Study and the Health Professionals Follow-Up Study. Biennial questionnaires were used to gather information on medical history and lifestyle factors, including smoking and alcohol consumption. B-vitamin and methionine intakes were derived from food-frequency questionnaires. Data on tumor molecular characteristics (including microsatellite instability, CpG island methylator phenotype, *KRAS*, *BRAF*, and *PIK3CA* mutations, and long interspersed nucleotide element 1 methylation level) were available for a subset of cases. We assessed colorectal cancer–specific mortality according to postdiagnostic intakes of one-carbon nutrients with the use of multivariable Cox proportional hazards regression models.

Results: In 1550 stage I–III colorectal cancer cases with a median follow-up of 14.9 y, we documented 641 deaths including 176 colorectal cancer–specific deaths. No statistically significant associations were observed between postdiagnostic intakes of folate or other one-carbon nutrients and colorectal cancer–specific mortality (multivariate P-trend ≥ 0.21). In an exploratory molecular pathologic epidemiology survival analysis, there was no significant interaction between one-carbon nutrients or alcohol and any of the tumor molecular biomarkers examined.

Conclusions: Higher postdiagnostic intakes of one-carbon nutrients are not associated with the prognosis in stage I–III colorectal cancer. Our findings do not support the hypothesis that higher folate intake after colorectal cancer diagnosis might increase risk of cancer-related death. *Am J Clin Nutr* 2015;102:1134–41.

Keywords: alcohol, colorectal carcinoma, micronutrients, public health, survivorship

INTRODUCTION

Dietary factors are believed to play an important role in colorectal cancer risk. One-carbon nutrients, including folate, methionine, and vitamins B-6 and B-12, are essential for nucleotide biosynthesis, DNA repair, and the epigenetic regulation of gene expression (1). Although a number of epidemiologic studies have suggested an inverse association between intakes of one-carbon nutrients and colorectal cancer risk (2–9), these associations have not generally been replicated in interventional studies that have examined adenoma recurrence as an endpoint (10–13). One adenoma prevention study reported an association between supplementation with high-dose folic acid (a synthetic folate) at 1 mg/d and increased risk of advanced or multiple adenomas (10). In addition, data from 2 randomized, double-blind, placebo-controlled trials of B vitamins in the secondary prevention of cardiovascular events suggested increased risk of cancer-related and all-cause mortality in individuals assigned to received 0.8 mg folic acid with vitamin B-12 supplementation (14). Several preclinical studies have supported a tumor-promoting

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effect of folic acid on established neoplasia (15–18). In a genetically predisposed mouse model of intestinal tumorigenesis, exposure to folic acid promoted tumor growth only when microscopic evidence of neoplasia was already apparent in the form of aberrant crypt foci, and not before (16). Therefore, it has been suggested that folate may have divergent roles during the initiation and evolution phases of colorectal carcinogenesis (18, 19).

The fortification of enriched cereal-grain products with folic acid is mandatory in the United States, Canada, and >70 other countries, and some authors have argued that the adoption of fortification by European countries is overdue (20). Despite the undisputed impact of mandatory folic acid fortification on the incidence of neural tube defects, the health effects of higher folic acid intake in subgroups who are not considered primary targets of fortification are largely unknown (18). Some observers have highlighted the temporal association between a transient increase in colorectal cancer incidence and the introduction of folic acid fortification in the United States in the 1990s (21). Therefore, the possibility that higher total intake of folate, comprising natural food folate and folic acid, may cause the progression of pre-existing malignant lesions is a major clinical and public health concern (18, 19).

After colorectal cancer resection, occult, residual cancer cells may later manifest as disease recurrence, which is associated with high mortality (22). Although a limited number of studies have evaluated postdiagnostic dietary factors and survival in colorectal cancer patients (23–25), to our knowledge, no previous study has specifically examined survival in relation to postdiagnostic intakes of one-carbon nutrients. Therefore, we tested the hypothesis that higher intakes of one-carbon nutrients might be associated with colorectal cancer–specific survival in patients from 2 large US cohort studies who had undergone resection for nonmetastatic colorectal cancer. Because alcohol is known to perturb folate metabolism, we also assessed the association of alcohol consumption on colorectal cancer survival. In addition, with the use of a molecular pathologic epidemiology database, which was available for a subset of cases, we performed exploratory analyses to test for differential associations between one-carbon nutrients, alcohol, and survival according tumor molecular features including epigenetic changes.

