

Markers of vitamin B6 status and metabolism as predictors of incident cancer: The Hordaland Health Study

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Dietary intake and/or circulating concentrations of vitamin B6 have been associated with risk of cancer, but results are inconsistent and mechanisms uncertain. Pyridoxal 5'-phosphate (PLP) is the most commonly used marker of B6 status. We recently proposed the ratio 3-hydroxykynurenine/xanthurenic acid (HK/XA) as an indicator of functional vitamin B6 status, and the 4-pyridoxic acid (PA) / (pyridoxal (PL) + PLP) ratio (PAr) as a marker of vitamin B6 catabolism during inflammation. We compared plasma PLP, HK/XA and PAr as predictors of cancer incidence in a prospective community-based cohort in Norway. This study included 6,539 adults without known cancer at baseline (1998–99) from the Hordaland Health Study (HUSK). HR and 95% CI were calculated for the risk of overall and site-specific cancers using multivariate Cox proportional hazards regression with adjustment for potential confounders. After a median follow-up time of 11.9 years, 963 cancer cases (501 men and 462 women) were identified. Multivariate-adjusted Cox-regression showed no significant relation of plasma PLP or HK/XA with risk of incident cancer. In contrast, PAr was significantly associated with risk of cancer with HR (95% CI) = 1.31 (1.12–1.52) per two standard deviation (SD) increment ($p < 0.01$). Further analysis showed that PAr was a particular strong predictor of lung cancer with HR (95% CI) = 2.46 (1.49–4.05) per two SD increment ($p < 0.01$). The present results indicate that associations of vitamin B6 with cancer may be related to increased catabolism of vitamin B6, in particular for lung cancer where inflammation may be largely involved in carcinogenesis.

Pyridoxal-5'-phosphate (PLP), pyridoxal (PL) and 4-pyridoxic acid (PA) are the main B6 vitamers in the circulation. PLP is the active form that is involved in over 140 enzy-

matic reactions including amino acid metabolism, lipid metabolism, gluconeogenesis, one-carbon metabolism, heme and neurotransmitter biosynthesis.^{1,2} PL is the transport form that crosses cellular membranes whereas PA is a vitamin B6 catabolite excreted into the urine.³ The liver is the primary site for vitamin B6 metabolism.³

Key words: vitamin B6, inflammation, metabolism, cancer, risk

Abbreviations: BMI: body mass index; CRP: C-reactive protein; FFQ: food frequency questionnaire; GAM: generalized additive model; HK/XA: 3-hydroxykynurenine/xanthurenic acid; HUSK: Hordaland Health Study; KTR: kynurenine/tryptophan ratio; PA: 4-pyridoxic acid; PAr: 4-pyridoxic acid/(pyridoxal + pyridoxal 5'-phosphate) ratio; PL: pyridoxal; PLP: pyridoxal 5'-phosphate

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Patients with a variety of diseases associated with inflammation or acute phase response have consistently been reported to have low concentrations of plasma PLP but normal erythrocyte PLP.⁴ These conditions include cardiovascular disease and cancer. Furthermore, plasma PLP was inversely associated with C-reactive protein (CRP) in cohorts of healthy subjects,⁴ whereas plasma PA was positively associated with CRP and other measures of activated cellular immunity in cardiovascular patients.⁵

Observational studies have suggested that dietary vitamin B6 intake and circulating concentrations are inversely associated with risk of cancer in the colon/rectum, lung, prostate, breast and pancreas.^{1,6–8} Several mechanisms have been suggested, including anti-inflammatory effects, effects on angiogenesis, DNA methylation, synthesis and repair and humoral and cell-mediated immune responses.¹ Randomized placebo-controlled trials using vitamin supplements, however, have mostly failed to decrease cancer risk.^{9,10}

What's new?

Vitamin B6 status is reflected in the measure of its active form, pyridoxal 5'-phosphate (PLP). Studies disagree, however, as to whether or not PLP measures are meaningful in relation to cancer risk, which has necessitated a search for additional markers of vitamin B6 status. In this study, inflammation-related changes in vitamin B6 catabolism were captured effectively by a novel marker, the 4-pyridoxic acid (PA) / (pyridoxal (PL) + PLP) ratio (PAr). Analyses based on the detection of PAr suggest that increased vitamin B6 metabolism and disposal are linked to increased cancer risk, particularly for lung cancer.

