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Prediagnostic plasma folate and the risk of death in patients with colorectal cancer

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Abstract

Purpose—Although previous studies have demonstrated an inverse relationship between folate intake and colorectal cancer risk, a recent trial suggests that supplemental folic acid may accelerate tumorigenesis among patients with a history of colorectal adenoma. Therefore, high priority has been given to research investigating the influence of folate on cancer progression in patients with colorectal cancer.

Methods—To investigate whether prediagnostic levels of plasma folate are associated with colorectal cancer-specific and overall mortality, we performed a prospective, nested observational study within two large U.S. cohorts, the Nurses' Health Study and Health Professionals Follow-up Study. We measured folate levels among 301 participants who developed colorectal cancer 2 or more years after their plasma was collected and compared participants using Cox proportional hazards models by quintile of plasma folate.

Results—Higher levels of plasma folate were not associated with an increased risk of colorectal cancer-specific or overall mortality. Compared with participants in the lowest quintile of plasma folate, those in the highest quintile experienced a multivariable-adjusted hazard ratio for colorectal-cancer-specific mortality of 0.42 (95% CI, 0.20–0.88) and overall mortality of 0.46 (95% CI, 0.24–0.88). When the analysis was limited to participants whose plasma was collected within 5 years of cancer diagnosis, no detrimental effect of high plasma folate was noted. In subgroup analyses, no subgroup demonstrated worse survival among participants with higher plasma folate levels.

Conclusion—In two large prospective cohorts, higher prediagnostic levels of plasma folate were not associated with an increased risk of colorectal cancer-specific or overall mortality.

Keywords

Colorectal cancer; cancer-specific mortality; folate; prospective cohort study

INTRODUCTION

Numerous epidemiologic studies have demonstrated an association between higher folate intake and a lower risk of colorectal adenoma and cancer^{1–3}. However, a recent placebo-controlled, randomized clinical trial noted no reduction in colorectal adenoma risk with supplemental folic acid, among subjects with prior colorectal adenoma⁴. Moreover, when compared to placebo, supplemental folic acid was associated with a 67% increase in the risk of advanced lesions and a more than 2-fold increase in the risk of developing at least 3 adenomas. The authors questioned whether supplemental folic acid may promote the growth of early precursor lesions present in the colonic mucosa. Animal studies have suggested that the timing of folic acid supplementation may be of critical importance; folic acid administration prior to the existence of preneoplastic lesions may prevent tumor development, whereas, administration of folic acid after the development of neoplastic foci may promote tumor progression⁵.

With mandatory food fortification with folic acid in the United States in 1998⁶ and the increasing use of folate-containing supplements, particularly in older populations⁷, a possible growth-promoting effect of folic acid on colorectal neoplasia is particularly concerning. Moreover, patients with cancer tend to consume more supplements than healthy individuals^{8,9}, possibly placing a significant population of patients at increased risk for cancer recurrence and progression. Thus, the investigation of folate's effects on patients with colorectal cancer represents a high priority research area¹⁰. In the current study, we prospectively examined the influence of prediagnostic levels of plasma folate on patients diagnosed with colorectal cancer participating in two large, prospective cohort studies.

MATERIALS AND METHODS

Study Population

The Nurses' Health Study (NHS) began in 1976, when 121,700 female nurses between 30 and 55 years of age completed a baseline questionnaire about their lifestyles and medical histories. Subsequently, these women have completed a self-administered, mailed questionnaire biennially to update information on their lifestyle, medical history, and diet. A total of 32,826 women between 43 and 69 years of age returned a mailed blood collection kit by overnight courier in 1989 and 1990. The Health Professionals Follow-up Study (HPFS) was initiated in 1986 when 51,529 U.S. men aged 40–75 years responded to a mailed questionnaire. Subsequently, these men have completed a self-administered, mailed questionnaire biennially to update information on their lifestyle, medical history, and diet. Blood was collected from 18,225 men and returned in a mailed blood collection kit by overnight courier in 1993 through 1995. In both cohorts, blood samples were centrifuged on arrival and separated into plasma, white blood cells, and red blood cells. In addition, approximately 95% of samples were received by overnight courier within 24 hours of blood collection. The current study was approved by the Human Research Committee at the Brigham and Women's Hospital, Boston, MA, and all participants provided consent.

