Vitamin B-6 and colorectal cancer risk: a prospective population-based study using 3 distinct plasma markers of vitamin B-6 status[1,2]

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ABSTRACT

Background: Higher plasma concentrations of the vitamin B-6 marker pyridoxal 5′-phosphate (PLP) have been associated with reduced colorectal cancer (CRC) risk. Inflammatory processes, including vitamin B-6 catabolism, could explain such findings.

Objective: We investigated 3 biomarkers of vitamin B-6 status in relation to CRC risk.

Design: This was a prospective case-control study of 613 CRC cases and 1190 matched controls nested within the Northern Sweden Health and Disease Study (n = 114,679). Participants were followed from 1985 to 2009, and the median follow-up from baseline to CRC diagnosis was 8.2 y. PLP, pyridoxal, pyridoxic acid (PA), 3-hydroxykynurenine, and xanthurenic acids (XAs) were measured in plasma with the use of liquid chromatography–tandem mass spectrometry. We calculated relative and absolute risks of CRC for PLP and the ratios 3-hydroxykynurenine:XA (HK:XA), an inverse marker of functional vitamin B-6 status, and PA:(PLP + pyridoxal) (PAr), a marker of inflammation and oxidative stress and an inverse marker of vitamin B-6 status.

Results: Plasma PLP concentrations were associated with a reduced CRC risk for the third compared with the first quartile and for PLP sufficiency compared with deficiency [OR: 0.60 (95% CI: 0.44, 0.81) and OR: 0.55 (95% CI: 0.37, 0.81), respectively]. HK:XA and PAr were both associated with increased CRC risk [OR: 1.48 (95% CI: 1.08, 2.02) and OR: 1.50 (95% CI: 1.10, 2.04), respectively] for the fourth compared with the first quartile. For HK:XA and PAr, the findings were mainly observed in study participants with <10.5 y of follow-up between sampling and diagnosis.

Conclusions: Vitamin B-6 deficiency as measured by plasma PLP is associated with a clear increase in CRC risk. Furthermore, our analyses of novel markers of functional vitamin B-6 status and vitamin B-6–associated oxidative stress and inflammation suggest a role in tumor progression rather than initiation. *Am J Clin Nutr* 2017;105:897–904.

Keywords: biomarkers, colorectal cancer, inflammation, metabolite ratios, vitamin B-6

INTRODUCTION

Pyridoxal 5′-phosphate (PLP)[11] is the active coenzyme form of vitamin B-6 and the most commonly used biomarker of vitamin B-6 status in epidemiologic studies, including investigations of colorectal cancer (CRC) risk (1). PLP has been proposed to influence carcinogenesis through its role in angiogenesis, one-carbon metabolism, cell proliferation, and inflammation (2).

In prospective case-control studies, individuals with higher plasma concentrations of PLP had a 30–50% lower risk of CRC (1, 3–7). In contrast, cohort studies of vitamin B-6 intake and randomized controlled trials of vitamin B-6 supplementation have been inconclusive (1, 8). The results for PLP have been explained by residual confounding because of other factors related to a healthy lifestyle, such as lower smoking rates, higher physical activity, and higher intake of other micronutrients in individuals with higher PLP concentrations (1). Moreover, the inverse association between plasma PLP concentrations and CRC risk may not be an expression of anticancer properties of vitamin B-6 itself but rather a reflection of inflammatory activity, which affects both vitamin B-6 status and CRC risk (9).

Inflammation promotes CRC development (10, 11). The inflammatory biomarker C-reactive protein (CRP) is associated with low PLP status (12, 13) but also increased CRC risk (10, 14–16). Low plasma PLP is also observed in inflammatory bowel

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[2] Supplemental Tables 1–4 and Supplemental Figures 1–3 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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[11] Abbreviations used: CRC, colorectal cancer; CRP, C-reactive protein; HK:XA, 3-hydroxykynurenine:xanthurenic acid ratio; MSP, Mammary Screening Project; PA, pyridoxic acid; PAr, pyridoxic acid:(pyridoxal 5′-phosphate + pyridoxal) ratio; PLP, pyridoxal 5′-phosphate; VIP, Västerbotten Intervention Program; XA, xanthurenic acid.

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disease (17), a CRC risk factor (17, 18). Accordingly, inflammation is one of the main predictors of reduced circulating vitamin B-6 concentrations (19). These observations suggest that the discrepancy between studies on intake compared with plasma concentrations of vitamin B-6 may be partly explained by the reduction of PLP concentrations because of inflammatory activity in persons at an increased risk of CRC.