METHODS

Study population and assessment of covariates

We used the databases of the following 2 prospective cohort studies: the Nurses' Health Study (NHS)[13] (121,701 women followed-up since 1976) (26) and the Health Professionals Follow-Up Study (HPFS) (51,529 men followed-up since 1986) (26). Study participants were sent biennial questionnaires to gather information on health and lifestyle factors and to identify diagnoses of cancer. Intakes of alcohol and various nutrients, including folate, methionine, and vitamins B-6 and B-12, were derived from self-administered semiquantitative food-frequency questionnaires (FFQs), which queried the average frequency of consumption of specific food items over the preceding 12 mo. The usual brand of cold breakfast cereal and the frequency, brand, and dose of dietary supplements, including single vitamins and multivitamin formulations, were also queried in the questionnaires (27, 28). FFQs were completed in 1980, 1984, 1986, and every 4 y thereafter in the NHS and at baseline (1986) and every 4 y thereafter in the HPFS. With the use of FFQ data, daily nutrient intakes were calculated for each participant and adjusted for total caloric intake (27). Total folate intake was the sum of food folate plus folic acid from fortified foods and supplements expressed as dietary folate equivalents (one dietary folate equivalent = 1 μg food folate = 0.6 μg folic acid) (29). Total intakes of vitamins B-6 and B-12 were the sum of dietary and supplemental sources. The use of FFQ data for the assessment of nutrient intakes has previously been validated (30, 31). For the calculation of daily alcohol consumption, we assumed an ethanol content of 13.1 g for a 380-mL serving of beer, 11.0 g for a 120-mL glass of wine, and 14.0 g for a standard measure of spirits. Because the FFQs captured dietary patterns over the preceding year, and to minimize assessment during the period of recuperation from surgery, or adjuvant oncologic therapy, we used questionnaires returned between 1 and 4 y after diagnosis for the assessment of postdiagnostic dietary intakes. Similarly, to reduce bias as a result of the nonreporting of dietary data and altered dietary behaviors in the period around cancer recurrence or death, patients with documented metastatic disease (stage IV) at presentation were excluded.

Case ascertainment, assessment of mortality, and tumor tissue collection

We included cases of incident colorectal cancer diagnosed up to 2006. The National Death Index was used to identify deaths and additional colorectal cancer cases. With permission from participants (or their next of kin), we attempted to confirm all colorectal cancer diagnoses and collected clinical and pathologic information related to colorectal cancer (including cancer stage) through a study physician review of medical records. Participants were followed until death or January 2012, whichever came first. The ascertainment of deaths was accomplished through the reporting by family members or postal authorities and by searching the National Death Index. The cause of death was assigned by study physicians. We collected paraffin-embedded tissue blocks from hospital where participants had undergone tumor resection or a diagnostic biopsy. All colorectal cancer cases with available tissue underwent a histopathologic review by a pathologist (SO). No significant differences in demographic features or exposures existed between cases with and without available tumor tissue (26).

Tumor molecular analyses

For cases with available tissue, DNA was extracted and subjected to molecular analyses as previously described (26, 32–35). Polymerase chain reaction (PCR) and pyrosequencing were performed for the assessment of mutational status in *BRAF* (codon 600), *KRAS* (codons 12, 13, 61, and 146), and *PIK3CA* (exons 9 and 20) (26, 35). Microsatellite instability (MSI) status

[13] Abbreviations used: CIMP, CpG island methylator phenotype; FFQ, semiquantitative food frequency questionnaire; HPFS, Health Professionals Follow-Up Study; LINE-1, long interspersed nucleotide element 1; MSI, microsatellite instability; PCR, polymerase chain reaction; NHS, Nurses' Health Study.

was assessed with PCR with the use of 10 microsatellite markers (32). CpG island methylator phenotype (CIMP) status was determined with the use of real-time PCR (MethyLight) on bisulfite-treated DNA (33). Methylation levels in long interspersed nucleotide element 1 (LINE-1) were evaluated with the use of bisulfite PCR and pyrosequencing (34).

Ethics

Informed consent was obtained from all study subjects. The institutional review boards of Harvard School of Public Health and Brigham and Women's Hospital approved the study.

Statistical analysis

All statistical analyses were conducted with SAS software (version 9.3; SAS Institute). All *P* values were 2 sided. Our primary hypothesis test was the analysis of colorectal cancer–specific survival in relation to increasing intake of each one-carbon nutrient (folate, methionine, and vitamins B-6 and B-12) or alcohol with the use of the combined cohort of men and women. For primary hypothesis testing, a *P* value for significance was set at 0.01 ($0.05 \div 5$) to adjust for multiple hypothesis testing. In the primary analysis, quintiles of postdiagnostic one-carbon nutrient intake (or 3 categories for alcohol consumption) were used and tested for a linear trend with the use of the median value for each category as a continuous variable in Cox proportional hazards models. All other analyses were secondary, and any positive findings were interpreted cautiously considering multiple hypothesis testing. No analysis in this study was planned at the inception of the cohort studies; thus, all analyses were, by definition, post hoc. For postdiagnostic energy-adjusted intakes of one-carbon nutrients, we used sex-specific quintiles. Alcohol consumption was divided into ordinal categories (no alcohol and 0.1–14.9 and ≥15 g/d). To test for differences in the distribution of categorical data, a chi-square or Fisher's exact test was performed, whereas the Kruskal-Wallis test was used to compare means for continuous variables across quintiles of one-carbon nutrients or alcohol categories.