Identification of Case Patients

When a participant (or next of kin for decedents) reported a diagnosis of colorectal cancer on a follow-up questionnaire, we asked permission to obtain hospital records and pathology reports. Study physicians blinded to exposure data reviewed medical records. For

nonrespondents, we searched the National Death Index (NDI) to discover deaths and ascertain diagnoses of colorectal cancer. In the NHS and HPFS, we included all participants diagnosed with colon or rectal cancer between the date of blood collection and May 31, 2000 or January 31, 2002, respectively. One participant from the NHS and HPFS were excluded due to unsuccessful measurements of plasma folate. Due to the possibility of subclinical cancer leading to alterations in folate intake and plasma folate levels, we excluded from our analysis those participants whose cancer was diagnosed within 2 years of blood collection.

Measurement of Mortality

Women were observed until death or June 2005, whichever came first. Men were observed until death or January 2005. Ascertainment of deaths included reporting by family or postal authorities. Names of persistent nonresponders were searched in the NDI. More than 98% of deaths have been identified by these methods¹¹. Physician reviewers assigned cause of death.

Laboratory Analyses

All plasma folate measurements were made by radioassay (Bio-Rad, Richmond, CA) at the Jean Mayer U.S. Department of Agriculture Human Nutrition Research Center on Aging at Tufts University. In the NHS, plasma folate levels were measured in two batches: in 1998 for cases diagnosed in 1990–1994 and in 2003 for cases diagnosed in 1996–2000. In the HPFS, samples were run as a single batch in 2005 for all cases of colorectal cancer identified between 1994 and 2002. To assess laboratory precision, each batch included masked replicate plasma samples. The mean coefficients of variation for plasma folate were 7.6% for HPFS and < 10% for the two batches in the NHS.

Covariates

Tumor stage, grade of differentiation, and location (colon or rectum) were extracted from the medical record. Starting in 1993, women were asked about colorectal cancer treatment in a supplemental questionnaire in the NHS. Further covariates were obtained from the questionnaire prior to the measurement of plasma folate.

Statistical Analyses

The primary exposure was a participant's prediagnostic level of plasma folate. Participants were divided into groups, based on quintiles of plasma folate. To account for slight variations in folate laboratory values over time, we generated quintiles of plasma folate separately for HPFS and the two batches in the NHS. Baseline characteristics were determined for participants in each quintile and differences across quintiles were evaluated with analysis of variance (ANOVA) for continuous variables and chi-squared for categorical variables.

Cox proportional hazards models were used to calculate hazards ratios with 95% confidence intervals for colorectal cancer-specific and overall mortality, according to quintile of plasma folate. Follow-up time was calculated from the date of colorectal cancer diagnosis to the date of death or to June 2005, whichever came first in the NHS, and to the date of death or January 2005, whichever came first in the HPFS. In the analyses of colorectal-cancer specific mortality, non-colorectal cancer-specific deaths were censored at the time of death. Two-tailed *P*-values for linear trend tests across categories were calculated by modeling the median value of each category as a continuous variable. The proportionality of hazards assumption was satisfied by evaluating time-dependent variables, which were the cross-product of plasma folate categories with log time.

To provide increased power for subgroup analyses, plasma folate was categorized into tertiles. Tests of interaction between tertiles of plasma folate and potential effect modifiers were assessed by entering into the model the cross-product of the plasma folate tertile and the dichotomized covariate. All statistical analyses were performed using the SAS 9.1 statistical package (SAS Institute, Cary, North Carolina) and all *P*-values are two sided.

RESULTS

Among the 301 eligible participants with colorectal cancer, there were 122 deaths, of which 95 were colorectal cancer-specific deaths. Plasma folate levels were assessed at a median of 6.0 years prior to colorectal cancer diagnosis (range, 2.1 to 10.7 years). Baseline patient characteristics by quintile of plasma folate are shown in Table 1. Participants with higher plasma folate levels were noted to have a higher total intake of folate, a higher rate of regular multivitamin use, and a higher total intake of vitamin D and calcium.

