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Higher vitamin B6 status is associated with improved survival among patients with stage I–III colorectal cancer

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ABSTRACT

Background: Folate-mediated 1-carbon metabolism requires several nutrients, including vitamin B6. Circulating biomarker concentrations indicating high vitamin B6 status are associated with a reduced risk of colorectal cancer (CRC). However, little is known about the effect of B6 status in relation to clinical outcomes in CRC patients.

Objectives: We investigated survival outcomes in relation to vitamin B6 status in prospectively followed CRC patients.

Methods: A total of 2031 patients with stage I–III CRC participated in 6 prospective patient cohorts in the international FOCUS (folate-dependent 1-carbon metabolism in colorectal cancer recurrence and survival) Consortium. Preoperative blood samples were used to measure vitamin B6 status by the direct marker pyridoxal 5′-phosphate (PLP), as well as the functional marker HK-ratio (HKr)[3′-hydroxykynurenine: (kynurenic acid + xanthurenic acid + 3′-hydroxy anthranilic acid + anthranilic acid)]. Using Cox proportional hazards regression, we examined associations of vitamin B6 status with overall survival (OS), disease-free survival (DFS), and risk of recurrence, adjusted for patient age, sex, circulating creatinine concentrations, tumor site, stage, and cohort.

Results: After a median follow-up of 3.2 y for OS, higher preoperative vitamin B6 status as assessed by PLP and the functional marker HKr was associated with 16–32% higher all-cause and disease-free survival, although there was no significant association with disease recurrence (doubling in PLP concentration: HR_{OS}, 0.68; 95% CI: 0.59, 0.79; HR_{DFS}, 0.84; 95% CI: 0.75, 0.94; $HR_{Recurrence}$, 0.96; 95% CI: 0.84, 1.09; HKr: HR_{OS}, 2.04; 95% CI: 1.67, 2.49; HR_{DFS}, 1.56; 95% CI: 1.31, 1.85; $HR_{Recurrence}$, 1.21; 95% CI: 0.96,1.52). The association of PLP with improved OS was consistent across colorectal tumor site (right-sided colon: HR_{OS}, 0.75; 95% CI: 0.59, 0.96; left-sided colon: HR_{OS}, 0.71; 95% CI: 0.55, 0.92; rectosigmoid junction and rectum: HR_{OS}, 0.61; 95% CI: 0.47, 0.78).

Conclusion: Higher preoperative vitamin B6 status is associated with improved OS among stage I–III CRC patients. *Am J Clin Nutr* 2022;116:303–313.

Keywords: colorectal cancer, vitamin B6, PLP, recurrence, one-carbon metabolism, HKr, PAR, rectal cancer, survivorship, colon cancer

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Introduction

Colorectal cancer (CRC) is the third most common cancer and second leading cause of cancer deaths in men and women worldwide, with an estimated 1,800,977 new cases diagnosed and 861,663 deaths reported annually (1). After a diagnosis of CRC, patients may be motivated to improve their diet, exercise habits, and other health behaviors. Yet, evidence-based dietary guidelines specifically for cancer patients are still lacking, as knowledge about how diet may modulate cancer progression is currently imprecise and incomplete (2–6). Pyrixodal-5′-phosphate (PLP)—the bioactive form of vitamin B6—is a long-time established direct marker of vitamin B6. PLP acts as a prosthetic group for a wide range of classified enzymatic activities (7) and plays a crucial role in diverse cellular processes. Vitamin B6 is an essential vitamin which exerts a vital coenzymatic activity in key metabolic circuitries, including the synthesis and catabolism of amino acids (e.g., homocysteine) (8) such that alterations in vitamin B6 bioavailability may impact disease pathogenesis. Vitamin B6 has also been associated with inflammatory processes and biomarkers of inflammation. Major sources of vitamin B6 in the diet are fish, beef, poultry, starchy vegetables, noncitrus fruits, milk products, beans, and nuts. Also, the intake of vitamin B6 could be increased by the use of various dietary supplements (9, 10). Vitamin B6 exerts a vital coenzymatic activity in key metabolic circuitries, including the synthesis and catabolism of amino acids (e.g., homocysteine), (8) such that alterations in vitamin B6 bioavailability may impact disease pathogenesis. Vitamin B6 has also been associated with inflammatory processes and biomarkers of inflammation. Indeed, clinical and preclinical studies have supported the notion that vitamin B6 plays a critical role in early carcinogenesis and cancer progression, as well as chemotherapeutic challenges (11–16). Given the clinical relevance of vitamin B6 metabolism and systemic imbalance of vitamin B6 as a consequence of carcinogenesis and tumor progression (16), a further understanding of the putative links between vitamin B6 and cancer are warranted.

A more recently established marker for functional vitamin B6 status is the HK ratio [3′-hydroxykynurenine (HKr):(hynurenic acid + xanturenic acid + 3′ anthranilic acid + anthranilic acid)]. This ratio is supposed to capture cofactor saturation of 2 PLP-dependent enzymes – kynurenine transaminase (KAT) and kynureninase (KYNU) in the tryptophan catabolic pathway. Previous studies have demonstrated that HKr is a specific indicator of intracellular vitamin B6 status.

The purpose of this study, which comprised 6 prospective patient cohorts in the international FOCUS (biomarkers related to folate-dependent 1-carbon metabolism in colorectal cancer recurrence and survival) Consortium, was to evaluate biomarkers of vitamin B6 status and function in preoperative circulating samples from patients diagnosed with stage I–III CRC with respect to survival and disease recurrence.

