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Prediagnostic plasma vitamin B6 (pyridoxal 5'-phosphate) and survival in patients with colorectal cancer

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Abstract

Purpose—Higher plasma pyridoxal 5'-phosphate (PLP) levels are associated with a decreased incidence of colorectal cancer, but the influence of plasma PLP on survival of patients with colorectal cancer is unknown. We prospectively examined whether prediagnostic plasma PLP levels are associated with mortality among colorectal cancer patients.

Conflict of interest

The authors declare that they have no conflict of interest.

Methods—We included 472 incident cases of colorectal cancer identified in the Nurses' Health Study, the Health Professionals Follow-up Study, and the Physicians' Health Study from 1984 to 2002. The patients provided blood samples two or more years before cancer diagnosis. Stratified Cox proportional hazards models were used to calculate hazard ratios (HR) with 95% confidence interval (CI) adjusted for other risk factors for cancer survival.

Results—Higher plasma PLP levels were not associated with a significant reduction in colorectal cancer-specific (169 deaths) or overall mortality (259 deaths). Compared with patients who had less than 45 pmol/ml of plasma PLP (median: 33.6 pmol/ml), those who had 110 pmol/ml or higher levels (median: 158.8 pmol/ml) had multivariable HRs of 0.85 (95% CI 0.50–1.45, P trend = 0.37) and 0.87 (95% CI 0.56–1.35, P trend = 0.24) for colorectal cancer-specific and overall mortality. Higher plasma PLP levels, however, seemed to be associated with better survival among patients who had lower circulating 25-hydroxyvitamin D₃ levels (< 26.5 ng/ml) (P interaction = .005).

Conclusions—Higher prediagnostic plasma PLP levels were not associated with an improvement on colorectal cancer survival overall. Further research is needed to clarify the influence of vitamin B6 on colorectal cancer progression and survival.

Keywords

vitamin B6; pyridoxal 5' phosphate (PLP); colorectal cancer; mortality; survival; prospective study

Introduction

Colorectal cancer is the third leading cause of cancer death in each sex and second overall in men and women combined. In 2011, 141,210 men and women are estimated to be newly diagnosed with colorectal cancer; 49,380 deaths will be attributable to the disease in the United States [1]. The number of patients who survive colorectal cancer is increasing, partly due to earlier detection and more-effective treatment methods. Colorectal cancer survival is dependent on several tumor characteristics such as tumor stage and differentiation [2]. However, not all patients with morphologically identical colorectal cancers who receive comparable therapeutic strategies have the same clinical outcomes, the variability of which may be due partly to differences in nutritional or other lifestyle factors [3]. Although a growing body of evidence suggests that dietary modification is likely to have a substantial overall impact on risk of colorectal cancer [4], few studies have examined the association between diet and outcomes in colorectal cancer patients.

Vitamin B6 is one of the nutrients that have been shown to be associated with colorectal cancer risk [5]. In circulation, it exists mainly as pyridoxal 5'-phosphate (PLP: the biologically active form of vitamin B6) which is considered as the best single measure because it reflects tissue stores [6]. A recent meta-analysis of prospective studies showed that high levels of plasma PLP were significantly associated with lower risk of colorectal cancer [5]. Vitamin B6 is critical for nucleotide synthesis and DNA methylation through its role in one-carbon metabolism, and thus vitamin B6 deficiency can lead to chromosome breaks, alterations in gene expression, and genomic instability [7]. Several studies have also found that PLP was an effective inhibitor of many enzymes, including RNA polymerase [8], reverse transcriptase [9], and DNA polymerase [10], overexpression of which cause cell proliferation and oncogenic transformation. In addition, vitamin B6 has been shown to reduce oxidative stress [11], nitric oxide synthesis [12], inflammation [13, 14], cell proliferation [12, 15], and have antiangiogenic properties [16, 17], all of which are associated with carcinogenesis in general and colorectal cancer in particular.

Despite the potential beneficial role of vitamin B6 on colorectal cancer progression, the influence of vitamin B6 on survival in patients with established colorectal cancer has not been investigated. Therefore, we prospectively examined the association of prediagnostic levels of plasma PLP on survival of participants diagnosed with colorectal cancer in three large prospective cohort studies: a female cohort, the Nurses' Health Study, and two male cohorts, Health Professionals Follow-up Study and Physicians' Health Study.

Materials and Methods

Study populations

The Nurses' Health Study (NHS) was initiated in 1976, when 121,700 female nurses aged 30–55 years returned a mailed questionnaire on risk factors for cancer and cardiovascular disease [18, 19]. The Health Professionals Follow-up Study (HPFS) began in 1986 when 51,529 male health professionals aged 40–75 years returned a mailed questionnaire on risk factors for cancer, cardiovascular disease, and diabetes. In these two cohorts, participants have updated information on their lifestyle and medical history through biennial follow-up questionnaires. The Physicians' Health Study (PHS) was initiated in 1982 as a randomized controlled trial of aspirin and β -carotene in the primary prevention of cardiovascular disease and cancer among 22,071 healthy male physicians aged 40–84 years, and the physicians provided information on their lifestyle and medical history at baseline.