Higher levels of plasma folate were not associated with an increase in colorectal cancer-specific or overall mortality (Table 2). In fact, higher levels of plasma folate were associated with reduced mortality. Compared with participants in the bottom quintile of plasma folate, participants in the top quintile had a multivariable-adjusted hazard ratio for colorectal cancer-specific mortality of 0.42 (95% CI, 0.20–0.88; *P* for trend, 0.01) and overall mortality of 0.46 (95% CI, 0.24–0.88; *P* for trend 0.02). Six covariates most influenced the adjusted hazard ratios in the multivariable-adjusted models: stage of disease, receipt of chemotherapy, time period of diagnosis, level of physical activity, total vitamin D intake, and postmenopausal hormone use.

We repeated our analysis after categorizing participants into deciles of plasma folate, to consider the possibility that more extreme levels of plasma folate might promote cancer progression. As compared to patients in the lowest decile (median plasma folate, 2.4 ng/ml), those in highest decile (median plasma folate, 19.7 ng/ml) had a multivariable-adjusted hazard ratio for colorectal cancer-specific mortality of 0.38 (95% CI, 0.15–0.97). Similarly, participants in the highest decile of plasma folate had a reduced risk for overall mortality, when compared with participants in the lowest decile (HR, 0.38; 95% CI, 0.17–0.81).

We also examined the influence of plasma folate on survival in those participants with plasma folate levels obtained closest to their time of diagnosis, i.e. within 5 years of plasma collection. When compared to those in the first tertile, participants in the second and third tertiles of plasma folate demonstrated hazard ratios for colorectal cancer-specific mortality of 0.49 (95% CI, 0.26–0.94) and 0.29 (95% CI, 0.13–0.65), respectively (*P* for trend = 0.004). Similarly, hazard ratios for overall mortality among participants in the second and third tertiles compared with those in the first tertile were 0.58 (95% CI, 0.32–1.04) and 0.56 (95% CI, 0.29–1.07), respectively (*P* for trend = 0.15).

In light of the apparent survival improvement associated with higher plasma folate, we considered the possibility that patients with occult cancer recurrences and limited survival may possess lower plasma folate levels. Consequently, we performed an analysis after excluding participants whose diagnosis was within 4 years of plasma collection (Table 3). The inverse association between plasma folate and both colorectal cancer-specific and overall mortality was maintained after these more stringent exclusion criteria.

For the analysis of three-year colorectal cancer-specific survival, plasma folate was also categorized into tertiles (Figures 1 and 2). The proportion of patients with colorectal cancer-specific death at three years was 35% for the first tertile, 24% for the second tertile and 19% for the third tertile of plasma folate (*P*, log rank test = 0.03; Figure 1).

We examined the influence of plasma folate levels across strata of other predictors of colon cancer-specific mortality (Table 4). The inverse relation between prediagnostic plasma folate levels and cancer-specific and overall mortality remained similar across strata of clinical covariates. Since mandatory folate fortification began in 1998, we evaluated whether the inverse association between plasma folate and mortality was different among those participants diagnosed with colorectal cancer before or after December 1997. The benefit of high plasma folate was more pronounced among participants diagnosed before 1998 when compared to those diagnosed in 1998 or later (P , interaction = 0.01), although a detrimental effect of folate was not noted in either group.

To evaluate plasma folate levels in a more homogenous population of patients with resected disease, we repeated our analyses after the exclusion of participants with either stage IV (metastatic) disease or with unknown stage. When compared to participants in the first tertile of plasma folate, those in the second and third tertiles had hazard ratios for colorectal-cancer specific mortality of 0.50 (95% CI, 0.22–1.15) and 0.42 (95% CI, 0.16–1.11), respectively.