Methods

Study population

Data from stage I–III CRC patients in this study derive from 6 international cohort studies collaborating within the FOCUS Consortium. With FOCUS, we study associations between folate and folate-mediated 1-carbon metabolism biomarkers and patient-reported outcomes such as quality of life (17) as well as clinical outcomes among patients aged 18 y and older diagnosed with primary CRC. The FOCUS Consortium has been previously described (11, 18). Briefly, the FOCUS Consortium comprises CRC patients from the ColoCare Study (19) (study sites: University Hospital Heidelberg, Germany; Huntsman Cancer Institute, University of Utah, USA; and Fred Hutchinson Cancer Research Center, USA), the COLON Study (20), the Colorectal

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ANH, JO, NH, EK, MPW, AG, and CMU contributed equally to this work.

Supplemental Tables 1–7 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: AA, anthranilic acid; CORSA, Colorectal Cancer Study of Austria; CRC, colorectal cancer; DFS, disease-free survival; EnCoRe, Energy for life after colorectal cancer; FOCUS, folate-dependent 1-carbon metabolism in colorectal cancer recurrence and survival; HAA, 3′, hydroxyantranilic acid; HK, 3′, hydroxykynurenine; HKr, HK-ratio [HK/(KA + XA + AA + HAA)]; KA, kynurenic acid; KAT, kynurenine transaminase; KYNU, kynureninase; OS, overall survival; PA, 4-pyridoxic acid; PAr, 4-pyridoxic acid ratio; PL, pyridoxal; PLP, pyridoxal 5′-phosphate; XA, xanthurenic acid.

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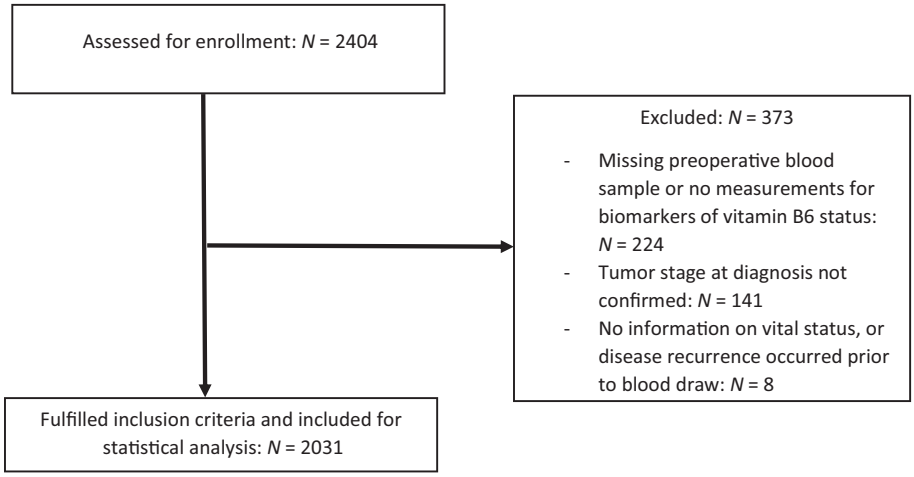

FIGURE 1 Study enrollment flow chart for the FOCUS consortium.

Cancer Study of Austria (CORSA), (21) and the Energy for life after ColoRectal cancer (EnCoRe) Study, the Netherlands (22). The ColoCare Study has a recruitment rate of 80% and the ENCORE study has a recruitment rate of 46%. Recruitment rates for COLON and CORSA were unavailable at the time of this report.

Detailed data on clinical and sociodemographic features, including CRC treatment regimens, disease stage/site, and follow-up were prospectively captured. Comprehensive data on lifestyle features [including BMI (in kg/m^2) and smoking history] were also collected when available and harmonized across all study sites. Data on these covariates were collected at the time of diagnosis. Among the 2404 CRC patients within the FOCUS Consortium, 373 patients were excluded: 224 patients did not have preoperative (baseline) blood samples or measurements for circulating functional markers of vitamin B6 status, 141 patients did not have confirmed stage I/II/III CRC, and 8 patients lacked information on vital status or with a disease recurrence prior to baseline blood draw (**Figure 1**). The final cohort included 2031 patients diagnosed with stage I–III CRC. All patients provided study-specific informed consent, and each study site protocol was approved by the institutional review board of each participating study.

Measurement of biomarkers of vitamin B6 status

Baseline blood samples from CRC patients were processed in identical settings across all study centers as previously described (11, 18), and metabolic profiling of biomarkers for all patients in the FOCUS Consortium was performed at BEVITAL AS (www.bevital.no). B6 vitamins and functional markers of vitamin B6 status (23), reflecting the metabolic effects of vitamin B6 serving as an enzyme cofactor, including the following: PLP (nmol/L), pyridoxal (PL) (nmol/L), 4-pyridoxic acid (PA) (nmol/L), 3′-hydroxykynurenine (HK) (nmol/L), kynurenic acid (KA) (nmol/L), 3′-hydroxyanthranilic acid (HAA) (nmol/L), anthranilic acid (AA) (nmol/L), xanthurenic acid (XA) (nmol/L), and creatinine (umol/L) were analyzed by LC-MS. PLP is the biologically active form of vitamin B6, PL is the 4-carboxyaldehyde form of vitamin B6, and PA is the end product of vitamin B6 catabolism. The HKr [HK/(KA + XA + AA + HAA)] is a sensitive and specific indicator of intracellular vitamin B6 status that provides superior discrimination (24). The 4-pyridoxic acid ratio (PAr) index [PA/(PLP + PL)] is a marker of B6 catabolism and an indicator of systemic inflammation (25, 26).