Two ratios of plasma metabolites have been proposed as more robust indicators of vitamin B-6 status. The 3-hydroxykynurenine: xanthurenic acid (XA) ratio (HK:XA) is based on the role of PLP in tryptophan catabolism via the PLP-dependent kynurenine pathway. HK:XA is the ratio of 2 downstream catabolites of tryptophan, 3-hydroxykynurenine and XA. It is an indicator of functional vitamin B-6 status (20, 21). Pyridoxic acid (PA):(PLP + pyridoxal) ratio (PAr) is the ratio of plasma concentrations of the vitamin B-6 catabolite 4-PA and the sum of the active forms PLP and pyroxidal, i.e., PAr = PA:(PLP + pyridoxal). It is a more robust biomarker of systemic inflammation than PLP and efficiently discriminates high inflammatory status measured by CRP and other inflammatory biomarkers (22). Both ratios are negatively correlated with PLP concentrations. A recent study found that PAr but not HK:XA was associated with overall cancer risk, mainly in lung and colorectal cancers (23).

We investigated circulating markers of vitamin B-6 status, including plasma PLP concentrations and the novel biomarkers PAr and HK:XA, in relation to CRC risk in a large, population-based, nested case-control study with a long follow-up time.

METHODS

Study participants

This was a nested case-control study within the Northern Sweden Health and Disease Study. Participants from 2 population-based cohorts were included: the Västerbotten Intervention Program (VIP) (78.2% of the study participants) and Mammography Screening Project (MSP) (21.8% of the study participants). These cohorts have been described in detail previously (24). In the VIP, established in 1985, residents of Västerbotten County were invited to participate in a health survey upon turning 30, 40, 50, and 60 y of age. The health survey consisted of a medical examination and laboratory tests, donation of a fasting blood sample for future research, and completion of an extensive participant-administered lifestyle questionnaire. As of 31 March 2009, the stop date for the case identification for this study, the VIP included 115,147 blood samples from 85,877 individuals (**Supplemental Figure 1**). The population-based nature of the VIP cohort is supported by comparisons of cancer incidence rates (25), and the selection bias has been found to be low (26). In the MSP, established in 1995 and concluded in 2006, women residing in Västerbotten County aged ~50–70 y were invited to complete a lifestyle questionnaire and donate a blood sample for future research while attending mammography screening. The MSP included 54,401 blood samples from 28,802 women.

CRC case participants diagnosed between 17 October 1986 and 31 March 2009 were identified by linkage with the essentially complete Cancer Registry of Northern Sweden. All cases and tumor data were verified by a single pathologist who specialized in gastrointestinal pathology. Patient records were used to verify the tumor site. Exclusion criteria were previous cancer diagnosis other than nonmelanoma skin cancer, location of primary tumor outside the colorectum, insufficient volume of stored plasma sample available, prioritization to other studies, serious infectious disease, or no matching control available. Two control participants were randomly selected for each case matched by sex, age at and year of blood sampling and data collection, cohort, and fasting status. The exclusion criteria for the controls were the same as for the cases. In addition, the control patients were all alive and with no diagnosed cancer other than nonmelanoma skin cancer at the time of diagnosis of their index cases. After exclusions (81 cases and 41 controls), 613 cases and 1190 controls were included for data analyses (Supplemental Figure 1).

The study protocol and data handling procedures were approved by the Umeå University Research Ethics Committee. All participants gave written informed consent at the time of recruitment to the VIP or MSP cohort. All samples and data were deidentified.

Blood sampling and analysis

The blood samples for this study were collected in sample tubes containing EDTA that were separated into plasma, buffy coat, and erythrocyte fractions. The samples were then divided and cryopreserved at −80°C at a central location. Samples were frozen within 1 h of collection either at −80°C or −20°C for ≤1 wk before being transferred to a −80°C freezer. Samples in the VIP were collected in the morning, and only 34 of 1409 study participants (2%) had fasted for <4 h and 295 (21%) had fasted for <8 h. In the MSP, blood samples were collected throughout the day, and 379 of 393 study participants (96%) had fasted for <4 h.

All biochemical analyses were performed at Bevital AS in 2011. Plasma concentrations of riboflavin, creatinine, and vitamin B-6 species were measured with LC-MS methods (27). Folate and vitamin B-12 concentrations were determined with the use of a microbiological method with the use of *Lactobacillus casei* and *L. leichmannii*, respectively, adapted to a microtiter plate format and carried out by a robotic workstation (28, 29). Interassay CVs for the plasma analyses were 7.1 for PLP, 12.7 for PA, 6.3 for pyridoxal, 9.7 for 3-hydroxykynurenine, and 14.6 for XA (30). The investigators and laboratory staff were blinded to case and control status.