The Kaplan-Meier method and log-rank test were used to test for differences in the survival time distribution. For colorectal cancer–specific mortality, deaths from other causes were censored. Mortality HRs and 95% CIs were computed with the use of Cox proportional hazards regression models. To control for confounding, in addition to postdiagnostic one-carbon nutrient and alcohol intakes, variables that were available for inclusion in the multivariate models included the time interval between diagnosis and the return of FFQs (continuous; mo), age at diagnosis (continuous; y), BMI (in kg/m^2; ≥30 compared with <30), family history of colorectal cancer in any first-degree relative (present compared with absent), year of diagnosis (continuous), postdiagnostic aspirin use (regular users compared with nonusers), postdiagnostic multivitamin use of any frequency (users compared with nonusers), postdiagnostic smoking status (current smokers or former smokers compared with never smokers), postdiagnostic physical activity (≥18 compared with <18 metabolic equivalent task score hours per week), tumor location (proximal compared with distal colorectum), tumor differentiation (well to moderate compared with poor), and prediagnostic intake of the variable of interest. In the subgroup with available molecular data, additional covariates included tumor MSI status (high compared with low or stable), LINE-1 methylation (continuous), CIMP status (high compared with low or negative), and *BRAF*, *KRAS*, and *PIK3CA* mutations. Indicator variables were used when there were missing covariate data. In multivariate models, we controlled for sex (cohort) and disease stage (I, II, III, or unknown) by stratification with the use of the strata option in the SAS proc phreg command. A backward-elimination procedure with a threshold of $P = 0.1$ was used to select variables in the final multivariate models. Additional adjustment for a history of prediagnostic lower endoscopy did not materially alter our effect estimates. Statistical interactions were assessed with the use of a likelihood-ratio test to compare the fit of Cox models with and without the inclusion of an interaction term, which was the cross-product of the variables of interest. The proportionality of hazards assumptions were fulfilled for one-carbon nutrient and alcohol intakes by assessing time-dependent covariates, which were the cross-products of variables of interest and survival time, in Cox multivariate models (all $P > 0.07$). The *Q* statistic was used to test for heterogeneity in mortality hazards estimates between study cohorts. On the basis of a sensitivity analysis (in which we included all participants with FFQs returned ≤4 y of diagnosis) and a 2-sided type I error rate of 0.05, we calculated that we would have 80% power to detect a statistically significant linear trend across one-carbon nutrient quintiles equivalent to a cancer-specific mortality HR of 1.78 or an overall mortality HR of 1.48 for the highest compared with lowest quintiles of intake.

RESULTS

Characteristics of colorectal cancer patients

We aimed to test the hypothesis that higher postdiagnostic intakes of one-carbon nutrients might be associated with shorter colorectal cancer patient survival with the use of the databases of the NHS and HPFS cohorts. We identified 1550 stage I–III colorectal cancer cases (1063 women in the NHS and 487 men in the HPFS) with available postdiagnostic FFQ data. The median time interval between diagnosis and the return of FFQs was 29.5 mo. Characteristics of colorectal cancer cases are summarized according to quintiles of postdiagnostic total folate intake in **Table 1** and for other one-carbon nutrients and alcohol in **Supplemental Tables 1–4** (**Supplemental Tables 5–9** show representative quintiles for women and men separately). For B vitamins (folate and vitamins B-6 and B-12), subjects with higher intakes tended to be older, were diagnosed after 1996, and were current multivitamin users. For methionine, those with higher intakes tended to be younger, were diagnosed before 1996, and had higher BMI. Within the cohort of 623 cases with available tumor molecular pathology data, there were no substantial differences in tumor molecular markers according to one-carbon nutrient intakes.