Blood samples were collected by 32,826 women in the NHS (1989–1990), by 18,225 men in the HPFS (1993–1995), and by 14,916 men in the PHS (1982–1984) in tubes with heparin (NHS) or EDTA (HPFS and PHS) through overnight courier in chilled containers. In the NHS and HPFS, the blood specimens were sent as whole blood to investigators, while in the PHS, they were immediately centrifuged and separated for plasma by the participants, and sent back to investigator. The blood specimens were stored in liquid nitrogen freezer at -130°C .

Individuals in this analysis were participants with pathologically confirmed colorectal adenocarcinoma diagnosed after the date of blood collection until May 31, 2000 for NHS, January 31, 2002 for HPFS, and July 31, 2000 for PHS. Participants were excluded if they had reported any cancer (other than nonmelanoma skin cancer) prior to the colorectal cancer diagnosis. Because of possibility of subclinical cancer leading to alterations in plasma PLP levels, we excluded from our analysis those participants whose cancer was diagnosed within 2 years of blood collection (79 patients). Therefore, a total of 472 colorectal cancer patients (150 patients for NHS, 137 patients for HPFS, and 185 patients for PHS) were included in this analysis.

The NHS and PHS were approved by the Institutional Review Boards (IRB) of the Brigham and Women's Hospital (Boston, MA), and the HPFS was approved by the IRB of the Harvard School of Public Health (Boston, MA).

Identification of colorectal cancer

Participants were asked whether they had a diagnosis of colorectal cancer by biennial (NHS and HPFS) and annual (PHS) follow-up questionnaires and then confirmed through review of medical records by the study investigators (NHS and HPFS) or an end-point committee (PHS). When a participant reported a colorectal cancer diagnosis, hospital records and pathology reports were requested, and physicians blinded to exposure data reviewed medical records and recorded information on tumor stage, histology, and location.

Measurement of mortality

Participants in the study were observed until death, May 31, 2010 (NHS), January 31, 2010 (HPFS), or March 1, 2011 (PHS). Ascertainment of deaths included reporting by family or postal authorities. Names of persistent nonresponders were searched in the National Death Index. More than 98% of deaths have been identified by these methods [20, 21]. Physicians blinded to exposure status assigned cause of death after review of death certificates, information from the family, and medical records [22].

Laboratory analyses

The stored plasma was thawed, and PLP, an active form of vitamin B6, was measured by an enzymatic procedure based on radioactive tyrosine and the apoenzyme tyrosine decarboxylase [23, 24]. During the assay process, masked replicate plasma samples were included in a manner identical to that for the regular sample to assess laboratory precision. The mean intraassay coefficients of variation for PLP from these blinded quality controls were less than 10% in each study. To control for two other biomarkers, plasma folate and 25-hydroxyvitamin D₃ (25(OH)D), which have been shown to influence survival, previously [25, 26], we measured concentrations of plasma folate using a radioassay kit (Bio-Rad, Richmond, CA) in the NHS and HPFS or a microbiological method [27] in the PHS, and plasma 25(OH)D using radioimmunoassay, as described previously [28]. DNA was extracted from blood using a commercially available process based on the absorption of DNA to a silica membrane after lysis with a proprietary agent (Qiagen, Chatsworth, CA). To examine a potential interaction between plasma PLP level and a common polymorphism in the methylenetetrahydrofolate reductase (*MTHFR C677T*) gene, a key enzyme in the provision of methyl groups to the body by converting 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which influences circulating levels of 5-methyl-tetrahydrofolate, the TaqMan assay was used [29, 30]. All assays were conducted at the Jean Mayer U.S. Department of Agriculture Human Nutrition Research Center on Aging at Tufts University.

Covariates

Prognostic factors known to influence cancer mortality were extracted from the medical records, including age at diagnosis, tumor stage, grade of differentiation, primary tumor location, and year of diagnosis. Starting in 1993, women were asked about colorectal cancer treatment in a supplemental questionnaire in the NHS. For all 3 cohorts, further covariates were obtained from the questionnaire closest but prior to blood collection, including body mass index (BMI: kg/m²), physical activity, smoking status, aspirin use, postmenopausal hormone use (NHS only), and alcohol consumption.

Statistical analyses

The primary exposure was a participant's prediagnostic level of plasma PLP. Participants were categorized as four groups, <45 (reference), 45 to <70, 70 to <110, or ≥110 pmol/ml, which approximates the quartiles of plasma PLP based on the distribution in the study population. Stratified Cox proportional hazards models were used to calculate hazard ratios (HRs) with 95% confidence intervals (CIs) for colorectal cancer-specific mortality as a primary endpoint and overall mortality as a secondary endpoint [31]. Using the Cox regression model stratified by cohort allows different underlying baseline hazards of death across cohort, and simultaneously control for other risk factors or potential confounders for cancer survival. In the analysis of colorectal cancer-specific mortality, deaths from other causes were censored at the time of death. Follow-up time was calculated from the date of colorectal cancer diagnosis to the date of death or to May 31, 2010 for NHS, January 31, 2010 for HPFS or to March 1, 2011 for PHS, whichever came first.