Several steps in tryptophan catabolism via the kynurenine pathway are vitamin B6 dependent. The ratio of two downstream metabolites, 3-hydroxykynurenine (HK) and xanthurenic acid (XA), *i.e.* the HK/XA, was recently proposed as an indicator of functional vitamin B6 status.¹¹

Other potentially useful substrate product ratios measured in plasma include the established inflammatory marker kynurenine/tryptophan ratio (KTR)⁵ and the PA/(PL+PLP) ratio (PAr).¹² The latter is a strong indicator of inflammation, in particular activated cellular immunity, indicating that increased vitamin B6 catabolism may partly explain low PLP concentrations in inflammatory conditions.^{4,13,14} Elevated PAr may also reflect increased oxidative stress,¹⁵ which is closely related to cancer pathogenesis.¹⁶

The aim of this study was to compare associations of different vitamin B6 measures, including PLP, HK/XA and PAr, with cancer risk in a prospective, community-based cohort in Norway.

Subjects and Methods**Study subjects**

The design of the Hordaland Health Study (HUSK) has been described in detail in previous papers^{17,18} and on the website: <http://husk-en.b.uib.no>. The baseline survey was conducted in 1997–1999 as collaboration between the University of Bergen, the National Institute of Public Health and the Municipal Health Services in Hordaland, Western Norway. Men and women born during 1925–1927 and 1950–1951 living in the city of Bergen and its surrounding areas were invited to participate. The present study cohort was confined to 7,051 participants recruited in the period of April 1998 to June 1999. Information on socio-demographic variables, health status and lifestyle factors were collected by self-administered questionnaires.

Of the 7,051 participants, we excluded 426 participants who were diagnosed with cancer (other than non-melanoma skin cancer) before enrollment. Participants with missing data on blood measurements of PLP, PL, PA, HK and XA ($n = 86$) were also excluded. A total of 6,539 participants (2,918 men and 3,621 women) were included in the final analysis.

The study protocol was approved by the Regional Committee for Medical and Health Research Ethics in Western Norway (REK-Vest) and the Norwegian Data Inspectorate. Written informed consent was obtained from all participants.

Laboratory analyses

Non-fasting blood samples were collected at baseline. Aliquots of plasma were frozen at -80°C until later analyses. Plasma B6 vitamers (PLP, PL and PA), neopterin, tryptophan, kynurenine, HK, XA were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS).^{19,20} Serum creatinine was measured colorimetrically using the alkaline picrate method with reagents from Roche (Basle, Switzerland),¹⁷ before the samples were frozen. PAr was calculated by dividing plasma PA concentrations by the sum of plasma concentrations of PL and PLP.¹² KTR was the ratio of the concentrations of kynurenine in nmol divided by tryptophan in μmol . Plasma high-sensitive CRP was determined by an immuno-MALDI-MS method.²¹ All biochemical analyses were performed at Bevital A/S (www.bevital.no), except for serum creatinine which was done at Ullevål University Hospital, Oslo. Within-day coefficients of variation (CVs) for B6 vitamers, neopterin, kynurenine and tryptophan were 2.5–4.7% and between-day CVs were 4.8–11.1%.¹⁹

Follow-up and outcome assessment

The cohort participants were followed from the baseline survey throughout the year of 2010. Incident cancer cases were ascertained through linkage with the Cancer Registry of Norway. Cancer incidence diagnoses were coded according to the third edition of the International Classification of Diseases for Oncology (ICD-O-3)²² and the 10th revision of the International Statistical Classification of Diseases, Injuries and Causes of Death (ICD-10) (<http://apps.who.int/classifications/icd10/browse/2010/en>). Only the first primary neoplasm was included in the analysis. Information on mortality was collected from the Cause of Death Registry at Statistics Norway and coded according to ICD-10.

Additional data

Smoking status was categorized as: never smokers, former smokers, light smokers (≤ 10 cigarettes per day) and heavy smokers (> 10 cigarettes per day). Dietary data were collected with the use of a validated self-administered food frequency questionnaire (FFQ).^{23,24} The FFQ included 169 food items and offered alternatives for frequency, number of units consumed and portion sizes to capture the habitual diet information during the past year. Intake of vegetables and fruits was obtained from FFQ, and daily alcohol intake was calculated using a nutrient database and software system developed at