DISCUSSION

In this prospective, nested observational study, we found no evidence that participants with colorectal cancer and higher prediagnostic levels of plasma folate are at an increased risk for colorectal cancer-specific or overall mortality. No detrimental effect of plasma folate was found either when comparing participants in the top quintile to those in the bottom quintile or when comparing participants in the highest decile to those in the lowest decile of plasma folate. In addition, no harmful effect of high plasma folate on mortality was noted after analyzing those participants with plasma folate levels drawn within five years of their cancer diagnosis.

Instead of a harmful effect, we observed a statistically significant inverse relationship between plasma folate and the risk of colorectal cancer-specific and overall mortality, after controlling for other risk factors for colorectal cancer mortality. The reduction in mortality appeared to be non-linear, with a similar improvement in survival for those participants in the third through fifth quintile of plasma folate. The inverse relationship between plasma folate and mortality was noted in all evaluated subgroups, although this relationship was attenuated in participants diagnosed with colorectal cancer after the date of mandatory food fortification with folic acid.

Multiple case-control and observational cohort studies suggest a reduction of 30% to 40% in colorectal cancer risk for participants with high levels of folate intake compared to those with low levels^{1–3}. In four studies of plasma folate and colorectal cancer risk, two demonstrated an inverse relationship between levels of plasma folate and colorectal cancer risk^{12–15}. In contrast, the Polyp Prevention Study randomized participants with a prior history of colorectal polyps to receive placebo or 1 mg/day of folic acid, and demonstrated no reduction in recurrent colorectal polyps in those participants receiving supplemental folic acid⁴. Somewhat unexpectedly, those participants receiving folic acid had an increased risk for advanced lesions and multiple polyps. In an accompanying editorial by Ulrich and Potter¹⁰, and a recent paper by Mason and colleagues¹⁶, concern has been expressed regarding the implications of high folate levels for colorectal tumor recurrence and progression.

Folate is suspected to influence tumorigenesis through its role as a conveyor of one-carbon units in the synthesis of purines, thymidylate, and methionine⁵. When intake of folate is inadequate, DNA biosynthesis and methylation are impaired, which can lead to chromosome

breaks, alterations in gene expression, and genomic instability¹⁷. Thus, in the absence of pre-neoplastic lesions, inadequate folate may promote cancer development.

Animal studies have suggested that supraphysiologic doses of folic acid and folate administered after the development of neoplastic foci may in fact promote colorectal tumorigenesis⁵. Since neoplastic cells divide more rapidly than their normal counterparts, they require higher rates of DNA synthesis, which in turn, requires the presence of folate and other one-carbon donors. In neoplastic cells, the lack of adequate folate is thought to impair rapid rates of division and lead to a decrease in tumor growth¹⁸. Therefore, plausible mechanisms exist to explain a possible benefit and a possible harm to high levels of folate intake, particularly in relation to timing and dose.

We evaluated whether higher prediagnostic levels of plasma folate were associated with a worse outcome in patients with colorectal cancer. Several aspects of this study lend credibility to its findings, including the prospective and longitudinal updating of covariate information; prospective measurement of plasma folate two or more years prior to colorectal cancer diagnosis, reducing the likelihood of bias due to reverse causation; high follow-up rates in both cohorts; strict quality control measures with low coefficients of variance for measurements of plasma folate; large sample size for a plasma-based study in colorectal cancer; and similar results in two different prospective cohorts.

In addition, the inverse association of plasma folate with mortality was attenuated among participants whose cancers were diagnosed after the date of food fortification with folic acid, providing further support for a true effect of folate on mortality. Among citizens of the United States and Canada, plasma folate levels increased by approximately 50% after the initiation of food fortification^{19,20}. Therefore, an increase in total folate intake among participants diagnosed after 1997 would likely obscure the inverse relationship between prediagnostic plasma folate and mortality in this group.

Our analysis has several limitations. We have only one measurement of plasma folate made at a single point in time. However, previous evidence suggests that plasma folate levels predict long-term exposure to this vitamin²¹. Additionally, the mean plasma folate level among participants in the highest quintile (17.1 ng/ml) and decile (21.5 ng/ml) of our study were lower than that seen in the treatment group of the Polyp Prevention Study, in which participants receiving 1 mg/day of folic acid were noted to have a mean plasma folate level of 32.8 ng/ml⁴. Therefore, we cannot entirely rule out the possibility that no adverse effect on mortality was seen among participants with high plasma folate in our study, because plasma folate levels in our population were lower than those among patients in randomized clinical trials of supplemental folic acid.