We developed three models: 1) age-adjusted model, 2) age and clinical, tumor characteristics-adjusted model (multivariable model 1), and 3) age, clinical, tumor characteristics, and lifestyle factors-adjusted model (multivariable model 2). The multivariable model 1 was stratified by cohort and adjusted for age at diagnosis, cancer stage, grade of tumor, tumor location, receipt of chemotherapy, and time period of diagnosis. The multivariable model 2 was further adjusted for BMI, physical activity, smoking status, alcohol intake, aspirin use, postmenopausal hormone use, plasma folate levels, and plasma 25(OH)D levels.

We also conducted sensitivity analyses by excluding patients whose cancer were diagnosed within 4 or 6 years of blood collection to minimize the possibility that prediagnostic cancer influenced plasma PLP levels or excluding patients with either stage IV (metastatic) disease or unknown stage to assess plasma PLP levels in a more homogeneous population of patients with resected disease. The two-tailed *p* values for linear trend tests across categories of PLP values were calculated by modeling the median value of each category as a continuous variable. We also examined the potential nonlinear relationship between PLP levels and the HR of death, non-parametrically with restricted cubic splines [32]. Tests for non-linearity used the likelihood ratio test, comparing the model with only the linear term to the model with the linear and the cubic spline terms. When no significant non-linear relation was found, we calculated HRs for a 20 pmol/ml increment of PLP after exclusion of patients who had excessive plasma PLP levels (i.e., beyond 2.5 standard deviation for log-transformed PLP levels: 7 patients). The proportionality of hazards assumption was satisfied by evaluating time-dependent variables which are the cross-product of plasma PLP categories with log transformed of follow-up time.

In addition, we examined whether the associations between plasma PLP levels and mortality differed by several colorectal cancer risk factors including alcohol intake, smoking, BMI, physical activity, folate and vitamin D status. Test of interaction between plasma PLP and the potential effect modifiers were assessed by entering in the model the cross product of PLP as a continuous variable and the dichotomized covariate. All statistical analyses were performed using the SAS version 9.2. statistical software (SAS Institute Inc, Cary, NC), and all *p* values are two-sided.

RESULTS

A total of 259 deaths including 169 colorectal cancer-specific deaths were identified among the 472 eligible participants diagnosed with colorectal cancer (32% females, 68% males) over 49,943 person-months of follow-up. Plasma PLP levels were assessed at a median of 7.0 years (range, 2.1–17.3 years) before cancer diagnosis. The median follow-up time of participants was 8.9 years (range, 0.02–25.7 years). The median level of plasma PLP of the entire study population was 64.8 pmol/ml (range, 10.3–492.1). Only 3% of the study population (15 female patients) were below 20 pmol/ml of PLP, which is a cutpoint usually used for vitamin B6 deficiency [33]. Baseline characteristics (age- and gender-standardized) according to categories of prediagnostic plasma PLP are shown in Table 1. Males or multivitamin users had higher plasma PLP levels than females or no multivitamin user. Overall, participants with higher PLP levels tended to have lower BMI, and were less likely to be current smokers. Participants with higher plasma PLP levels also had higher levels of plasma folate and slightly higher plasma 25(OH)D levels.

The age-adjusted HR comparing 110 pmol/ml or higher PLP with less than 45pmol/ml was 1.01 (95% CI 0.63–1.63; *P* trend = 0.89) for colorectal cancer-specific mortality. After adjustment for clinical variables, the HR for the extreme categories was stronger, mostly after adjustment for stage of cancer (HR = 0.83, 95% CI 0.51–1.35; *P* trend = 0.28). After

further adjustment for lifestyle factors, the HR remained non-significant (HR = 0.85, 95% CI 0.50–1.45; P trend = 0.37). Similar results for overall mortality were found, with patients in the highest category of PLP having a multivariable HR of 0.87 (95% CI 0.56–1.35; P trend = 0.24) as compared with those in the lowest category of PLP. We found no evidence of non-linear relationship between plasma PLP and mortality based on spline analyses (P values for nonlinearity were 0.63 and 0.67 for colorectal cancer-specific and overall mortality, respectively). The multivariable HRs for a 20 pmol/ml increment of PLP were 0.96 (95% CI 0.91–1.03) and 0.96 (95% CI 0.91–1.01) for colorectal cancer-specific and overall mortality. This lack of association was consistent across cohorts: for colorectal cancer-specific mortality, the multivariable HRs for a 20 pmol/ml increment of PLP in NHS, HPFS, and PHS were 0.99 (95% CI 0.87–1.13), 0.98 (95% CI 0.85–1.12), and 1.06 (95% CI 0.95–1.18), respectively; and for overall mortality, the multivariable HRs were 0.99 (95% CI 0.89–1.10), 0.96 (95% CI 0.87–1.06), and 1.02 (95% CI 0.94–1.12), respectively.

In the sensitivity analyses of excluding participants who were diagnosed within 6 years after blood collection, multivariable HRs for the extreme categories of PLP were slightly stronger, but failed to reach statistical significance (HR for colorectal cancer-specific mortality = 0.71, 95% CI 0.34–1.49; P trend = 0.22; HR for overall mortality = 0.78, 95% CI 0.43–1.45; P trend = 0.16) (Table 3). Overall non-significant associations were found when we restricted our analysis to participants diagnosed with stage I, II, and III of cancer as well.