Postdiagnostic intake of one-carbon nutrients and colorectal cancer survival

In 1550 stage I–III colorectal cancer cases followed-up over a median 14.9 y (for censored cases; IQR: 9.6 y), there were 641 deaths of which 176 were attributable to colorectal cancer. We first assessed for heterogeneity in the associations of one-carbon nutrients with colorectal cancer–specific mortality between cohorts. On the basis of Cox regression analyses (**Supplemental Tables 10** and **11**), we observed no significant heterogeneity in effect estimates between cohorts with the use of the *Q* statistic (all *P*-heterogeneity ≥ 0.54 for comparison of multivariate

TABLE 1
Colorectal cancer case characteristics according to quintile of postdiagnostic folate intake[1]

		Quintile of postdiagnostic total folate intake, DFE/d					
	All cases	1	2	3	4	5	*P*
Women (NHS)	—	298 (119–381)[2]	474 (383–589)	775 (594–1031)	1231 (1035–1374)	1602 (1375–3775)	—
Men (HPFS)	—	374 (161–492)	592 (495–783)	1042 (789–1256)	1388 (1257–1521)	2021 (1531–4738)	—
Case characteristic							
Total cases, *n*	1550	309	311	310	311	309	—
Women, %	69	69	68	69	68	69	0.99
Age, y	66.1 ± 8.9[3]	62.1 ± 9.1	65.8 ± 8.6	65.7 ± 8.8	67.9 ± 8.2	68.7 ± 8.1	<0.001
Diagnosed after 1996, %	51	21	45	41	73	78	<0.001
Regular aspirin users, %	36	32	37	36	35	38	0.54
Multivitamin users,[4] %	57	12	24	71	86	90	<0.001
Smokers, current/former, %	60	63	59	58	62	58	0.57
With family history of colorectal cancer,[5] %	20	18	20	19	22	20	0.87
Folate intake, DFE/d	978 ± 638	314 ± 77	514 ± 88	865 ± 178	1273 ± 121	1927 ± 571	<0.001
Folate as food folate, %	46	85	64	39	24	19	<0.001
Vitamin B-6 intake,[6] mg/d	11.2 ± 27.7	5.0 ± 18.0	5.7 ± 18.1	9.4 ± 25.3	9.0 ± 17.7	26.8 ± 44.0	<0.001
Vitamin B-12 intake,[6] μg/d	27.9 ± 77.9	8.6 ± 17.7	10.8 ± 27.7	22.8 ± 73.6	30.8 ± 57.7	66.8 ± 135.5	<0.001
Methionine intake,[6] g/d	1.69 ± 0.43	1.75 ± 0.41	1.69 ± 0.45	1.71 ± 0.44	1.70 ± 0.44	1.62 ± 0.40	0.003
Alcohol intake, g/d	7.3 ± 12.2	9.6 ± 13.8	6.2 ± 11.2	7.1 ± 12.4	6.6 ± 11.3	6.7 ± 11.9	0.002
BMI, kg/m^2	26.4 ± 4.5	26.2 ± 4.4	26.9 ± 4.8	26.3 ± 4.7	26.2 ± 4.2	26.2 ± 4.4	0.56
Physical activity, MET score/wk	22.5 ± 31.4	20.0 ± 29.7	22.3 ± 32.7	23.1 ± 27.8	22.4 ± 31.0	24.3 ± 35.4	0.87
Histologically poorly differentiated, %	9.4	6.5	10	7.7	11	12	0.12
By tumor location, %							0.72
Right colon	35	30	33	33	38	38	
Left colon	29	28	31	31	27	28	
Rectum	20	21	20	20	18	20	
Unknown	16	20	16	15	16	13	
By disease stage, %							0.18
I	27	27	24	25	30	27	
II	25	26	26	25	23	28	
III	21	15	24	20	21	23	
Unknown	27	32	26	30	26	23	
Molecular cohort							
Total cases, *n*	623	102	130	119	129	143	—
KRAS mutation, %	37	36	38	37	36	37	0.99
BRAF mutation, %	12	6.9	12	12	12	14	0.58
PIK3CA mutation, %	16	14	16	17	15	17	0.94
CIMP high	16	13	15	15	18	17	0.86
MSI high	15	8.8	15	14	15	20	0.21
LINE-1 methylation	62.6 ± 9.2	61.1 ± 9.8	62.9 ± 9.5	62.7 ± 8.9	63.4 ± 8.5	62.6 ± 9.3	0.45

[1]Total energy-adjusted folate intake from food and supplemental sources in DFEs. When percentages are given, they indicate the proportion of cases with a specific clinical, pathologic, or molecular feature. A chi-square or Fisher's exact *P* value is given for comparison of categorical variables across quintiles of folate intake; the Kruskal-Wallis test was used to compare means for continuous characteristics across quintiles of folate intake. CIMP, CpG island methylator phenotype; DFE, dietary folate equivalent; HPFS, Health Professionals Follow-Up Study; LINE-1, long interspersed nucleotide element-1; MET, metabolic equivalent of task; MSI, microsatellite instability; NHS, Nurses' Health Study.
[2]Median; range in parentheses (all such values).
[3]Mean ± SD (all such values for a continuous characteristic).
[4]Multivitamin use, regardless of frequency.
[5]In one or more first-degree relatives (excluding offspring).
[6]Energy-adjusted intake from food and supplemental sources.