Study endpoints

The primary endpoint was overall survival (OS), defined as the time from baseline blood draw to the date of last follow-up or death from any cause. Disease-free survival (DFS) was defined as the time from baseline blood draw to first event: disease recurrence (locoregional or distant) or death from any cause. We also assessed risk of recurrence, defined as the time from baseline blood draw to the date of recurrence. Criteria for the diagnosis of CRC recurrence included histological confirmation or radiologic evidence with subsequent clinical progression. The date of recurrence was defined as the date of confirmatory imaging, or as applicable, the date of biopsy.

Statistical analysis

Baseline characteristics are presented by frequency and percentage (or mean and SD) of cases within the study population and by cohort. Statistical analysis was performed using biomarkers of vitamin B6 status as a continuous measure (log2-transformed concentrations) and in tertiles. Adjusted HRs and 95% CIs were estimated by multivariable Cox proportional hazards regression analysis. The final model was adjusted for potential confounders, including patient age (continuous, years), sex, circulating creatinine concentrations (continuous), tumor site [right colon (cecum to transverse colon), left colon (splenic flexure to sigmoid colon), and rectosigmoid junction/rectum], tumor stage (I/II/III), adjuvant therapy, and study cohort. Stratified analysis was performed by receipt (yes/no) and type of neoadjuvant therapy (chemotherapy and chemoradiation/radiation therapy) and by colorectal tumor site. Survival curves for OS, DFS, and risk of recurrence by PLP and HKr tertiles (tertiles defined on the basis of the total study population) were calculated using Kaplan–Meier methods, and

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TABLE 1 Demographic and primary tumor characteristics of the study population: FOCUS Consortium[1]

Characteristics	Study population	Study cohorts					
		COLON	EnCoRe	ColoCare FHCRC	ColoCare HCI	ColoCare HD	CORSA
Total, *n*	2031	1077	287	131	68	271	197
Sex							
Female	732 (36.0)	392 (36.4)	96 (33.4)	61 (46.6)	27 (39.7)	90 (33.2)	66 (33.5)
Male	1299 (64.0)	685 (63.6)	191 (66.6)	70 (53.4)	41 (60.3)	181 (66.8)	131 (66.5)
Age at diagnosis, y	65.4 (10.3)	66.2 (8.7)	66.6 (9.3)	58.0 (12.9)	61.4 (11.2)	64.2 (11.9)	67.6 (12.0)
BMI	27.1 (4.7)	26.5 (4.0)	28.3 (4.7)	28.9 (7.5)	29.2 (7.7)	26.7 (4.1)	27.7 (4.3)
Smoking history							
Current	247 (12.2)	120 (11.1)	38 (13.2)	8 (6.1)	4 (5.9)	47 (17.3)	30 (15.2)
Former	1027 (50.6)	622 (57.8)	153 (53.3)	44 (33.6)	18 (26.5)	120 (44.3)	70 (35.5)
Never	647 (31.9)	300 (27.9)	90 (31.4)	48 (36.6)	36 (52.9)	82 (30.3)	91 (46.2)
Unknown	110 (5.4)	35 (3.2)	6 (2.1)	31 (23.7)	10 (14.7)	22 (8.1)	6 (3.0)
Tumor stage[2]							
I	558 (27.5)	280 (26.0)	86 (30.0)	30 (22.9)	16 (23.5)	65 (24.0)	81 (41.1)
II	608 (29.9)	330 (30.6)	59 (20.6)	42 (32.1)	18 (26.5)	107) (39.5)	52 (26.4)
III	849 (41.8)	463 (43.0)	142 (49.5)	59 (45.0)	34 (50.0)	99 (36.5)	52 (26.4)
Tumor site[2]							
Right colon	596 (29.3)	330 (30.6)	74 (25.8)	29 (22.1)	24 (35.3)	72 (26.6)	67 (34.0)
Left colon	655 (32.3)	387 (35.9)	100 (34.8)	34 (26.0)	16 (23.5)	59 (21.8)	59 (29.9)
Rectosigmoid junction/rectum	771 (38.0)	360 (33.4)	113 (39.4)	67 (51.1)	23 (33.8)	140 (51.7)	68 (34.5)
Neoadjuvant therapy[3]							
No	1549 (76.3)	824 (76.5)	207 (72.1)	88 (67.2)	46 (67.6)	206 (76.0)	178 (90.4)
Yes	467 (23.0)	253 (23.5)	80 (27.9)	42 (32.1)	15 (22.1)	63 (23.2)	14 (7.1)
Type of neoadjuvant therapy							
Chemotherapy	11 (0.5)	5 (2.0)	0 (0.0)	0 (0.0)	3 (20.0)	2 (3.2)	1 (7.1)
Radiation therapy	181 (8.9)	138 (54.5)	20 (25.0)	1 (2.4)	1 (6.7)	18 (28.6)	3 (21.4)
Chemoradiation	275 (13.5)	110 (43.5)	60 (75.0)	41 (97.6)	11 (73.3)	43 (68.3)	10 (71.4)
Adjuvant therapy[3]							
None	1181 (58.1)	777 (72.1)	191 (66.6)	57 (43.5)	16 (23.5)	0 (0)	140 (71.1)
Yes	753 (37.1)	251 (23.3)	95 (33.1)	71 (54.2)	29 (42.6)	256 (94.5)	51 (25.9)
Type of adjuvant therapy							
Chemotherapy	550 (27.1)	234 (93.2)	94 (98.9)	65 (91.5)	29 (100.0)	85 (33.2)	43 (84.3)
Radiation therapy	169 (8.3)	3 (1.2)	0 (0.0)	0 (0.0)	0 (0.0)	164 (64.1)	2 (3.9)
Chemoradiation	27 (1.3)	7 (2.8)	1 (1.1)	6 (8.5)	0 (0.0)	7 (2.7)	6 (11.8)
Unknown	7 (0.3)	7 (2.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Folate supplement[4]							
Yes	404 (23.8)	262 (25.1)	58 (20.2)	46 (74.2)	21 (35.0)	17 (6.9)	NA
Vitamin supplement							
B2	389 (43.7)	254 (23.6)	56 (19.5)	46 (74.2)	20 (33.3)	13 (5.3)	NA
B6	397 (43.6)	254 (23.6)	57 (19.9)	46 (74.2)	21 (35.0)	19 (7.7)	NA
B12	422 (24.9)	271 (26.0)	57 (19.9)	48 (77.4)	25 (41.7)	21 (8.5)	NA
Vital status							
Alive	1754 (86.4)	941 (87.4)	260 (90.6)	107 (81.7)	63 (92.6)	242 (89.3)	141 (71.6)
Deceased	277 (13.6)	136 (12.6)	27 (9.4)	24 (18.3)	5 (7.4)	29 (10.7)	56 (28.4)