We examined the association of plasma PLP levels across strata of other predictors of colorectal cancer-specific mortality (Table 4). Among participants who were below the median plasma 25(OH)D (< 26.5 ng/ml), we observed a significant inverse trend between plasma PLP and mortality (HR = 0.38, 95% CI 0.16–0.90 comparing extreme categories; P trend = 0.01), whereas plasma PLP was not associated with death among participants who were above the median (HR = 1.15, 95% CI 0.54–2.45 comparing extreme categories; P trend = 0.50) (P interaction = 0.005). Similarly, the association with plasma PLP was stronger among participants who were below the median plasma folate (< 5.8 ng/ml) (HR = 0.41, 95% CI 0.17–0.98 comparing extreme categories; P trend = 0.03), but the interaction with plasma folate was not significant (P interaction = 0.19). We additionally examined the association of plasma PLP stratified by genotype of *MTHFR*. Those with CT or TT variants were shown to have lower enzyme activity of *MTHFR*, producing lower circulating levels of 5-methyltetrahydrofolate [34]. The multivariable HR of colorectal cancer-specific mortality for a 20 pmol/ml increment in PLP tended to be stronger among participants who had *MTHFR*677 variants (HR = 0.92, 95% CI 0.83–1.02) compared with those with CC wild-type (HR=1.02, 95% CI 0.93–1.12) (P interaction = 0.09). The association between plasma PLP levels and cancer-specific mortality did not vary substantially by age at diagnosis, BMI, physical activity, alcohol intake, or smoking status. The results of stratified analyses for overall mortality were similar to those for overall mortality (Table 5).

We also assessed whether the association of plasma PLP on survival varied by different time intervals between blood collection and colorectal cancer diagnosis. The lack of association seemed similar for patients diagnosed within 7 years of plasma collection versus 7 years or more after collection (P values for interaction = 0.61 and 0.73 for colorectal cancer-specific and overall mortality, respectively).

DISCUSSION

In these large prospective studies, we did not observe a significant inverse association between prediagnostic plasma PLP levels and mortality overall. The results remained unchanged after excluding either patients diagnosed within 6 years of blood collection or

metastatic patients. It seemed that, however, higher PLP levels were associated with better survival among patients who had lower circulating vitamin D levels.

No previous study, to our knowledge, has evaluated the association of prediagnostic vitamin B6 status on survival in patients with established colorectal cancer. Experimental studies have documented a number of anticarcinogenic properties of vitamin B6, including reducing oxidative stress, inhibiting inflammation, proliferation, angiogenesis, and metastatic potential, some of which may relate to progression and survival [11–17]. In an *ex vivo* serum-free matrix culture model, higher concentrations of PLP caused complete inhibition of microvessel outgrowth in a dose-dependent manner, using rat aortic ring which had been incubated with PLP, suggesting antiangiogenic properties of vitamin B6 [16]. Some of the antitumor properties of vitamin B6 may involve an indirect effect on the level of DNA methylation because vitamin B6 is involved in the methyl donor cycle [7].

Two prospective epidemiological studies showed that the association with risk of colorectal cancer was stronger in the short follow-up periods than in the long follow-up periods [35, 36]. The Physicians' Health Study found a stronger inverse association with high PLP (above the median) in the earlier follow-up period, 1982–1992, (relative risk[RR]=0.37, 95% CI 0.19–0.71) than in the later follow-up period, 1993–2000 (RR=0.63, 95% CI 0.35–1.14) [35]. Similarly, another prospective cohort study in Europe found that the association of plasma PLP and risk of colorectal cancer was slightly stronger in individuals diagnosed within the first 3.6 years after enrollment (RR=0.55, 95% CI 0.39–0.77) than those diagnosed later (RR=0.67, 95% CI 0.48–0.94) [36]. However, the strong association in the earlier follow-up period could have been due to reverse causation, i.e., the phenomenon that preclinical disease influences exposure status. To minimize bias in the plasma PLP levels by the presence of preclinical disease, we initially excluded participants diagnosed with colorectal cancer within 2 years of blood collection, and continued to observe no significant association of high PLP, even when extending this restriction to 6 years, suggesting that our results were unlikely to be influenced by preclinical disease.

Although high vitamin B6 status may help delay tumor progression and thus prolong survival of colorectal cancer patients, it is also plausible that high vitamin B6 may accelerate growth of tumor among patients with established colorectal cancer, given its role of DNA synthesis in one-carbon metabolism. Vitamin B6 is needed to regenerate 5, 10-methylene tetrahydrofolate, which is involved in the conversion of uracil to thymidylate for DNA synthesis [7]. Since neoplastic cells divide more rapidly than their normal counterparts, they require higher rates of DNA synthesis, which in turn, requires the presence of high levels of one-carbon nutrients. Animal studies have suggested that supraphysiologic doses of folic acid and folate administered after the development of neoplastic foci may, in fact, promote colorectal tumorigenesis [37]. One recent prospective cohort study of survival in colorectal cancer patients found an inverse association, rather than a positive association, in the third (median: 6.9 ng/ml) through fifth quintiles (median: 15.1 ng/ml) of plasma folate, although the possibility of detrimental effect of very high folate status on mortality cannot be entirely ruled out [26]. The potential harmful effect of very high dose of folate may not be the same for vitamin B6 as a one-carbon nutrient since some evidence also exists that vitamin B6 may inhibit thymidylate synthase, the enzyme used to generate deoxythymidine monophosphate (dTTP) for use in DNA synthesis [38–40].