models). Thus, we combined cohorts to maximize he statistical power for additional analyses. In Kaplan-Meier analyses, participants in the third quintile of total folate intake appeared to experience shorter colorectal cancer–specific survival (log-rank $P = 0.022$) (**Supplemental Figure 1**); however, survival time did not differ appreciably between other quintiles nor were there any significant differences in overall survival (log-rank $P = 0.39$) (**Supplemental Figure 2**). Colorectal cancer–specific and the overall survival-time distributions did not differ according to quintiles of other one-carbon nutrients (all log-rank $P \geq 0.15$) (Supplemental Figures 1 and 2). In Cox regression models (**Table 2**), we did not observe significant associations between colorectal cancer–specific mortality and total intakes across one-carbon nutrient quintiles (all multivariate *P*-trend ≥ 0.21).

Postdiagnostic alcohol intake and colorectal cancer survival

On the basis of Cox regression analyses (**Supplemental Table 12**), we did not observe heterogeneity between cohorts in the associations of alcohol consumption (as a trend across the 3 ordinal categories) with colorectal cancer–specific mortality (Q statistic P-heterogeneity = 0.75 for comparison of multivariate models). In Kaplan-Meier analyses (**Supplemental Figure 3**), there was no significant association between alcohol intake categories and colorectal cancer–specific survival (log-rank P = 0.19). The overall survival time appeared shorter in nondrinkers (log-rank P = 0.027). In Cox regression models (**Table 3**), there was no significant association between increasing alcohol intake and colorectal cancer–specific mortality (multivariate P-trend = 0.33). In our secondary analysis, compared with nondrinkers, lower colorectal cancer–specific mortality was associated with alcohol consumption of 0.1–14.9 and ≥15 g/d [multivariate HRs: 0.51 (95% CI: 0.34, 0.76; P = 0.001) and 0.53 (95% CI: 0.28, 0.98; P = 0.044), respectively].

Sensitivity analyses

We excluded cases with FFQs completed ≤1 y of colorectal cancer diagnosis, which would have potentially captured dietary patterns during the period of surgery, recuperation, or active oncologic treatment. To confirm that the exclusion of these cases did not substantially alter the study findings, we conducted a sensitivity analysis including any FFQs completed ≤4 y of a diagnosis. The median time between a diagnosis and return of FFQs was reduced to 21 mo, and case number increased to 2083 participants (1426 women and 657 men) in whom there were a total of 871 deaths (274 colorectal cancer–specific deaths). Results of Cox regression analyses were similar to those of our main analysis (**Supplemental Tables 13** and **14**).

Because the biologic activity of folic acid may differ from that of natural food folate, we performed Cox regression analyses for food folate and folic acid (from fortified foods and supplements) separately (**Supplemental Table 15**). Neither food folate nor folic acid intake was associated with colorectal cancer–specific mortality (multivariate P-trend ≥ 0.50). Because the average total folate intake in the United States increased markedly after the introduction of the fortification of enriched cereal-grain products (29), which began in 1996 and was mandated by 1998, we conducted an additional analysis of total folate intake stratified by the date of diagnosis (**Supplemental Table 16**). The association between folate intake and colorectal cancer–specific mortality did not differ between patients diagnosed before 1996 and those diagnosed in or after 1996 (multivariate P-heterogeneity = 0.89 between strata of diagnosis date).

Analyses of interaction between one-carbon nutrients, alcohol, and tumor molecular biomarkers

We conducted an exploratory analysis with the use of a combined cohort of men and women (n = 623) with available tumor tissue data. We examined whether the relation between one-carbon nutrients or alcohol and colorectal cancer–specific mortality differed by tumor molecular subtypes classified by tumor status for MSI, CIMP, and LINE-1 methylation or *KRAS*, *BRAF*, or *PIK3CA* mutation. There was no appreciable statistical interaction between one-carbon nutrient intakes or alcohol intake and any of the tumor molecular biomarkers examined (all P-interaction ≥0.060; data not shown).