Statistical analyses

We used Mann-Whitney *U* tests, chi-square tests, or linear regressions to test for differences in variable distributions. Measures of RRs for CRC in relation to the vitamin B-6 biomarkers were calculated as ORs with the use of conditional logistic regression. The markers were analyzed in groups divided into quartiles. The cutoff values were based on the distribution of the controls or as standardized log-transformed continuous variables. The trend across quartiles was tested by modeling quartile variables as continuous variables (labeled 1–4). Plasma PLP was also analyzed as sufficient compared with deficient with a cutoff at 20 nmol/L. This concentration is the basis of the mean requirements in the United States (31, 32). Absolute risk estimates in the form of marginal risk differences between estimated risks in the quartile groups of vitamin B-6

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markers were computed with the weighted maximum likelihood estimator (33). Cumulative incidence data from the whole study cohort and within groups defined by sampling year, age, sex, and cohort were used (34). The cumulative incidence of CRC in the study cohort was ~830 cases/100,000 individuals from 1987 to 2009.

To evaluate dose-response and potential nonlinear relations, we modeled continuous log-transformed vitamin B-6 markers in relation to CRC risk with the use of restricted cubic splines (with 5 knots at the 5th, 25th, 50th, 75th, and 95th percentiles of the vitamin B-6 marker distributions). Nonlinearity was tested with a Wald test that compared the model with spline terms to a linear model.

To adjust for potential confounders, several variables with a plausible link to both exposure and outcome were considered. These included the plasma B vitamins folate, riboflavin, and vitamin B-12 (continuous, log-transformed); estimated glomerular filtration rate calculated by the Cockcroft-Gault formula (based on plasma creatinine concentrations, age, sex, and body weight) (35); smoking status; BMI (in kg/m^2); alcohol intake; dietary fiber intake; and recreational and occupational physical activity. In the conditional logistic regression models, 3 models were estimated for each vitamin B-6 marker. Model 1 represented an unadjusted model. Model 2 was adjusted for estimated glomerular filtration, smoking status, BMI, alcohol intake, and recreational and occupational physical activity. Model 3 was additionally adjusted for the plasma B vitamins folate, riboflavin (vitamin B-2), and cobalamin (vitamin B-12). For the risk differences, a crude estimate adjusted only for the matching variables and an adjusted estimate additionally adjusted for the variables corresponding to model 3 were calculated. For plasma B vitamins, 3-hydroxykynurenine, and XA, for which there were very few missing values (≤7 cases), missing data were excluded from the analyses. Missing values for other potential confounders were assigned to a separate category.

We estimated subgroup-specific ORs for CRC by tumor site and stage (with controls given the same value as their index case to retain matching), sex, age, and follow-up time from blood sampling to diagnosis with the use of conditional logistic regression. Heterogeneity was tested with a likelihood ratio test that compared a model in which the risk association could vary across endpoints to a model in which associations were held constant (36) or for sex and age-specific analyses that compared an interaction model to an additive model. Heterogeneity tests were conducted with the use of continuous vitamin B-6 markers (standardized after log transformation).

All tests were 2-sided, and $P < 0.05$ was considered statistically significant. All computations were conducted in R version 3.3.1 (R Foundation).

RESULTS

Baseline characteristics of cases and controls and tumor characteristics are presented in **Table 1**. The median age at diagnosis was 65.2 y (5th–95th percentile: 50.3–76.2 y). Baseline data for BMI, physical activity, and alcohol intake were comparable for cases and controls. There was a slightly higher frequency of ex-smokers among cases than among controls ($P = 0.01$). We observed a tendency for slightly lower PLP concentrations in cases than in controls ($P = 0.08$) and a tendency for slightly higher HK:XA in cases than in controls ($P = 0.06$). PAr was higher in cases than in controls ($P = 0.03$). The associations between potential confounders and the biomarkers are shown in **Supplemental Table 1** and **Supplemental Figure 2**. Overall, the effect size of the associations was weak, with the possible exception of lower PLP for ex-smokers and for participants with a BMI ≥30 and lower alcohol or dietary fiber intake. The vitamin B-6 biomarkers did not vary by storage time (**Supplemental Figure 3**).

ORs for CRC risk for the quartiles of PLP, HK:XA, and PAr are presented in **Figure 1**. The association between PLP and CRC risk was strongest for the third compared with the first (lowest) quartile [OR: 0.60 (95% CI: 0.44, 0.81), $P_{trend} = 0.07$; OR per 1-SD increase: 0.94 (95% CI: 0.83, 1.05)]. PLP sufficiency was associated with a lower risk of CRC compared with deficiency [OR: 0.55 (95% CI: 0.37, 0.81); risk difference: 476 fewer cases/100,000 individuals (95% CI: 859, 17)]. Higher HK:XA was associated with increased CRC risk for the fourth compared with the first quartile [OR: 1.48 (95% CI: 1.08, 2.02), $P_{trend} = 0.02$; OR per 1-SD increase: 1.14 (95% CI: 1.02, 1.28)]. The risk association for PAr was similar for the fourth compared with first quartile [OR: 1.50 (95% CI: 1.10, 2.04), $P_{trend} = 0.01$; OR per 1-SD increase: 1.18 (95% CI: 1.05, 1.31)]. The corresponding absolute risk differences were a decrease in incidence of 325 cases/100,000 individuals for the third compared with the first quartile of PLP (decrease of 45 cases/100,000 individuals per 1-SD increase) and an increase of 211 and 273 cases/100,000 individuals for the fourth compared with the first quartiles of HK:XA and PAr (increase of 68 and 131 cases/100,000 individuals per 1-SD increase), respectively. The inclusions of potential confounders did not markedly alter any risk estimates.