If vitamin B6 influences colorectal cancer progression via its role of DNA methylation in one-carbon metabolism, we would expect PLP levels to be more strongly associated with reduced risk of mortality among patients with low methyl status. In fact, we found a strong inverse association with high PLP in patients with low plasma folate and to a lesser extent, those with *MTHFR* variants (CT or TT) who had lower folate levels. We previously found

that the association of vitamin B6 intake appeared confined to low LINE-1 (long interspersed nucleotide element-1, a good indicator of global DNA methylation level) methylated colon cancer risk [41], which has been linked to poor colon cancer survival [42].

We found that higher PLP levels were associated with better survival among patients with low vitamin D status, but not with high vitamin D status. It has been proposed that binding of vitamin D receptor (VDR) by 1,25-dehydroxyvitamin D₃, the active metabolite of vitamin D, leads to transcriptional activation and repression of target genes, resulting in differentiation and apoptosis, and inhibition of inflammation, proliferation and angiogenesis [43]. We previously found that high 25(OH)D levels were significantly associated with better survival among colorectal cancer patients, especially obese individuals [25]. It is conceivable that a demonstrable influence of vitamin B6 on survival may be restricted to patients that have relatively low 25(OH)D status through anti-inflammatory or antineoplastic properties of vitamin B6. Since the sample size is small for the subgroup analysis and any clear explanations for the interaction between low vitamin D and a potential benefit for vitamin B6 do not exist for now, the observation with vitamin D might be merely due to a random sampling event. More large prospective studies should be conducted to support and confirm the potential interaction.

The important strengths of our study include the prospective measurement of plasma PLP two or more years before cancer diagnosis and high follow-up rates. Although some measurement error in the laboratory assays is inevitable, the relatively low coefficients of variability of our plasma PLP levels suggest that they were relatively reliable. In addition, we were able to control for many potential confounding factors due to the extensive information we have on participants' lifestyle factors, which have been validated [44, 45].

Despite the strengths of our study, several limitations should be acknowledged. We only had a single measurement of plasma PLP drawn before colorectal cancer diagnosis, which may not represent the levels at the time of diagnosis. Since we do have information on the time between blood draw and cancer diagnosis, we initially adjusted for the time interval as a continuous variable in the multivariable model. The results of the analysis showed that the relationship between plasma PLP and CRC-specific or overall mortality was not significantly affected by the time interval between blood collection and cancer diagnosis, and thus we did not include the time interval in the final multivariable model due to parsimony. Although exposure misclassification due to any measurement error in plasma PLP levels would attenuate the results toward the null, significant findings of the PLP measures linked to colorectal cancer incidence or other chronic diseases that we previously observed may indicate that the exposure misclassification is not large enough to hide any real associations [35, 46]. Although the range of PLP levels in this study was comparable or greater than the range of early studies [5], our study population had relatively good vitamin B6 status, which may have limited our ability to observe an overall significant association with PLP for colorectal cancer survival. We had limited information on which patients received chemotherapy. However, adjusting for tumor stage and period of diagnosis in our multivariable models overcome this lack of data on chemotherapy use. In addition, there were no significant differences between plasma PLP and chemotherapy use, conditional on tumor stage. When we conducted a stratified analysis by tumor stage, multivariable HRs for a 20 pmol/ml increment of PLP among stage I/II, a majority of which would be unlikely to receive chemotherapy, and stage III/IV were similar (HRs = 0.93 and 0.94, respectively).

In summary, higher prediagnostic plasma PLP levels were not associated with a significant improvement on colorectal cancer survival overall. However, among individuals with poor vitamin D status, higher PLP levels may be associated with better survival. To our knowledge, this is the first study to examine the influence of vitamin B6 status on survival in

patients with colorectal cancer. Further research is needed to clarify the influence of vitamin B6 on colorectal cancer progression and survival.