(*Continued*)

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TABLE 1 (*Continued*)

Characteristics	Study population	Study cohorts					
		COLON	EnCoRe	ColoCare FHCRC	ColoCare HCI	ColoCare HD	CORSA
Disease recurrence							
None	1699 (83.7)	926 (86.0)	256 (89.2)	106 (80.9)	51 (75.0)	191 (70.5)	169 (85.8)
Yes	266 (13.1)	146 (13.6)	31 (10.8)	22 (16.8)	9 (13.2)	34 (12.5)	24 (12.2)
Unknown	66 (3.2)	5 (0.5)	0 (0.0)	3 (2.3)	8 (11.8)	46 (17.0)	4 (2.0)

[1]Values are presented as *n* or *n*(%). Sixteen patients had a CRC that cound not be distinguished between stage I–II or stage II–III disease. CORSA, Colorectal Cancer Study of Austria; CRC, colorectal cancer; EnCoRe, Energy for life after colorectal cancer; FHCRC, Fred Hutchinson Cancer Research Center; HCI, Huntsman Cancer Institute, HD, Heidelberg; NA, not applicable.

[2]Nine patients had an unspecified colorectal tumor site.

[3]Fifteen patients had unknown information on neoadjuvant therapy and 97 patients had unknown information on adjuvant therapy.

[4]Information on supplement use was missing for $n = 333$ (folate); $n = 1122$ (vitamin B2); $n = 1122$ (vitamin B6), and $n = 333$ (vitamin B12).

the log-rank test was used to compare survival between groups. All analyses were performed using SAS version 9.4 software. A P value of < 0.05 was considered to be statistically significant.

Results

Baseline characteristics

Baseline characteristics of CRC patients of the total study population and by cohort are presented in **Table 1**. We have summarized patient characteristics and compared patients excluded from the study as well as patients who have been included in the study (**Supplemental Table 1**). Nearly two-thirds of the population was male and the mean ± SD age at cancer diagnosis was 65.4 ± 10.3 y; ≥60% of CRC patients had a BMI (in kg/m^2) classified as overweight or obese (>25) at cancer diagnosis (1293 of 2031; 63.7%), with a BMI in this cohort classified as overweight (mean: 27.1; SD: 4.7). Current and former smokers accounted for 63% of the study population (1274 of 2031). Approximately 2 out of every 5 patients were diagnosed with stage III CRC (849 of 2031; 41.8%), and/or were diagnosed with CRC in the rectosigmoid junction and rectum (771 of 2031; 38.0%). Nearly one-fourth of all patients received neoadjuvant therapy (23.0%), almost all patients underwent CRC resection (98%; data not shown), and approximately one-third of cases (37.1%) within the FOCUS Consortium received adjuvant therapy.

Association between Circulating Direct and Functional Markers of Vitamin B6 Status with Cancer Survival and Recurrence

Median follow-up time from time of baseline blood draw was 3.2 y for OS. During follow-up, 426 of the 2031 CRC patients had a disease recurrence ($n = 266$) and/or died ($n = 277$), and 67.3% (179 of 266) of disease recurrences occurred within the first 2 y after CRC diagnosis (179 of 266; 67.3%). A doubling of the PLP concentration was associated with a 32% longer survival after adjusting for other covariates (doubling in concentration of PLP: HR_{OS}, 0.68; 95% CI: 0.59, 0.79; HR_{DFS}, 0.84; 95% CI: 0.75, 0.94) (**Table 2**; **Figure 2**). A doubling of the HKr concentration was associated with a 2-fold higher risk of death after adjusting for other covariates (doubling in concentration of HKr: HR_{OS}, 2.04; 95% CI: 1.67, 2.49; HR_{DFS}, 1.56; 95% CI: 1.31, 1.85; Table 2; Figure 2). However, PLP and HKr were not statistically significantly associated with risk of recurrence in adjusted models (e.g., PLP: $HR_{Recurrence}$, 0.96; 95% CI: 0.84, 1.09; HKr: $HR_{Recurrence}$, 1.21; 95% CI: 0.96, 1.52). We further examined associations with the PA ratio. Low values of these indices [e.g., low B6 catabolism (PAr)] were associated with a 55% to 104% longer survival in adjusted models (PAr index: HR_{OS}, 1.55; 95% CI: 1.28, 1.87; HR_{DFS}, 1.33; 95% CI: 1.14, 1.54; HKr: HR_{OS}, 2.04; 95% CI: 1.67, 2.49; HR_{DFS}, 1.56; 95% CI: 1.31, 1.85). Similarly to PLP and HKr, no statistically significant associations of the PAr index were observed with the risk of disease recurrence. Consideration of adjuvant therapy into the model did not statistically significantly alter our findings, as a doubling of the PLP concentration remained associated with a 30% longer survival but was not associated with disease recurrence in adjusted models, as follows: PLP: HR_{OS}, 0.70; 95%