Dose-response relations of the vitamin B-6-markers in relation to CRC risk modeled with restricted cubic splines are presented in **Figure 2**. Although linearity could not be rejected for any marker in the spline regressions, the association for PLP had nonlinear tendencies manifested as a U-shaped association with a particularly high risk for low concentrations ($P_{nonlinearity} = 0.05$).

In **Figure 3**, we present ORs according to follow-up time between sampling and CRC diagnosis. The association for PLP seemed to be similar across follow-up times. P_{trend} was significantly linearly associated with CRC risk for HK:XA and PAr in the subgroups with <10.5 y of follow-up but not for the HK:XA and PAr subgroups with ≥10.5 of follow-up. Similarly, a per 1-SD increase was significantly associated with increased CRC risk in the HK:XA and PAr subgroups with <10.5 y of follow-up but not in the subgroups with ≥10.5 y of follow-up. However, overall heterogeneity for the associations between all follow-up groups was only significant for HK:XA ($P_{heterogeneity} = 0.02$). We observed no clear differences in associations between site, stage, sex, or age, and no overall tests of heterogeneity were significant (**Supplemental Tables 2–4**). However, a 1-SD increase of PAr was significantly associated with left-sided CRC (Supplemental Table 2).

DISCUSSION

In this prospective study, the risk of CRC was highest in participants with the lowest plasma PLP concentrations. High HK:XA, indicating low functional vitamin B-6 status, and PAr, a marker of inflammatory and oxidative stress as well as low vitamin B-6 status, were both associated with an increased CRC risk. For both HK:XA and PAr, the associations were mainly

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TABLE 1
Baseline characteristics of colorectal cancer cases and their matched controls and the tumor characteristics of cases[1]

	Cases		Controls		
	n	Value[3]	*n*	Value[3]	*P* value[2]
Sex					
Men	253	41	487	41	
Women	360	59	703	59	
Age at sampling, y	613	59.8 (40.1–67.8)	1190	59.70 (40.0–67.8)	
Follow-up time, y	613	8.2 (1.3–16.4)	—	—	
eGFR, mL · min^{-1} · 1.73 m^{-2}	596	62.0 (52.3–73.0)	1149	61.7 (50.9–72.3)	0.48
Smoking status					0.01
Current	114	19	239	20	
Ex-smoker	136	22	197	17	
Never	361	59	753	63	
BMI, kg/m^2	591	25.7 (23.5–28.2)	1147	25.6 (23.3–28.1)	0.43
Alcohol intake,[4] g/d	385	2.4 (0.2–5.8)	754	2.3 (0.3–5.7)	0.94
Dietary fiber intake,[4,5] g/2000 kcal	359	22.4 (13.7–33.5)	697	18.9 (9.1–33.7)	0.33
Recreational physical activity[4,6]					0.92
1	221	45	354	40	
2	117	25	240	27	
3	81	17	168	19	
4	29	6	68	7	
5	30	7	64	7	
Occupational physical activity[4,7]					0.94
1	90	21	163	20	
2	77	18	155	19	
3	111	27	223	27	
4	102	25	229	28	
5	36	9	45	6	
Tumor site[8]					
Right colon	183	30	—	—	
Left colon	215	35	—	—	
Rectum	214	35	—	—	
Stage[9]					
I–II	308	53	—	—	
III–IV	276	47	—	—	
Plasma concentrations					
PLP, nmol/L	608	35.9 (17.5–99.9)	1186	38.2 (19.0–107.1)	0.08
Pyridoxal, nmol/L	606	8.5 (6.8–10.9)	1184	8.6 (6.8–11.1)	0.99
PA, nmol/L	608	20.6 (15.9–26.6)	1186	20.0 (15.9–26.3)	0.97
PAr	606	0.46 (0.24–0.93)	1184	0.44 (0.25–0.82)	0.03
3-hydroxykynurenine, nmol/L	606	32.5 (27.3–40.3)	1182	33.0 (27.0–40.1)	0.53
XA, nmol/L	608	11.8 (8.4–15.7)	1186	12.4 (8.8–16.9)	0.05
HK:XA	606	2.8 (1.5–7.1)	1182	2.7 (1.4–6.5)	0.06
Folate, nmol/L	613	7.3 (4.9–10.4)	1190	7.2 (4.6–10.2)	0.49
Vitamin B-2, nmol/L	608	10.8 (7.4–16.0)	1186	11.8 (7.9–17.9)	0.002
Vitamin B-12, nmol/L	603	413 (337–498)	1173	426 (353–501)	0.02