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Table 1

Baseline characteristics of participants diagnosed with colorectal cancer according to categories of prediagnostic plasma pyridoxal 5'-phosphate (PLP)^a

	Plasma PLP, pmol/ml			
	<45	45–69	70–109	110
No of participants, n	126	135	108	103
Median plasma PLP, pmol/ml	33.6	54.4	83.3	158.8
Age at blood draw (SD)	59.6 (9.5)	59.9 (8.0)	59.7 (8.4)	61.5 (9.0)
Age at diagnosis, years (SD)	67.4 (9.3)	67.6 (8.1)	67.9 (8.1)	68.7 (8.7)
Sex, %				
Female	63	23	24	14
Male	37	77	76	86
Body mass index, kg/m ² (SD)	25.9 (3.8)	25.5 (3.5)	25.1 (3.4)	25.0 (2.8)
Plasma folate, ng/ml (SD)	4.9 (3.5)	7.2 (5.7)	8.5 (6.6)	9.7 (6.0)
Plasma 25(OH)D, ng/ml (SD)	24.8 (9.7)	27.7 (9.3)	27.0 (9.5)	28.2 (8.5)
Physical activity 4 th quartile, %	16	24	28	20
Alcohol consumption, %				
Non-drinkers	17	11	19	15
<1 drink/d	55	62	46	58
1 drink/d	22	20	25	18
2 drinks/d	6	7	10	9
Smoking status, %				
Never	44	45	35	39
Past	37	45	55	56
Current	19	10	10	5
Postmenopausal hormone use ^b , %				
Premenopausal	14	11	3	22
Never	37	27	39	28
Past	23	31	27	21
Current	27	31	31	29
Aspirin (> once per week), %	44	40	38	40
Regular multivitamin use, %	10	24	36	72
Tumor location, %				
Colon	80	69	77	74
Rectum	20	31	23	26
Tumor differentiation, %				
Well	9	12	15	6
Moderate	55	56	52	52
Poor	13	11	11	19
Unknown	23	21	22	23
Stage of disease, %				
I	23	29	29	23

	Plasma PLP, pmol/ml			
	<45	45–69	70–109	110
II	25	20	20	14
III	25	19	21	34
IV	13	13	13	17
Unknown	14	19	17	12
Receipt of chemotherapy, %				
Yes	9	1	5	11
No	4	8	10	7
Unknown	88	91	85	82

SD standard deviation

^aValues that are not percentages are means. Data were directly standardized to age and gender distributions of the entire cohort.

^b Among women only.

Age-adjusted and multivariable-adjusted hazard ratios (HRs) for mortality according to prediagnostic plasma pyridoxal 5'-phosphate (PLP)

Table 2

	Plasma PLP, pmol/ml				P trend ^e	HR for a 20 pmol/ml increment ^b
	<45	45–69	70–109	110		
Median plasma PLP, pmol/ml	33.6	54.4	83.3	158.8		
No. at risk	126	135	108	103		
Colorectal cancer-specific mortality						
No. of deaths	45	50	36	38		
Age-adjusted model ^c	1.00	0.96 (0.62–1.47)	0.85 (0.53–1.34)	1.01 (0.63–1.63)	0.89	0.99 (0.94–1.05)
Multivariable model 1 ^d	1.00	1.13 (0.73–1.75)	0.97 (0.60–1.56)	0.83 (0.51–1.35)	0.28	0.96 (0.91–1.02)
Multivariable model 2 ^e	1.00	1.09 (0.68–1.76)	1.01 (0.61–1.68)	0.85 (0.50–1.45)	0.37	0.96 (0.91–1.03)
Overall mortality						
No. of deaths	69	80	55	55		
Age-adjusted model ^c	1.00	0.97 (0.69–1.36)	0.80 (0.55–1.15)	0.88 (0.60–1.30)	0.49	0.98 (0.93–1.02)
Multivariable model 1 ^d	1.00	1.14 (0.80–1.62)	0.92 (0.63–1.36)	0.84 (0.56–1.25)	0.19	0.96 (0.92–1.01)
Multivariable model 2 ^e	1.00	1.20 (0.82–1.75)	1.01 (0.67–1.52)	0.87 (0.56–1.35)	0.24	0.96 (0.91–1.01)

CI confidence interval

^aCalculated by modeling the median value of plasma PLP categories as a continuous variable.

^bPatients with excessive plasma PLP levels (i.e., beyond 2.5 standard deviation for log-transformed PLP levels; 7 subjects) were not included.

^cStratified by cohort and adjusted for age at diagnosis (in years as a continuous variable).

^dMultivariable model 1: stratified by cohort and adjusted for age at diagnosis (in years as a continuous variable), cancer stage (I to IV or unknown), grade of tumor differentiation (well differentiated, moderately differentiated, poorly differentiated, or unknown), tumor location (colon or rectum), receipt of chemotherapy (yes, no, or unknown), and time period of diagnosis (1984–1994, 1995–1998, or 1999–2002).

^eMultivariable model 2: multivariable model 1 plus further adjusted for body mass index (in kg/m² as a continuous variable), physical activity (quartiles, metabolic equivalent task, MET/wk for NHS and HPFS; quartiles, times/week for PHS), smoking status (never, past, or current), alcohol consumption (never, <1/d, 1/d, or ≥2/d), aspirin use (or >1/wk), postmenopausal hormone use (premenopausal, never user, past user, or current user), plasma folate levels (in ng/ml as a continuous variable), and plasma 25-hydroxyvitamin D3 levels (in ng/ml as a continuous variable).