DISCUSSION

Epidemiologic, clinical, and preclinical data have led to the concern that exposure to higher levels of total folate or folic acid may promote the progression of premalignant lesions or accelerate the evolution of preclinical cancers (18, 19). Therefore, we had hypothesized that, in patients who had undergone colorectal cancer resection, exposure to higher total folate intake might increase risk of cancer-related mortality. However, our survival analysis did not provide evidence for an association between higher postdiagnostic total folate intake and disease-specific survival in patients with stage I–III colorectal cancer. Intakes of one-carbon nutrients for participants in the highest quintiles were at least equivalent to the dose of supplemental vitamins used in some adenoma-prevention studies (10, 14). Although several studies have reported inverse associations between colorectal cancer or adenoma incidence and intakes of folate, methionine, and vitamins B-6 and B-12 (2–5, 7–9), we showed no convincing associations between postdiagnostic intakes of these one-carbon nutrients and colorectal-cancer mortality. Our data are in agreement with a previous study of prediagnostic plasma folate and colorectal-cancer survival in 301 participants from the same cohorts (36) and are also compatible with a recent null study of folate intake and recurrence after definitively treated prostate cancer (37). Collectively, these data support a model whereby intakes of one-carbon nutrients influence the neoplastic initiation but not neoplasitc progression and later phases of tumor evolution (19). This hypothesis is strengthened by data from longitudinal studies that highlighted the importance of the timing of folate and vitamin B-6 exposure in relation to colorectal cancer risk (28, 38).

Several previous studies have suggested that dietary folate intake may influence epigenetic events during colorectal carcinogenesis, especially aberrant promoter methylation (39–41). We previously showed that low folate intake and high alcohol consumption were associated with higher risk of LINE-1 hypomethylated colorectal cancer (42). In our exploratory survival analyses, which were restricted to cases with available tumor tissue, we showed no evidence of an interaction between one-carbon nutrients and tumor molecular biomarker, including CIMP and LINE-1 methylation status.

Higher alcohol intake has generally been associated with higher risk of colorectal cancer (43), whereas light-to-modest alcohol consumption may be associated with lower all-cause mortality, in particular mortality from cardiovascular events (44). Although we did not detect a significant linear trend for the association between alcohol intake and mortality, in our secondary analysis, compared with nondrinkers, consumption of alcohol after diagnosis appeared to be associated with lower colorectal cancer–specific mortality. A similar association has been reported for breast cancer survival in women (45). One important source of bias in analyses of postdiagnostic alcohol consumption and cancer survival is that individuals who suffer cancer recurrence and declining health may be less inclined to drink alcohol. It remains plausible that an influence of alcohol consumption after colorectal cancer diagnosis exists; however, this hypothesis warrants evaluations in additional populations.

Our study possessed some limitations. The inclusion of data from only FFQs returned between 1 and 4 y postdiagnosis meant

TABLE 2
Colorectal cancer–specific and overall mortality according to postdiagnostic one-carbon nutrient intakes[1]