[1] eGFR, estimated glomerular filtration rate; HK:XA, 3-hydroxykynurenine:xanthurenic acid ratio; PA, pyridoxic acid; PAr, pyridoxic acid:(PLP + pyridoxal); PLP, pyridoxal 5′-phosphate; VIP, Västerbotten Intervention Program; XA, xanthurenic acid.
[2] Test for difference in distribution between cases and controls. Mann-Whitney *U* tests were used for continuous variables and chi-square tests were used for categorical variables.
[3] Values are percentages or medians (5th–95th percentiles).
[4] Variables were available only for the VIP cohort.
[5] Estimated from self-administered semiquantitative food-frequency questionnaires designed to measure intakes from the previous year in mass per day divided by total energy intake.
[6] Self-reported on a scale from 1 to 5 ranging from no exercise during leisure time to exercise >3 times/wk.
[7] Self-reported on a scale from 1 to 5 ranging from low to high physical effort at work.
[8] Could not be determined for 1 case.
[9] Could not be determined for 29 cases.

observed in participants with follow-up times of <10.5 y between sampling and CRC diagnosis.

Although the relation between plasma PLP concentrations and CRC risk was nonlinear in our study, the highest risk was consistently observed in participants with the lowest PLP, assessed as the lowest quartile, as concentrations under the clinically relevant cutoff for deficiency (20 nmol/L), and in the regression spline models. Our results are thus in line with previous

	Cases/ controls	OR Model 1	OR Model 2	OR Model 3	RD Crude (cases/100 000)	RD Adjusted (cases/100 000)
PLP						
Q1 (<28.0)	190/297	ref	ref	ref	ref	ref
Q2 (28.0 to <38.2)	146/296	0.76 (0.58, 1.01)	0.79 (0.59, 1.06)	0.79 (0.58, 1.06)	−201 (−405, 1)	−139 (−350, 84)
Q3 (38.2 to <51.4)	119/296	0.61 (0.46, 0.82)	0.60 (0.45, 0.82)	0.60 (0.44, 0.81)	−378 (−569, −187)	−325 (−527, −110)
Q4 (≥51.4)	153/297	0.80 (0.61, 1.05)	0.78 (0.58, 1.03)	0.80 (0.59, 1.09)	−161 (−351, 30)	−115 (−341, 120)
P_{trend}		0.05	0.03	0.07		
Per 1SD increase		0.92 (0.83, 1.02)	0.92 (0.82, 1.02)	0.94 (0.83, 1.05)	−58 (−126, 7)	−45 (−125, 36)
B−6 deficient (<20)	64/78	ref	ref	ref	ref	ref
B−6 sufficient (≥20)	544/1108	0.58 (0.41, 0.82)	0.55 (0.38, 0.79)	0.55 (0.37, 0.81)	−499 (−865, −82)	−476 (−859, −17)
HK:XA						
Q1 (<2.0)	134/295	ref	ref	ref	ref	ref
Q2 (2.0 to <2.7)	148/295	1.14 (0.85, 1.52)	1.09 (0.80, 1.47)	1.13 (0.83, 1.53)	32 (−168, 229)	64 (−119, 242)
Q3 (2.7 to <3.7)	139/295	1.06 (0.78, 1.42)	1.05 (0.77, 1.43)	1.08 (0.79, 1.49)	6 (−197, 194)	37 (−167, 233)
Q4 (≥3.7)	182/296	1.42 (1.05, 1.91)	1.45 (1.07, 1.97)	1.48 (1.08, 2.02)	215 (−5, 423)	211 (−6, 412)
P_{trend}		0.04	0.02	0.02		
Per 1SD increase		1.12 (1.01, 1.25)	1.14 (1.02, 1.27)	1.14 (1.02, 1.28)	64 (−16, 138)	68 (−19, 146)
PAr						
Q1 (<0.35)	126/296	ref	ref	ref	ref	ref
Q2 (0.35 to <0.44)	152/296	1.20 (0.90, 1.60)	1.20 (0.90, 1.62)	1.25 (0.92, 1.69)	133 (−49, 311)	170 (−24, 353)
Q3 (0.44 to <0.57)	155/296	1.24 (0.93, 1.64)	1.26 (0.94, 1.68)	1.29 (0.96, 1.74)	178 (3, 353)	189 (6, 366)
Q4 (≥0.57)	173/296	1.40 (1.05, 1.87)	1.44 (1.07, 1.94)	1.50 (1.10, 2.04)	263 (65, 457)	273 (58, 477)
P_{trend}		0.03	0.02	0.01		
Per 1SD increase		1.14 (1.03, 1.26)	1.15 (1.03, 1.28)	1.18 (1.05, 1.31)	122 (37, 206)	131 (37, 217)