Table 3

Sensitivity analyses of colorectal cancer-specific and overall mortality according to prediagnostic plasma pyridoxal 5'-phosphate (PLP)^a

	No. of Patients	No. of Deaths	Plasma PLP, pmol/ml				<i>P</i> trend ^b	HR for a 20 pmol/ml increment ^c
			<45	45–69	70–109	110		
			HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)		
Colorectal cancer-specific mortality								
Time since plasma collection								
> 4 years	377	132	1.00	1.21 (0.69–2.12)	1.28 (0.73–2.26)	0.78 (0.42–1.45)	0.19	0.95 (0.88–1.02)
> 6 years	289	96	1.00	1.13 (0.58–2.19)	1.07 (0.53–2.14)	0.71 (0.34–1.49)	0.22	0.95 (0.87–1.03)
Stage I/II/III patients	329	91	1.00	0.77 (0.37–1.58)	0.71 (0.34–1.48)	0.83 (0.40–1.70)	0.88	0.98 (0.91–1.06)
Overall mortality								
Time since plasma collection								
> 4 years	378	204	1.00	1.39 (0.90–2.15)	1.17 (0.74–1.85)	0.78 (0.46–1.30)	0.07	0.94 (0.88–1.00)
> 6 years	290	149	1.00	1.36 (0.80–2.31)	1.07 (0.61–1.85)	0.78 (0.42–1.45)	0.16	0.94 (0.88–1.02)
Stage I/II/III patients	329	164	1.00	0.93 (0.56–1.53)	0.74 (0.44–1.27)	0.85 (0.49–1.47)	0.62	0.97 (0.91–1.03)

HR hazard ratio, CI confidence interval

^a Adjusted for the same covariates for the multivariable model 2 in Table 2.

^b Calculated by modeling the median value of plasma PLP categories as a continuous variable.

^c Patients with excessive plasma PLP levels (i.e., beyond 2.5 standard deviation for log-transformed PLP levels) were not included.

Table 4

Stratified analyses of colorectal cancer-specific mortality according to prediagnostic plasma pyridoxal 5'-phosphate (PLP)^a

Characteristics	No. of Patients	No. of Deaths	Plasma PLP, pmol/ml				P trend ^b	HR for a 20 pmol/ml increment ^c	P interaction ^d	
			<45	45–69	70–109	110				
Age at diagnosis ^e										
<68 years	238	90	1.00	0.95 (0.46–1.93)	1.07 (0.50–2.28)	0.82 (0.37–1.79)	0.60	0.96 (0.88–1.05)	0.41	
68 years	234	79	1.00	1.52 (0.69–3.32)	1.09 (0.50–2.41)	1.41 (0.62–3.17)	0.64	1.00 (0.91–1.10)		
Body mass index ^e										
<25 kg/m ²	235	87	1.00	0.67 (0.33–1.36)	0.77 (0.37–1.58)	0.65 (0.31–1.38)	0.43	0.94 (0.85–1.03)	0.91	
25 kg/m ²	237	82	1.00	1.22 (0.57–2.62)	1.20 (0.50–2.85)	0.89 (0.37–2.18)	0.52	0.99 (0.88–1.10)		
Physical activity ^e										
Low	228	80	1.00	1.41 (0.66–2.99)	1.31 (0.62–2.79)	0.94 (0.41–2.16)	0.58	0.96 (0.88–1.05)	0.99	
high	244	89	1.00	1.10 (0.56–2.15)	0.85 (0.38–1.88)	0.92 (0.42–2.02)	0.74	1.00 (0.91–1.10)		
Alcohol intake										
<1 drink/d	340	118	1.00	0.86 (0.48–1.54)	0.93 (0.49–1.75)	0.74 (0.39–1.40)	0.39	0.96 (0.89–1.03)	0.67	
1 drink/d	132	51	1.00	0.88 (0.31–2.51)	1.08 (0.38–3.07)	0.99 (0.28–3.50)	0.93	1.01 (0.88–1.16)		
Smoking status										
Never	197	70	1.00	0.95 (0.43–2.12)	0.62 (0.23–1.71)	0.73 (0.28–1.89)	0.50	0.98 (0.86–1.11)	0.80	
Past/current	275	99	1.00	1.15 (0.59–2.24)	1.17 (0.59–2.32)	0.71 (0.34–1.52)	0.20	0.92 (0.85–1.00)		
Plasma 25(OH)D, ng/ml ^e										
<26.5 ng/ml	244	88	1.00	1.00 (0.51–1.94)	0.87 (0.40–1.87)	0.38 (0.16–0.90)	0.01	0.83 (0.74–0.93)	0.005	
26.5 ng/ml	228	81	1.00	0.92 (0.45–1.90)	0.78 (0.35–1.74)	1.15 (0.54–2.45)	0.50	1.03 (0.95–1.11)		
Plasma folate, ng/ml ^e										
<5.8 ng/ml	236	92	1.00	0.94 (0.50–1.77)	0.68 (0.33–1.41)	0.41 (0.17–0.99)	0.03	0.86 (0.76–0.98)	0.19	
5.8 ng/ml	236	77	1.00	1.25 (0.52–3.00)	1.51 (0.62–3.67)	1.66 (0.71–3.92)	0.26	1.01 (0.94–1.09)		
MTHFR677										
CC wild-type	193	75	1.00	0.92 (0.39–2.19)	0.71 (0.30–1.68)	1.09 (0.47–2.54)	0.66	1.02 (0.93–1.12)	0.09	
CT/TT variants	233	83	1.00	0.94 (0.46–1.95)	1.21 (0.56–2.63)	0.55 (0.23–1.32)	0.16	0.92 (0.82–1.02)		