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TABLE 2 Associations between functional and direct biomarkers of vitamin B6 status and OS, DFS, and risk of recurrence among $n = 2031$ patients with stage I–III CRC: FOCUS Consortium[1]

		Death from any cause (OS)			DFS			Recurrence		
Biomarker	Median (IQR) concentration	Total events/total patients	Crude HR (95% CI)	Adj HR (95% CI)	Total events/total patients	Crude HR (95% CI)	Adj HR (95% CI)	Total events/total patients	Crude HR (95% CI)	Adj HR (95% CI)
PLP, nmol/L										
Continuous	39.6 (26.8–61.2)	277/2031	0.64 (0.56,0.73)	0.68 (0.59,0.79)	419/1965	0.80 (0.72,0.89)	0.84 (0.75,0.94)	265/1963	0.96 (0.84,1.08)	0.96 (0.84,1.09)
T1	<31.2		ref	ref		ref	ref		ref	ref
T2	31.2–52.1		0.53 (0.40, 0.70)	0.60 (0.45, 0.81)		0.69 (0.55, 0.86)	0.74 (0.58,0.94)		0.91 (0.68, 1.22)	0.89 (0.66,1.22)
T3	>52.1		0.38 (0.28, 0.51)	0.46 (0.33,0.63)		0.59 (0.46, 0.74)	0.65 (0.51,0.84)		0.87 (0.64, 1.16)	0.88 (0.66,1.20)
HKr										
Continuous	0.4 (0.3, 0.5)	275/2017	2.07 (1.74,2.46)	2.04 (1.67,2.49)	416/1951	1.51 (1.29,1.76)	1.56 (1.31,1.85)	263/1949	1.09 (0.89,1.34)	1.21 (0.96,1.52)
T1	<−1.636		ref	ref		ref	ref		ref	ref
T2	1.635 to 1.222		1.14 (0.81, 1.62)	1.10 (0.77,1.57)		0.93 (0.72, 1.20)	0.97 (0.75,1.26)		0.87 (0.64, 1.18)	0.95 (0.70,1.29)
T3	>−1.222		2.33 (1.72, 3.15)	2.16 (1.56,2.98)		1.42 (1.13, 1.79)	1.45 (1.13,1.86)		1.02 (0.77, 1.37)	1.16 (0.85,1.59)

[1]Model adjusted using Cox proportional hazards regression for age, sex, tumor stage and site, creatinine, and study cohort. Adj, adjusted; DFS, disease-free survival; HKr, HK-ratio [HK/(KA + XA + AA + HAA)]; OS, overall survival; PLP, pyridoxal 5′-phosphate; T, tertile.

CI: 0.60, 0.80; HR_{DFS}, 0.84; 95% CI: 0.75, 0.94; $HR_{Recurrence}$, 0.94; 95% CI: 0.82, 1.07; HKr: HR_{OS}, 2.02; 95% CI: 1.65, 2.48; HR_{DFS}, 1.57; 95% CI: 1.32, 1.88; $HR_{Recurrence}$, 1.25; 95% CI: 0.99, 1.58) (**Supplemental Tables 2** and **3**).

Stratified analyses

We examined the effect of preoperative concentrations of biomarkers of vitamin B6 status in circulation on CRC outcome across strata of other potential confounders and effect modifiers. We performed stratified analysis by receipt of neoadjuvant therapy and by tumor site to investigate if either of these covariates are in fact effect modifiers. We have observed comparable results for patients who received neoadjuvant therapy compared with those who did not. In stratified analyses by tumor site we have observed similar associations for right-sided colon, left-sided colon, and cancer of the rectum (including rectosigmoid junction). For example, a doubling in PLP concentration among patients with tumors located in the right-sided colon, left-sided colon, and rectosigmoid junction/rectum consistently yielded a 25%, 29%, and 39% longer survival, respectively (right-sided colon: HR_{OS}, 0.75; 95% CI: 0.59, 0.96; left-sided colon: HR_{OS}, 0.71; 95% CI: 0.55, 0.92; rectosigmoid junction and rectum: HR_{OS}, 0.61; 95% CI: 0.47, 0.78; **Table 3**). On the contrary, doubling of HKr concentrations among patients with tumor located in the right-sided colon, left-sided colon, and rectosigmoid was associated with a 2-fold higher risk of death (right-sided colon: HR_{OS}, 2.37; 95% CI: 1.66, 3.37; left-sided colon: HR_{OS}, 1.91; 95% CI: 1.25, 2.91; rectosigmoid junction and rectum: HR_{OS}, 2.18; 95% CI: 1.60, 2.99; Table 3). Similar patterns were observed for PLP concentrations and tumor site for DFS, although associations for cancers of the right-sided colon and left-sided colon were not statistically significant (right-sided colon HR_{DFS}: 0.87; 95% CI: 0.70, 1.08; left-sided colon HR_{DFS}: 0.83; 95% CI: 0.68, 1.02; rectosigmoid junction and rectum HR_{DFS}: 0.82; 95% CI: 0.69, 0.97). No significant associations of PLP, the PAr index, and HKr were observed with risk of recurrence by tumor site (Table 3 and **Supplemental Table 4**). In this analysis, the association between PLP concentrations and OS was consistent across neoadjuvant therapy status (**Supplemental Table 5**). Higher concentrations of PLP were associated with 39% and 26% greater OS among patients who received neoadjuvant therapy (HR_{OS}: 0.61; 95% CI: 0.46, 0.82) as well as those who received no neoadjuvant therapy (HR_{OS}, 0.74; 95% CI: 0.63, 0.86), respectively, in adjusted models (Supplemental Table 5). In addition, associations between PLP and the HKr with survival and recurrence are presented by cohort in **Supplemental Table 6**.