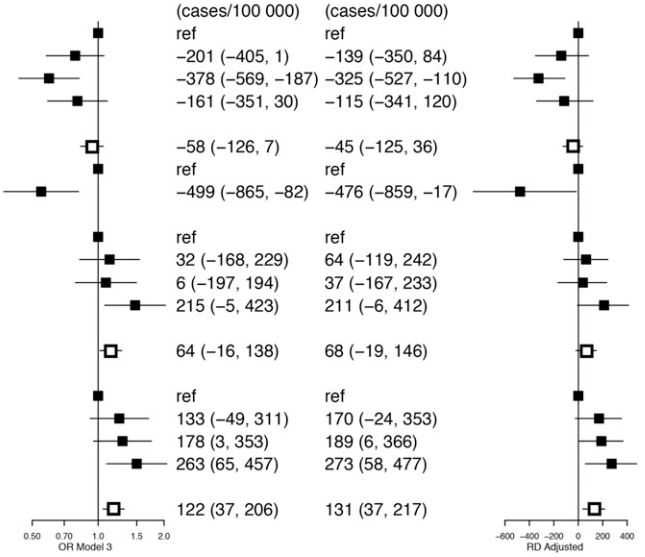

FIGURE 1 ORs and 95% CIs calculated by conditional logistic regression and marginal RDs and bootstrapped 95% CIs calculated by the weighted maximum likelihood estimation for colorectal cancer risk by PLP, HK:XA, and PAr in quartiles (with cutoffs based on the distribution of the controls) and per 1-SD increase of plasma concentrations (modeling standardized log-transformed variables) (log mean/SD—PLP: 3.68/0.57; HK:XA: 1.05/0.49; PAr: −0.79/0.38). Model 1 was unadjusted. Model 2 was adjusted for eGFR, BMI (in kg/m^2), smoking status, alcohol intake, dietary fiber intake, and recreational and occupational physical actvity. Model 3 was additionally adjusted for log-transformed plasma folate, riboflavin, and vitamin B-12 concentrations. Crude RDs were adjusted only for the matching variables. Adjusted RDs were adjusted for the same variables as in model 3. eGFR, estimated glomerular filtration rate; HK:XA, 3-hydroxykynurenine:xanthurenic acid ratio; PAr, pyridoxic acid:(PLP + pyridoxal) ratio; PLP, pyridoxal 5′-phosphate; Q, quartile; ref, reference; RD, risk difference.

findings (1, 3–7). In our study, PLP status above sufficiency was not associated with any additional lowering of CRC risk. Plasma PLP is influenced by dietary vitamin B-6 intake and supplementation, plasma albumin and alkaline phosphatase concentrations, kidney function, smoking, and alcohol intake (21, 37), which may contribute to PLP variability (22). PAr is less prone to confounding by a high vitamin B-6 intake (22), which may explain the linear association between PAr and CRC risk. Our results for PAr are in accordance with a previous cohort study that included 158 CRC cases (23). To our knowledge, HK:XA has not previously been studied in relation to CRC risk. The positive association between HK:XA and CRC risk in this study was apparent primarily in the fourth quartile, suggesting that metabolic alterations in the kynurenine pathway may contribute to the increased CRC risk at low PLP concentrations. HK:XA seems to be a valuable complement to PLP in investigations of vitamin B-6 and disease risk.