HR hazard ratio, CI confidence interval, 25(OH)D 25-hydroxycholecalciferol or 25-hydroxyvitamin D3

- ^a Adjusted for the same covariates for the multivariable model 2 in Table 2.
- ^b Calculated by modeling the median value of plasma PLP categories as a continuous variable.
- ^c Patients with excessive plasma PLP levels (i.e., beyond 2.5 standard deviation for log-transformed PLP levels) were not included.
- ^d Calculated by entering into the model the cross-product of plasma PLP as a continuous variable and the dichotomized covariate.
- ^e Cutpoints chosen based on median values.

Table 5
Stratified analyses of overall mortality according to prediagnostic plasma pyridoxal 5'-phosphate (PLP)^a

Characteristics	No. of Patients	No. of Deaths	Plasma PLP, pmol/ml				P trend ^b	HR for a 20 pmol/ml increment ^c	P interaction ^d	
			<45	45–69	70–109	110				
										HR (95% CI)
Age at diagnosis ^e										
<68 years	238	115	1.00	0.84 (0.45–1.57)	0.80 (0.41–1.57)	0.82 (0.41–1.65)	0.70	0.96 (0.88–1.04)	0.68	
68 years	234	144	1.00	1.58 (0.89–2.82)	1.10 (0.60–2.01)	0.97 (0.51–1.84)	0.37	0.96 (0.89–1.03)		
Body mass index ^e										
<25 kg/m ²	235	123	1.00	0.80 (0.45–1.44)	0.86 (0.48–1.55)	0.72 (0.37–1.38)	0.42	0.94 (0.87–1.02)	0.52	
25 kg/m ²	237	136	1.00	1.26 (0.71–2.25)	0.84 (0.43–1.64)	0.74 (0.37–1.52)	0.13	0.93 (0.85–1.01)		
Physical activity ^e										
low	228	131	1.00	0.92 (0.52–1.63)	0.86 (0.48–1.55)	0.69 (0.36–1.32)	0.25	0.95 (0.88–1.02)	0.99	
high	244	128	1.00	1.51 (0.86–2.64)	1.18 (0.60–2.31)	1.19 (0.60–2.35)	0.91	0.98 (0.91–1.06)		
Alcohol intake										
<1 drink/d	340	181	1.00	0.89 (0.57–1.39)	0.76 (0.45–1.26)	0.63 (0.37–1.06)	0.07	0.93 (0.82–1.05)	0.94	
1 drink/d	132	78	1.00	1.41 (0.60–3.33)	2.11 (0.90–4.94)	1.57 (0.56–4.38)	0.63	0.94 (0.88–1.00)		
Smoking status										
Never	197	99	1.00	0.83 (0.44–1.55)	0.38 (0.17–0.87)	0.53 (0.24–1.15)	0.12	0.93 (0.82–1.05)	0.99	
Past/current	275	160	1.00	1.36 (0.80–2.31)	1.25 (0.73–2.16)	0.89 (0.48–1.65)	0.31	0.94 (0.89–1.01)		
Plasma 25(OH)D, ng/ml ^e										
<26.5 ng/ml	244	142	1.00	0.89 (0.54–1.46)	0.82 (0.47–1.45)	0.39 (0.19–0.77)	0.005	0.85 (0.78–0.93)	0.002	
26.5 ng/ml	229	117	1.00	1.39 (0.75–2.59)	0.95 (0.47–1.93)	1.49 (0.76–2.92)	0.33	1.02 (0.95–1.09)		
Plasma folate, ng/ml ^e										
<5.8 ng/ml	236	137	1.00	1.17 (0.70–1.97)	0.80 (0.44–1.47)	0.61 (0.30–1.27)	0.09	0.89 (0.80–0.99)	0.51	
5.8 ng/ml	236	122	1.00	0.93 (0.48–1.78)	1.07 (0.53–2.17)	1.07 (0.54–2.13)	0.73	0.99 (0.93–1.06)		
MTHFR677										
CC wild-type	193	99	1.00	1.04 (0.52–2.09)	0.74 (0.36–1.52)	1.02 (0.50–2.12)	0.98	1.00 (0.92–1.09)	0.18	
CT/TT variants	233	129	1.00	1.19 (0.66–2.16)	1.16 (0.61–2.18)	0.62 (0.31–1.25)	0.06	0.90 (0.82–0.98)		

HR hazard ratio, CI confidence interval, 25(OH)D 25-hydroxycholecalciferol or 25-hydroxyvitamin D3

^a Adjusted for the same covariates for the multivariable model 2 in Table 2.

^b Calculated by modeling the median value of plasma PLP categories as a continuous variable.

^c Patients with excessive plasma PLP levels (i.e., beyond 2.5 standard deviation for log-transformed PLP levels) were not included.

^d Calculated by entering into the model the cross-product of plasma PLP as a continuous variable and the dichotomized covariate.

^e Cutpoints chosen based on median values.