We had limited information on which participants received chemotherapy. Most standard chemotherapy regimens for patients with colorectal cancer include a fluoropyrimidine, a class of drug that inhibits thymidylate synthetase²². Folinic acid, a reduced folate, improves the efficacy of the fluoropyrimidine 5-fluorouracil²³, indicating that folate stores may influence tumor responsiveness to chemotherapy. In our analyses, we attempted to overcome this lack of data on chemotherapy use by controlling for date of diagnosis and stage in our multivariate analyses, which are both strong predictors of the receipt of chemotherapy. We also performed a stratified analysis by stages I/II and III/IV and continued to see an effect of plasma folate on mortality even among patients with stage I or II disease, the majority of whom would be unlikely to receive chemotherapy. Additionally, among those participants with available information regarding chemotherapy use, no significant differences were noted in the percent of patients receiving chemotherapy by quintile of plasma folate.

Finally, we cannot entirely exclude the possibility that lower levels of plasma folate may be reflective of other occult predictors for poor prognosis. However, our findings were consistent after adjusting for other potential risk factors for colorectal cancer mortality and among participants with either stage I/II or stage III/IV disease. We also continued to observe an inverse relationship between plasma folate and mortality after extending the time between plasma collection and cancer diagnosis to 4 years. Furthermore, we would expect few patients to have undetected recurrences over extended periods of time, given the relatively brief natural history of recurrent colorectal cancer.

In summary, this prospective, nested observational study did not observe a detrimental effect of high levels of plasma folate on mortality among patients with colorectal cancer. Given the large public health implications of folic acid supplementation and the recent concerning results from randomized trials, further studies are needed to better elucidate the role of folate in malignant transformation and progression.

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Figure 1.
Cumulative incidence curve of colorectal cancer-specific mortality by tertile of prediagnostic plasma folate

Table 1
Baseline characteristics of women and men with colorectal cancer according to quintile of prediagnostic plasma folate

Characteristic	Quintiles of Plasma Folate (%)					P-value [#]
	Q1 N = 60	Q2 N = 61	Q3 N = 60	Q4 N = 60	Q5 N = 60	
Median plasma folate (ng/ml)	3.2	4.9	6.9	9.1	15.1	
Age (years) [*]	69.5 (+/-8.1)	68.0 (+/-8.1)	68.0 (+/-8.2)	70.0 (+/-7.6)	71.5 (+/-8.2)	0.19
Gender						0.99
Female	53	52	52	52	52	
Male	47	48	48	48	48	
Caucasian	97	93	97	95	98	0.70
Body-mass index (kg/m ²) [*]	25.8 (+/-4.3)	25.4 (+/-3.8)	24.9 (+/-4.6)	25.3 (+/-3.5)	24.9 (+/-4.1)	0.81
Activity level (MET-hr/wk) [*]	14.8 (+/-17.9)	16.7 (+/-25.5)	20.4 (+/-28.7)	16.5 (+/-29.4)	15.6 (+/-41.2)	0.67
Smoking status						0.98
Never	40	44	43	43	38	
Past	47	46	45	50	50	
Current	13	10	12	7	12	
Alcohol consumption (gm/d) [*]	1.3 (+/-9.8)	3.5 (+/-10.9)	2.5 (+/-15.9)	2.7 (+/-13.4)	4.4 (+/-10.2)	0.49
Diabetes mellitus	2	5	12	5	3	0.14
Family history of colorectal cancer ^{II}	22	18	22	20	17	0.94
Aspirin use, > once per week	18	25	38	33	32	0.13
Post-menopausal hormone use [§]						0.38
Pre-menopausal	16	9	13	13	3	
Never used	38	41	42	39	16	
Current user	25	28	19	26	36	
Past user	22	22	26	23	45	
Regular multivitamin use	13	33	30	55	67	<0.0001
Total folate intake (mcg/d) [*]	300.5 (+/-97.1)	333.0 (+/-143.4)	364.0 (+/-197.7)	453.0 (+/-239.4)	632.0 (+/-432.1)	<0.0001
Total calcium intake (mg/d) [*]	715.5 (+/-424.1)	763.0 (+/-387.7)	773.5 (+/-442.8)	910.0 (+/-615.2)	970.0 (+/-506.1)	0.05
Total vitamin D intake (IU/d) [*]	208.8 (+/-160.1)	238.0 (+/-208.1)	302.5 (+/-243.0)	398.3 (+/-260.9)	524.3 (+/-302.3)	<0.0001