Sensitivity analyses

To consider the possibility that disease severity may affect dietary intake and thereby biomarker concentration as well as changes in inflammatory burden that may impact biomarker concentration, we repeated our primary analyses while excluding patients who developed disease recurrence or died within 60 d of blood draw ($n = 31$ patients), and our results remained largely unchanged (**Supplemental Table 7**). Higher concentrations of PLP remained associated with a 31% reduction in mortality after adjustment for other covariates (doubling in concentration

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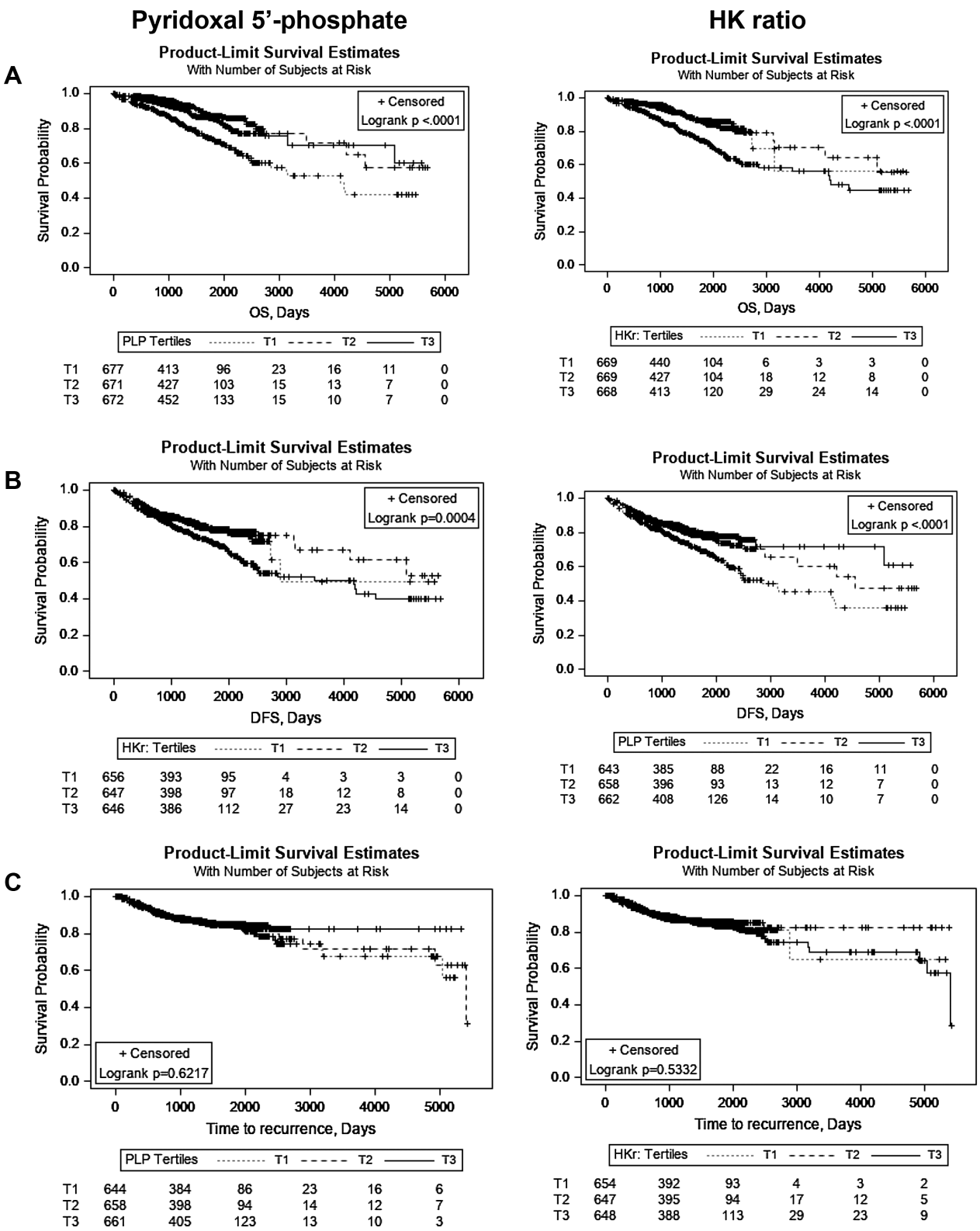

FIGURE 2 Unadjusted associations between PLP concentrations and HKr with overall survival (A), disease-free survival (B), and risk of recurrence (C) by tertiles among stage I–III colorectal cancer patients from the FOCUS Consortium. PLP, T1: 5.52–31.1 nmol/l; T2: 31.2–52.1 nmol/l; T3: >52.1 nmol/l. HKr, T1: −7.138 to −1.636; T2: −1.635 to −1.222; T3: >−1.222. FOCUS, folate-dependent 1-carbon metabolism in colorectal cancer recurrence and survival; PLP, pyridoxal 5′-phosphate; HAA, 3′-hydroxyantranilic acid; HKr, 3′-hydroxykynurenine: [hynurenic acid + xanturenic acid + 3′ anthranilic acid + anthranilic acid.