Postdiagnostic micronutrient intake, quintiles	Total cases, *n*	Colorectal cancer–specific mortality				Overall mortality			
		Event, *n*	Univariate HR (95% CI)	Sex- and stage-stratified HR (95% CI)	Multivariate sex- and stage-stratified HR (95% CI)	Event, *n*	Univariate HR (95% CI)	Sex- and stage-stratified HR ((95% CI)	Multivariate sex- and stage-stratified HR (95% CI)
Folate[2]									
1	309	36	1 (referent)	1 (referent)	1 (referent)	151	1 (referent)	1 (referent)	1 (referent)
2	311	39	1.17 (0.74, 1.85)	1.10 (0.69, 1.73)	1.17 (0.74, 1.88)	140	1.17 (0.93, 1.47)	1.15 (0.91, 1.45)	1.03 (0.81, 1.31)
3	309	50	1.54 (1.00, 2.37)	1.49 (0.97, 2.30)	1.63 (1.04, 2.56)	146	1.24 (0.99, 1.56)	1.24 (0.98, 1.56)	1.17 (0.92, 1.49)
4	311	22	0.70 (0.41, 1.20)	0.67 (0.39, 1.15)	0.76 (0.43, 1.35)	106	1.08 (0.84, 1.39)	1.06 (0.82, 1.37)	0.86 (0.66, 1.13)
5	310	29	0.96 (0.58, 1.56)	0.89 (0.54, 1.46)	1.04 (0.60, 1.82)	98	1.07 (0.83, 1.39)	1.05 (0.81, 1.36)	0.87 (0.65, 1.16)
P-trend			0.24	0.18	0.21		0.90	0.98	0.13
Vitamin B-6[3]									
1	309	36	1 (referent)	1 (referent)	1 (referent)	147	1 (referent)	1 (referent)	1 (referent)
2	312	36	0.96 (0.61, 1.53)	0.94 (0.59, 1.49)	0.95 (0.59, 1.51)	140	0.90 (0.72, 1.14)	0.88 (0.70, 1.11)	0.87 (0.69, 1.11)
3	309	35	0.99 (0.62, 1.58)	1.02 (0.64, 1.63)	1.08 (0.67, 1.74)	115	0.86 (0.67, 1.09)	0.85 (0.67, 1.09)	0.80 (0.62, 1.03)
4	311	32	0.97 (0.60, 1.56)	0.89 (0.55, 1.44)	0.94 (0.57, 1.55)	127	1.09 (0.86, 1.38)	1.04 (0.81, 1.32)	0.94 (0.73, 1.22)
5	309	37	1.07 (0.68, 1.70)	0.99 (0.62, 1.57)	0.93 (0.58, 1.49)	112	0.89 (0.70, 1.14)	0.83 (0.65, 1.07)	0.78 (0.59, 1.03)
P-trend			0.63	0.91	0.66		0.56	0.29	0.18
Vitamin B-12[3]									
1	321	37	1 (referent)	1 (referent)	1 (referent)	136	1 (referent)	1 (referent)	1 (referent)
2	298	39	1.14 (0.73, 1.78)	1.19 (0.75, 1.87)	1.23 (0.77, 1.95)	141	1.19 (0.94, 1.50)	1.23 (0.97, 1.57)	1.19 (0.93, 1.52)
3	311	31	0.87 (0.54, 1.40)	0.78 (0.48, 1.26)	0.70 (0.43, 1.14)	131	1.06 (0.83, 1.34)	1.02 (0.80, 1.30)	0.96 (0.74, 1.23)
4	310	38	1.11 (0.71, 1.75)	1.01 (0.64, 1.61)	0.88 (0.55, 1.42)	128	1.10 (0.87, 1.41)	1.08 (0.84, 1.38)	0.94 (0.72, 1.22)
5	309	31	1.01 (0.62, 1.63)	0.97 (0.59, 1.57)	1.04 (0.62, 1.74)	104	1.23 (0.95, 1.59)	1.24 (0.95, 1.61)	1.11 (0.82, 1.50)
P-trend			0.99	0.83	0.99		0.24	0.25	0.71
Methionine[3]									
1	307	40	1 (referent)	1 (referent)	1 (referent)	130	1 (referent)	1 (referent)	1 (referent)
2	310	25	0.59 (0.36, 0.97)	0.56 (0.34, 0.93)	0.57 (0.34, 0.95)	115	0.82 (0.64, 1.06)	0.80 (0.62, 1.03)	0.82 (0.63, 1.05)
3	313	32	0.72 (0.45, 1.14)	0.72 (0.45, 1.16)	0.82 (0.51, 1.32)	115	0.72 (0.56, 0.93)	0.72 (0.56, 0.93)	0.92 (0.71, 1.19)
4	313	35	0.79 (0.50, 1.24)	0.78 (0.50, 1.24)	0.79 (0.50, 1.27)	127	0.79 (0.62, 1.01)	0.79 (0.62, 1.01)	1.02 (0.79, 1.31)
5	307	44	0.99 (0.64, 1.51)	0.91 (0.59, 1.40)	0.90 (0.57, 1.41)	154	0.90 (0.71, 1.13)	0.89 (0.70, 1.13)	1.17 (0.92, 1.49)
P-trend			0.56	0.76	0.91		0.57	0.62	0.053

[1]Multivariate sex- and stage-stratified Cox regression models initially included postdiagnostic one-carbon nutrient intakes, prediagnostic intake of the variable of interest, age at diagnosis, year of diagnosis, BMI, family history of colorectal cancer in any first-degree relative, postdiagnostic aspirin use, postdiagnostic multivitamin use, postdiagnostic smoking status, postdiagnostic alcohol consumption, postdiagnostic physical activity, tumor location, tumor differentiation, and the time from diagnosis to questionnaire return. A backward elimination with a threshold of $P = 0.1$ was used to select variables in the final models. Tests for a linear trend across quintiles were calculated with the use of the median value for each quintile of nutrient intake as an ordinally scaled variable in the proportional hazards model.

[2]Total energy-adjusted folate intake from food and supplemental sources as dietary folate equivalents.

[3]Total energy-adjusted intake from food and supplemental sources.