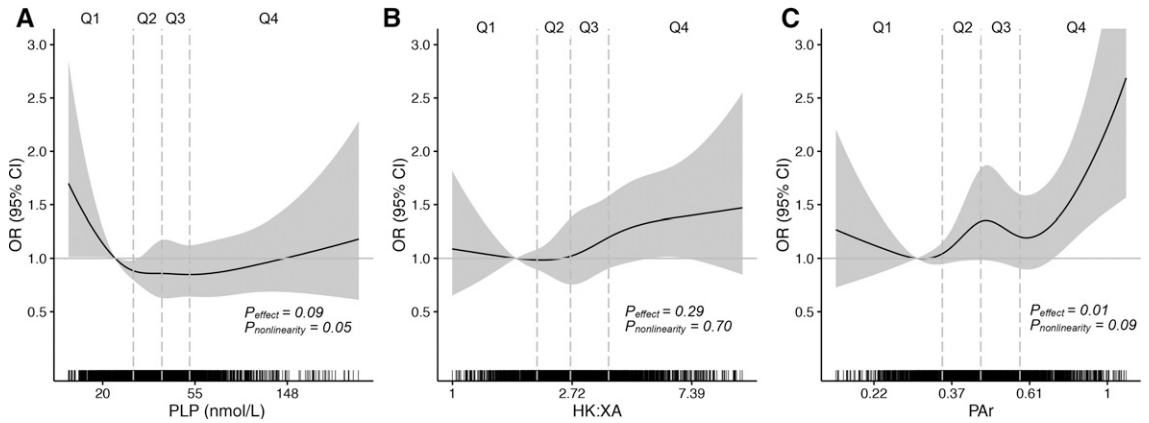

FIGURE 2 ORs and 95% CIs for colorectal cancer risk by log-transformed levels of vitamin B-6 markers (A) PLP, (B) HK:XA, and (C) PAr modeled with restricted cubic splines (with 5 knots at the 5th, 25th, 50th, 75th, and 95th percentiles of the vitamin B-6 marker distributions). The models were adjusted for the matching variables [age at and year of blood sampling (continuous), cohort, fasting status, and sex], eGFR, BMI (in kg/m^2), smoking status, alcohol intake, dietary fiber intake, recreational and occupational physical activity, and log-transformed plasma folate, riboflavin, and vitamin B-12 concentrations (model 3). The median within the first quartile group is the reference. The shaded area represents 95% CIs. The rug plot represents individual vitamin B-6 marker data points (log scale). Observations outside the 1st and 99th percentiles were omitted from the plot. Dashed vertical lines represent quartile cutoffs. A 2-sided Wald test was used to test effect and nonlinearity. The corresponding null hypotheses were no effect and that the effect was linear, respectively. eGFR, estimated glomerular filtration rate; HK:XA, 3-hydroxykynurenine:xanthurenic acid ratio; PAr, pyridoxic acid:(PLP + pyridoxal) ratio; PLP, pyridoxal 5′-phosphate; Q, quartile.