Characteristic	Quintiles of Plasma Folate (%)					P-value [#]
	Q1 N = 60	Q2 N = 61	Q3 N = 60	Q4 N = 60	Q5 N = 60	
Tumor location						0.26
Colon	75	77	63	80	70	
Rectum	25	23	37	20	30	
Tumor differentiation						0.52
Well	5	7	13	10	10	
Moderate	60	57	52	43	60	
Poor	12	13	7	8	8	
Unknown	23	23	28	38	22	
Stage of disease						0.08
I	17	16	28	33	30	
II	18	34	18	23	17	
III	18	25	18	20	23	
IV	20	13	10	12	18	
Unknown	27	12	25	12	12	
Chemotherapy received						0.80
Yes	13	10	15	10	7	
No	5	10	12	10	8	
Unknown	82	80	73	80	85	

* Median +/- standard deviation

^{II} History of colorectal cancer in a participant's mother, father, or sibling

[§] Among women only

[#] Covariates were analyzed using analysis of variance (ANOVA) for continuous variables and chi-squared for categorical variables

Age-adjusted and multivariable-adjusted hazard ratios and 95% confidence intervals for mortality by quintile of prediagnostic plasma folate

Table 2

Quintiles of Plasma Folate Hazard ratio (95% confidence interval)						P-value, for trend
	Q1	Q2	Q3	Q4	Q5	
Median plasma folate (ng/ml)	3.2	4.9	6.9	9.1	15.1	
No. at risk	60	61	60	60	60	
<i>Colorectal Cancer-Specific Mortality</i>						
No. of deaths	26	24	11	15	19	
Age-adjusted [§]	1.00	0.89 (0.51–1.54)	0.35 (0.17–0.71)	0.48 (0.25–0.91)	0.67 (0.37–1.22)	0.17
Multivariable-adjusted [†]	1.00	1.02 (0.56–1.84)	0.37 (0.17–0.82)	0.47 (0.22–1.01)	0.42 (0.20–0.88)	0.01
<i>Overall Mortality</i>						
No. of deaths	31	27	17	21	26	
Age-adjusted [§]	1.00	0.84 (0.50–1.40)	0.43 (0.24–0.78)	0.53 (0.30–0.92)	0.73 (0.43–1.23)	0.30
Multivariable-adjusted [†]	1.00	0.93 (0.54–1.63)	0.42 (0.21–0.81)	0.45 (0.23–0.86)	0.46 (0.24–0.88)	0.02

[§] Adjusted for age at diagnosis

[†] Adjusted for age at diagnosis, stage of disease, histologic differentiation, receipt of chemotherapy, tumor location (colon, rectum), time period of diagnosis (1991–1994, 1995–1998, 1999–2002), and patient characteristics from the most recent questionnaire prior to blood draw, including body-mass index (kg/m²), level of physical activity (MET-hrs/week), smoking status (current, past, never), aspirin use (or > 1x/week), alcohol consumption (gm/day), total vitamin D intake (IU/day), and post-menopausal hormone use (premenopausal, never used, current user, past user, male gender).