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TABLE 3 Associations between log2-transformed functional and direct biomarkers of vitamin B6 status with overall survival, disease-free survival, and risk of recurrence among $n = 2006$ colorectal cancer patients stratified by tumor site (right-sided colon, left-sided colon, rectosigmoid junction/rectum)[1]

	Right-sided colon		Left-sided colon		Rectosigmoid junction/rectum	
Vitamin B6 markers	Patient deaths/ total patients	Adj HR (95% CI)	Total events/ total patients	Adj HR (95% CI)	Total events/ total patients	Adj HR (95% CI)
PLP						
Continuous	93/594	0.75 (0.59, 0.96)	82/648	0.71 (0.55, 0.92)	95/764	0.61 (0.47, 0.78)
HKr						
Continuous	92/591	2.37 (1.66, 3.37)	81/642	1.91 (1.25, 2.91)	95/760	2.18 (1.60, 2.99)
PLP						
Continuous	112/572	0.87 (0.70, 1.08)	123/632	0.83 (0.68, 1.02)	117/738	0.82 (0.69, 0.97)
HKr						
Continuous	111/569	1.99 (1.45, 2.72)	122/626	1.44 (1.01, 2.05)	176/734	1.51 (1.17, 1.96)
PLP						
Continuous	65/572	1.02 (0.78, 1.34)	74/631	0.96 (0.75, 1.23)	125/737	0.93 (0.77, 1.13)
HKr						
Continuous	64/569	1.42 (0.91, 2.20)	74/625	1.19 (0.75, 1.88)	124/733	1.16 (0.83, 1.61)

[1]Model adjusted using Cox proportional hazards regression for age, sex, tumor site, creatinine, and cohort. Adj, adjusted; HKr, HK-ratio [HK/(KA + XA + AA + HAA)]; PLP, pyridoxal 5′-phosphate; T, tertile.

of PLP HR_{OS}: 0.69; 95% CI: 0.60, 0.80; HR_{DFS}: 0.85; 95% CI: 0.76, 0.95). Inversely, higher concentrations of the HKr and PAr index also remained associated with a 60% to 107% higher hazard of death or recurrence in adjusted models (HKr HR_{OS}: 2.07; 95% CI: 1.68, 2.55; HR_{DFS}: 1.54; 95% CI: 1.29, 1.84; and PAr index HR_{OS}: 1.60; 95% CI: 1.31, 1.95; HR_{DFS}: 1.31; 95% CI: 1.12, 1.53). We have observed similar associations for OS, DFS, and risk of recurrence after adjustment for BMI and smoking. For example, the association for PLP with OS in the fully adjusted model was comparable with the association in the fully adjusted model with additional adjustment for BMI: e.g., PLP fully adjusted model HR_{OS}: 0.68; 95% CI: 0.59, 0.79; additional adjustment for BMI HR_{OS}: 0.69; 95% CI: 0.60, 0.79 (data not shown). Similarly, the association for PLP with OS in the fully adjusted model was comparable with the association in the fully adjusted model with additional adjustment for smoking, e.g., PLP fully adjusted model HR_{OS}: 0.68; 95% CI: 0.59, 0.79; additional adjustment for smoking HR_{OS}: 0.69; 95% CI: 0.60, 0.80 (data not shown). We further performed stratified analysis by tumor stage. The direction of the association was independent of tumor stage at diagnosis. We observed somewhat stronger associations of PLP and HKr with clinical outcomes in stage I patients compared with stage III patients. However, the number of events in patients diagnosed with stage I tumors was quite low. For example, $n = 48$ deaths occurred in patients diagnosed with stage I tumors compared with $n = 145$ deaths in patients diagnosed with stage III tumors (data not shown). In stratified analysis by follow-up time (2, >2–5, or >5 y) we have observed comparable results. For example, associations of PLP with OS were similar across the invested strata: PLP $HR_{<2years}$: 0.62; 95% CI: 0.49, 0.77; $HR_{2\text{-}5years}$: 0.74; 95% CI: 0.60, 0.91; $HR_{>5years}$: 0.78; 95% CI: 0.55, 1.09 (data not shown).

Discussion

In this international study that included 6 prospective cohorts of patients with stage I–III CRC, higher vitamin B6 status, as assessed by the direct marker PLP and functional marker HKr, was associated with increased all-cause and DFS, although there was no association with disease recurrence. These associations were independent of confounders. The effect of circulating B6 vitamin (=PLP) and HKr concentrations persisted by neoadjuvant therapy status and across tumor sites. To our knowledge, this large consortium study is the first to investigate associations between direct and functional markers of vitamin B6 status and clinical outcomes among patients with stage I–III CRC.

With respect to dietary intake of vitamin B6, numerous reports have suggested a protective effect of dietary intake of vitamin B6 with regard to colorectal adenoma and cancer risk (13, 27–42). In particular, a prospective study by Gylling and colleagues identified vitamin B6 deficiency, measured by plasma concentrations of PLP, markers of functional vitamin B6 status, HK:XA, and PAr index, (a marker of B6 catabolism during inflammation), to be associated with increased CRC risk (25). More recently, we have identified and validated a novel marker of intracellular vitamin B6 status with higher sensitivity and specificity—the HKr (24). Given the ability of markers like HKr to provide unique insight into functional consequences of low intracellular PLP availability, these findings underscore the merit of utilizing a direct measure (PLP) together with a functional marker (HKr) of vitamin B6 status to elucidate the link between vitamin B6 and CRC.

Despite the apparent protective role of dietary and circulating vitamin B6 in CRC risk, prospective cohort studies investigating associations of vitamin B6 with CRC survival and recurrence have yielded inconsistent results. Prior studies have reported that higher plasma PLP concentrations from blood samples as well as questionnaire data on postdiagnostic intake of B vitamins, collected ≥2 y before cancer diagnosis, were not statistically significantly associated with a reduction in overall or cancer-specific mortality among CRC patients (43, 44). In contrast, our investigation of circulating and functional biomarkers of vitamin B6 status collected in samples from CRC patients just prior to surgery showed a substantial benefit of higher vitamin B6 status with respect to improved survival among patients. Reasons for these contradictory findings may include the timing of sample collection and dual role of vitamin B6 in carcinogenesis.