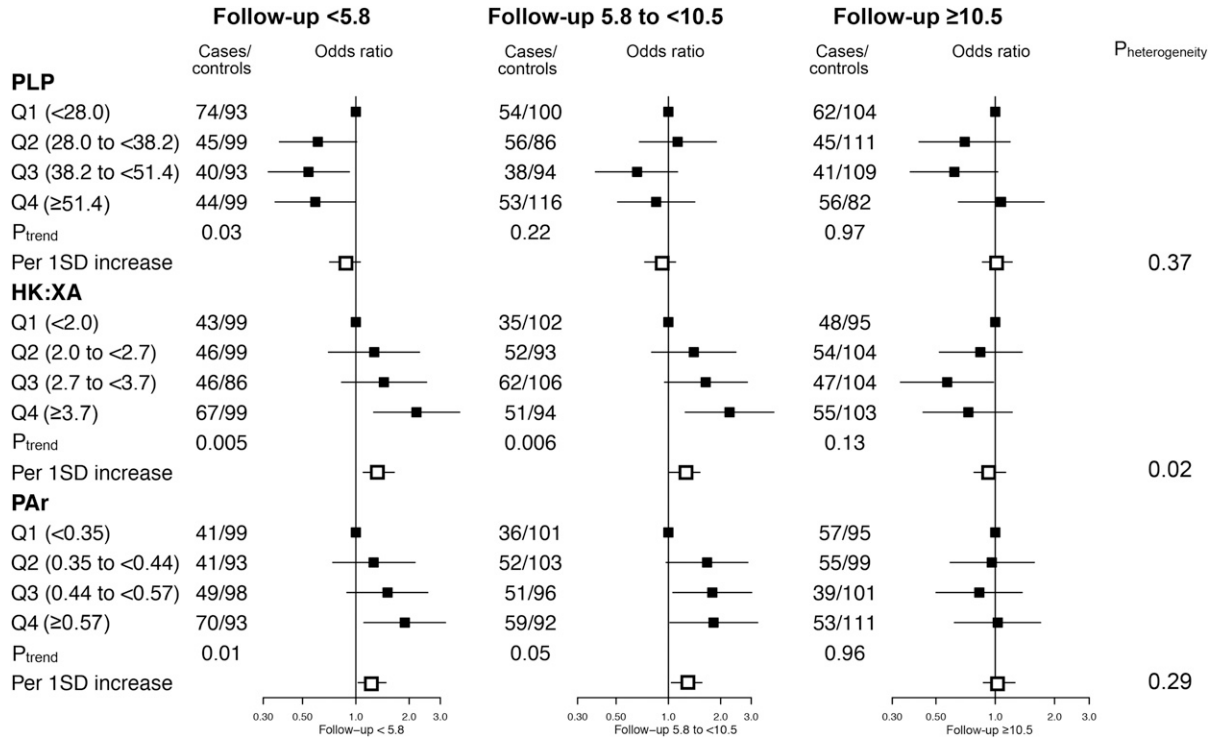

FIGURE 3 ORs and 95% CIs calculated by conditional logistic regression for colorectal cancer risk by quartiles and per 1-SD increase of plasma concentrations of PLP, HK:XA, and PAr, with cutoffs based on the distribution of the controls, for 3 groups of follow-up times. The models were adjusted for the matching variables [age at and year of blood sampling (continuous), cohort, fasting status, and sex], eGFR, BMI (in kg/m^2), smoking status, alcohol intake, dietary fiber intake, recreational and occupational physical activity, and log-transformed folate, riboflavin, and vitamin B-12 concentrations (model 3). Heterogeneity was tested for the per 1-SD estimates, with a 2-sided likelihood ratio test comparing a model that allowed for separate associations for tumor subgroups to a model that assumed a common association across subgroups. eGFR, estimated glomerular filtration rate; HK:XA, 3-hydroxykynurenine: xanthurenic acid ratio; PAr, pyridoxic acid:(PLP + pyridoxal) ratio; PLP, pyridoxal 5′-phosphate; Q, quartile.

Inflammatory activity is causally related to cancer development (38), particularly for CRC, for which inflammatory bowel disease is an established risk factor (18). Many environmental CRC risk factors, such as smoking, infections, obesity and diet, are associated with inflammatory activity (38). Inflammation may therefore be a common underlying carcinogenic mechanism for several risk factors that together contribute to the association between inflammatory markers, including PAr, and CRC risk. For PAr, an inverse marker of vitamin B-6 status, confounding by these risk factors may also be present, but adjusting the multivariable analyses had essentially no effect on the results. PAr was mainly associated with CRC risk in the subgroup with <10.5 y from baseline to CRC diagnosis. Given the slow progression of colorectal lesions, this finding is consistent with previous findings for the inflammatory marker CRP of a direct association with the risk of CRC but not colorectal adenoma (10, 14, 15, 39). Because undiagnosed cancerous lesions at baseline were unlikely to be present in the group with ≥10.5 y of follow-up, the putative role in CRC may occur once a lesion has been established (i.e., tumor progression) and not in the healthy mucosa (i.e., tumor initiation). However, for PLP, the risk relation was similar across follow-up groups, possibly reflecting the complexity in determinants of PLP status. Although our results did not differ significantly by tumor site, the association between PAr and CRC risk was strongest in cases with left-sided colon cancer, which is in line with several studies on different inflammatory biomarkers and CRC (14, 16, 40).

Cancer, including CRC, is known to reduce plasma concentrations of PLP (21). The underlying mechanisms may include increased catabolism and tissue redistribution (19, 22). Furthermore, vitamin B-6 concentrations are >2-fold higher in colorectal tumors than in normal mucosa (41), which might be caused by inflammation-related tissue redistribution or elevated metabolic demands of the growing tumor. Given the slow development of CRC, observed risk relations may therefore reflect an influence of undiagnosed disease at baseline on circulating markers (reverse causation). However, the opposite is also possible; i.e., vitamin B-6 and inflammation may influence the progression of established but undiagnosed CRC (10, 42). Although the study design did not allow us to distinguish between these 2 relations, in our subgroup analyses for PAr and HK:XA, participants with <5.8 y from sampling to CRC diagnosis (likely to have the greatest tumor burden) did not have a statistically stronger association to CRC risk than those with 5.8–10.5 y from baseline to diagnosis.

The main strength of our study was the use of 3 vitamin B-6 markers to allow a more comprehensive assessment of vitamin B-6 status and, to some extent, account for the many determinants of PLP variability. Other important strengths of our study include the population-based prospective design, large sample size, and long follow-up. A large proportion of the samples were collected after ≥8 h of fasting, and the sample handling procedures and laboratory analyses were of very high quality. We also present both relative and absolute risk estimates, which is of value in interpreting the clinical relevance of the results. We analyzed a single baseline blood sample per participant, which may not be representative of vitamin B-6 status over time and could result in

an underestimation of the true risk relations caused by regression dilution (43). We adjusted for several potentially important parameters, including kidney function, one-carbon metabolites, and several established risk factors for CRC. We were not able to control for several potential confounders, such as nonsteroidal anti-inflammatory drug use and hereditary CRC, but the use of biomarkers of functional vitamin B-6 status, including the inflammatory marker PAr, may have reduced the risk of confounding from inflammation. We were also not able to control for CRC screening because there was no formal CRC screening program in the source population, and opportunistic screening was found to be essentially nonexistent in a similar Swedish cohort (44).

In conclusion, in this nested case-control study, vitamin B-6 sufficiency compared with deficiency defined by PLP concentrations was associated with a clear decrease in CRC risk. The association was nonlinear, and PLP status above sufficiency was not associated with any additional benefit. Higher HK:XA, indicating lower functional vitamin B-6 status, and PAr, an inflammatory marker and inverse marker of vitamin B-6 metabolism during inflammation, were associated with increased CRC risk. The associations were consistent with a role in tumor progression rather than initiation.

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