TABLE 3
Colorectal cancer–specific and overall mortality according to postdiagnostic alcohol intake[1]

		Colorectal cancer–specific mortality				Overall mortality			
Category of postdiagnostic alcohol intake	Total cases, *n*	Event, *n*	Univariate HR (95% CI)	Sex- and stage-stratified HR (95% CI)	Multivariate sex- and stage-stratified HR (95% CI)	Event, *n*	Univariate HR (95% CI)	Sex- and stage-stratified HR (95% CI)	Multivariate sex- and stage-stratified HR (95% CI)
No alcohol	644	83	1 (referent)	1 (referent)	1 (referent)	287	1 (referent)	1 (referent)	1 (referent)
0.1–14.9 g/d	646	66	0.75 (0.55, 1.04)	0.70 (0.50, 0.97)	0.51 (0.34, 0.76)	246	0.79 (0.66, 0.94)	0.76 (0.64, 0.91)	0.83 (0.70, 0.99)
≥15 g/d	260	27	0.78 (0.51, 1.20)	0.79 (0.50, 1.25)	0.53 (0.28, 0.98)	108	0.91 (0.73, 1.14)	0.77 (0.61, 0.98)	0.91 (0.72, 1.16)
P-trend	—	—	0.37	0.46	0.33		0.70	0.09	0.41

[1]Multivariate, stage- and sex-stratified Cox regression models initially included postdiagnostic alcohol consumption; prediagnostic alcohol consumption; age at diagnosis; year of diagnosis; BMI; family history of colorectal cancer in any first-degree relative; postdiagnostic aspirin use; postdiagnostic multivitamin use; postdiagnostic smoking status; postdiagnostic physical activity; postdiagnostic folate, vitamin B-12, methionine, and vitamin B-6 intakes; tumor location; tumor differentiation; and the time from diagnosis to questionnaire return. A backward elimination with a threshold of $P = 0.1$ was used to select variables in the final models. Tests for a linear trend across alcohol categories were calculated with the use of the median value for each category as an ordinally scaled variable in the proportional hazards model.

that survival bias was inherent in selecting cases with available data. Nonetheless, the reduction of the interval to dietary assessment by including data from all FFQs returned ≤4 y of diagnosis reduced the median time between diagnosis and the return of the FFQs, but did not substantially alter the results. Another limitation was the unavailability of data on adjuvant therapy and cancer recurrence. However, because of the duration of follow-up available in our study, we believe cancer-specific and overall mortality were robust endpoints (46). Furthermore, it seems unlikely that chemotherapy use differed according to one-carbon nutrient intake or alcohol, and we stratified by disease stage, on which treatment decisions are largely based. However, it is likely that some patients who received adjuvant therapy were exposed to folinic acid, which is a folate analog, and we cannot dismiss this as a potential source of bias.

It is conceivable that high folate intake might be associated with unmeasured health-related behaviors such as more intensive disease follow-up. Althogh it is impossible to exclude residual confounding, note that data from meta-analyses and a recent randomized controlled trial have failed to show a reduction in disease-specific mortality in colorectal cancer patients who received more-intensive surveillance (47, 48). An additional caveat was that our study may have been unable to detect associations of small or modest magnitude between the exposures of interest and mortality. Our study gained several strengths from use of the database of 2 large, nationwide, prospective US cohorts. Dietary and lifestyle patterns often change after the diagnosis of cancer (49), and we were able to assess associations between postdiagnostic nutrient intakes, adjusting for prediagnostic intakes, with the use of prospectively-collected data. Furthermore, the accuracy of the FFQ data from these cohorts has been extensively validated (27, 30). The colorectal cancer cases from these cohorts were diagnosed and treated at hospitals across the Unites States and are more representative of colorectal cancer cases in the general population than those from a single academic institution. Nonetheless, additional studies are necessary to assess the generalizability of our findings and the possible effect modification by demographic, ethnic, socioeconomic, and other factors. Aside from broader arguments advocating the publication of null studies (50) and the absence of comparable data in the literature, we believe our findings represent an important contribution to what remains a contentious issue (15, 17–19, 37).

In conclusion, our findings suggest that higher intakes of one-carbon nutrients do not substantially influence risk of death after a diagnosis of nonmetastatic colorectal cancer. Our data fail to support the hypothesis that higher intake of total folate or folic acid increases risk of colorectal cancer mortality after tumor resection.

The authors' responsibilities were as follows—PL, RN, ZRQ, KM, JAM, ATC, CSF, and SO: interpreted the data; SO: conceived, designed, and supervised the study; PL and RN: performed the statistical analysis; PL, RN, and SO: drafted the manuscript; PL, RN, ZRQ, CSF, and SO: provided administrative, technical, or material support; and all authors: acquired the data, critically revised the manuscript, and assumed full responsibility for the analyses and interpretation of the data. ATC has previously served as a consultant for Bayer Healthcare, Millennium Pharmaceuticals, and Pfizer Inc. PL, RN, ZRQ, KM, YC, YS, SAK, KI, XZ, KW, EG, JAM, CSF, and SO reported no conflicts of interest related to the study.

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