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Although high vitamin B6 status may slow disease progression and lead to improved patient outcomes, it is also possible that high vitamin B6 prior to diagnosis may promote tumor growth among patients, given the role of vitamin B6 for DNA synthesis in 1-carbon metabolism. Supporting a role in patients with established digestive tract carcinoma, there is also recent clinical evidence that high-dose vitamin B6 may enhance antitumor potency of FU-based regimens (45). Further studies are warranted to disentangle the role of vitamin B6 in tumor progression in order to improve prognostic outcomes for patients with CRC.

Evidence has accumulated to suggest that the relation between 1-carbon nutrients and CRC progression may be mediated, in part, by inflammatory processes (12, 46, 47). These findings are concordant with our recent studies, both in healthy individuals and in patients with CRC, demonstrating that higher concentrations of PLP are associated with decreased circulating proinflammatory biomarker concentrations (11, 12). Indeed, PLP has been found to be redistributed from plasma to tissues, including the liver, during inflammation (48, 49). Consequently, functional markers of vitamin B6 status reflect vitamin function in tissues and are less affected by vitamin redistribution. A marker of B6 catabolism during inflammation, the PAr index, has also been previously linked to inflammatory modalities—including clinical conditions linked to low-grade inflammation (23, 25, 50), aldehyde and oxidative stress (51), immune activation, (52) and the acute inflammatory response (53). Low functional B6 status may reflect impaired function of PLP as a cofactor in various enzymatic reactions (54). Indeed, here we report that increasing values of the HKr and PAr were associated with a significantly higher hazard of death among CRC patients in adjusted models. This raises the question of which coinciding diseases may influence circulating concentrations of biomarkers of vitamin B6 status.

We acknowledge the strengths and limitations of our study. The use of 2 independent, complementary biomarkers is a major strength. Indeed, the results of this study demonstrate that findings for 1 functional and 1 direct biomarker are reflected and supported by each other. Our analyses were conducted using data from several European and US cohort studies from a large number of patients with stage I–III CRC. As these cohort studies do not all collect information about CRC-specific survival, we were unable to investigate whether vitamin B6 concentrations were associated with differences in CRC-specific survival or conflated with competing risks of death. The stability of folate and B12 over time has been demonstrated previously for short-term as well as long-term periods >6 mo and ≤13 y (55). The stability of vitamin B6 has been shown for a period >4 y (56). However, these data are not available for all of the measured biomarkers, and a single measurement may not accurately reflect average biomarker concentration over longer time periods. All cohorts have implemented standard operating procedures for active follow-up for patients to capture clinical outcomes such as OS and recurrence using chart abstraction and questionnaires. The follow-up time and follow-up procedures across cohorts vary to some extent. In order to account for these variations, we performed stratified analysis by follow-up time to investigate potential differences in risk estimates. All analyses were further adjusted by study site to address potential confounding. We addressed most relevant confounders; however, as in other studies, there is always the possibility of unmeasured confounding. Because technical factors (e.g., fasted/nonfasted and serum/plasma blood samples) were cohort specific, we also cannot exclude the possibility of residual confounding by these factors. However, we adjusted for study cohort and the associations between vitamin B6 status and survival persisted, even after we controlled for other covariates, and remained largely consistent in sensitivity analyses. For the present study selection bias is unlikely as cancer patients were recruited before surgery. This diverse population across Europe and the United States ensures broad generalizability and clinical applicability. It is possible that preoperative concentrations of vitamin B6 may have been influenced by preoperative treatments. However, this is unlikely, because samples were collected at least 2 wk posttreatment.

In conclusion, findings from this prospective, international consortium-wide study of patients with stage I–III CRC yield important clinical information. We observed significantly improved OS, but no decreased risk of recurrence, after CRC diagnosis among individuals with higher preoperative vitamin B6 status, consistent across colon and rectal cancer. Additional studies are warranted to explore the effects of vitamin B6 status among CRC patients to improve disease outcomes.

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The authors' responsibilities were as follows—CMU, AU, JB, HB, CIL, PMU, ABU, MS, NH, EK, MPW, AG, WMG, FJBvD, PSK, DEK, MH, JCC, and HB: designed research; ANH, JO, BG, CMU, AU, JB, HB, CIL, PMU, ABU, MS, NH, EK, MPW, AG, WMG, and SB: conducted research; TL, AB, AU, CAW, GK, PS, and EH: provided essential reagents or provided essential materials (applies to authors who contributed by providing animals, constructs, databases, etc., necessary for the research); ANH, JO, and TL: analyzed data or performed statistical analysis; ANH, CMU, and JO: wrote paper (only authors who made a major contribution); ANH, CMU, JO: had primary responsibility for final content; RK, EW, FJV, KV, HKvH, MCS, EAK, KK, JLK, TG, TGu, JHWdW, SB, MJLB, and SOB: involved in patient recruitment and follow-up; and all authors: read and approved the final manuscript. CMU has as cancer center director oversight over research funded by several pharmaceutical companies but has not received funding directly herself. The authors report no conflicts of interest.

Data Availability

Data described in the manuscript, code book, and analytic code have been generated from European-based consortia and as such are subject to regulations from multiple European countries, which limit our availability to share data. The consortium's funding has ended, and no centralized staff is available to support data requests. However, the FOCUS PIs have agreed to answer any queries or discuss potential projects with anyone interested in future collaborative research. For further questions please contact colocarestudy_admin@hci.utah.edu.

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