Multivariable-adjusted hazard ratios for mortality by tertile of prediagnostic plasma folate and time between plasma collection and cancer diagnosis

Table 3

Time since plasma collection	No. at Risk	No. of Deaths	Hazard Ratio [§] (95% confidence interval)	P-value, trend
			<div>Tertile 1</div> <div>Tertile 2</div> <div>Tertile 3</div>	
<i>Colorectal Cancer-Specific Mortality</i>				
2 years	301	95	1.00 0.60 (0.34–1.04) 0.40 (0.22–0.74)	0.005
4 years	220	62	1.00 0.50 (0.24–1.05) 0.58 (0.27–1.26)	0.22
<i>Overall Mortality</i>				
2 years	301	122	1.00 0.56 (0.35–0.91) 0.42 (0.25–0.71)	0.003
4 years	220	80	1.00 0.48 (0.25–0.91) 0.55 (0.28–1.06)	0.12

[§] Adjusted for age at diagnosis, stage of disease, histologic differentiation, receipt of chemotherapy, tumor location (colon, rectum), time period of diagnosis (1991–1994, 1995–1998, 1999–2002), and patient characteristics from the most recent questionnaire prior to blood draw, including body-mass index (kg/m²), level of physical activity (MET-hrs/week), smoking status (current, past, never), aspirin use (or > 1x/week), alcohol consumption (gm/day), total vitamin D intake (IU/day), and post-menopausal hormone use (premenopausal, never used, current user, past user, male gender).

Table 4

Stratified analyses of colorectal cancer-specific mortality by tertile of prediagnostic plasma folate

Characteristic	No. at Risk	No. of Deaths	Hazard Ratio [§] (95% Confidence Interval)			P-value, interaction
			Tertile 1	Tertile 2	Tertile 3	
Age*						0.51
< 70 years	150	46	1.00	0.36 (0.15–0.87)	0.45 (0.15–1.34)	
70 years	151	49	1.00	0.87 (0.37–2.02)	0.35 (0.13–0.92)	
Cohort						0.65
NHS (women)	157	51	1.00	0.49 (0.23–1.05)	0.38 (0.16–0.91)	
HPFS (men)	144	44	1.00	0.68 (0.29–1.59)	0.20 (0.07–0.64)	
Body-mass index						0.30
<25 kg/m ²	144	49	1.00	0.67 (0.27–1.69)	0.66 (0.22–1.95)	
25 kg/m ²	157	46	1.00	0.50 (0.22–1.11)	0.18 (0.07–0.47)	
Physical activity*						0.23
< 16 MET-hrs/wk	149	53	1.00	0.84 (0.37–1.92)	0.28 (0.11–0.74)	
16 MET-hrs/wk	152	42	1.00	0.56 (0.23–1.34)	0.68 (0.26–1.78)	
Multivitamin use						0.58
No	182	60	1.00	0.54 (0.27–1.11)	0.34 (0.12–0.97)	
Yes	119	35	1.00	0.74 (0.21–2.62)	0.43 (0.12–1.50)	
Alcohol use*						0.98
< 2.9 gm/d	151	41	1.00	0.51 (0.21–1.23)	0.12 (0.04–0.40)	
2.9 gm/d	150	54	1.00	0.62 (0.28–1.36)	0.58 (0.25–1.39)	
Stage of disease						0.98
I/II	142	18	1.00	0.41 (0.10–1.63)	0.21 (0.04–1.03)	
III/IV/unknown	159	77	1.00	0.59 (0.31–1.11)	0.44 (0.23–0.87)	
Year of diagnosis						0.01
1991–1997	146	52	1.00	0.31 (0.14–0.67)	0.16 (0.06–0.40)	
1998–2002	155	43	1.00	1.14 (0.46–2.83)	0.72 (0.27–1.94)	

* Cutpoints chosen based on median values for age, physical activity, and alcohol use.

§ Multivariable-adjusted hazard ratios adjusted for age at diagnosis, stage of disease, histologic differentiation, receipt of chemotherapy, tumor location (colon, rectum), time period of diagnosis (1991–1994, 1995–1998, 1999–2002), and patient characteristics from the most recent questionnaire prior to blood draw, including body-mass index (kg/m^2), level of physical activity (MET-hrs/week), smoking status (current, past, never), aspirin use (or > 1x/week), alcohol consumption (gm/day), total vitamin D intake (IU/day), and post-menopausal hormone use (premenopausal, never used, current user, past user, male gender). In each case, the stratification variable was excluded from the model.