Table 1. Baseline characteristics of the HUSK participants

Characteristics ¹	Overall (n = 6,539)		Incident cancer (n = 963)		Non-cancer (n = 5,576)		p
Male (%)	2,918	(44.6)	501	(52.0)	2417	(43.4)	<0.01
Age (%)							
46–49 years	3,590	(54.9)	271	(28.1)	3319	(59.5)	<0.01
70–74 years	2,949	(45.1)	692	(71.9)	2257	(40.5)	
BMI (kg m ⁻²)	25.4	(23.1–27.9)	25.6	(23.2–28.2)	25.4	(23.1–27.8)	0.11
Smoking (%) ²							
Never smokers	2,576	(40.9)	320	(34.5)	2256	(42.0)	<0.01
Former smokers	2,115	(33.5)	381	(41.0)	1734	(32.2)	
Light smokers	882	(14.0)	134	(14.4)	748	(13.9)	
Heavy smokers	733	(11.6)	94	(10.1)	639	(11.9)	
Alcohol intake (g day ⁻¹)	1.7	(0–6.8)	1.7	(0–6.9)	1.9	(0–6.9)	0.43
Intake of vegetables and fruits (g day ⁻¹)	392	(258–566)	373	(240–544)	398	(262–574)	0.02
Creatinine (μmol l ⁻¹)	79.8	(71.7–89.4)	82.0	(73.3–91.4)	79.5	(71.5–89.2)	<0.01
PLP (nmol l ⁻¹)	50.1	(35.8–75.1)	47.1	(32.9–75.6)	50.6	(36.2–75.0)	<0.01
PL (nmol l ⁻¹)	11.9	(9.1–16.6)	12.1	(9.2–18.0)	11.8	(9.0–16.5)	0.04
PA (nmol l ⁻¹)	22.3	(16.7–34.5)	24.0	(17.7–38.9)	22.1	(16.5–33.7)	<0.01
HK/XA ratio	2.0	(1.5–2.8)	2.2	(1.6–3.0)	2.0	(1.5–2.7)	<0.01
Par	0.38	(0.29–0.51)	0.43	(0.32–0.59)	0.38	(0.29–0.50)	<0.01
Neopterin (nmol l ⁻¹)	7.6	(6.3–9.2)	8.2	(6.8–10.0)	7.5	(6.3–9.1)	<0.01
KTR (nmol μmol ⁻¹)	22.4	(18.4–27.7)	24.7	(20.2–30.1)	22.1	(18.1–27.2)	<0.01
CRP (mg l ⁻¹)	1.5	(0.7–3.6)	2.1	(0.9–4.2)	1.4	(0.6–3.4)	<0.01

Abbreviations: BMI, body mass index; CRP, C-reactive protein; HK/XA, 3-hydroxykynurenine/xanthurenic acid; HUSK, Hordaland Health Study; KTR, kynurenine/tryptophan ratio; PA, 4-pyridoxic acid; Par, 4-pyridoxic acid/(pyridoxal + Pyridoxal 5'-phosphate) ratio; PL, pyridoxal; PLP, pyridoxal 5'-phosphate.

¹Values are given as medians (interquartile ranges) or numbers (percentages).

²Numbers of participants do not sum to the total numbers because of missing data.

the Department of Nutrition, University of Oslo.²³ Following a standard protocol, height and weight were measured in light clothing, without shoes, to the nearest 1 cm and 0.5 kg, respectively. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg m⁻²).

Statistical analysis

Statistical analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC) and R (version 2.15 for Windows, www.r-project.org). Log-transformation was applied to plasma concentrations of PLP, HK/XA, Par, neopterin, KTR and CRP to normalize their distribution. Continuous variables are reported as medians (interquartile ranges) and categorical variables as numbers (percentages). Chi-square tests were used to compare differences between incident cancer cases and non-cases for categorical and Wilcoxon–Mann–Whitney tests for continuous variables. Pearson correlation was calculated to examine the relations between plasma PLP and HK/XA.

We used Cox proportional hazards models to estimate the hazard ratios (HRs) and their 95% confidence intervals (CIs) for the risk of overall and specific cancers. Follow-up began at baseline and ended at the date of cancer diagnosis, death, emi-

gration or December 31, 2010, whichever came first. The proportionality assumption of the models was tested and plotted based on Schoenfeld residuals,²⁵ and no evidence of violation was found. Multivariate models were adjusted for gender, age (46–49 years vs. 70–74 years), BMI (continuous), smoking (never, former, light smokers or heavy smokers), alcohol intake (continuous), intake of vegetables and fruits (continuous) and creatinine (continuous). Plasma concentrations of PLP, HK/XA and Par were analyzed both as categorical variables (quartiles) and continuous measures (per 2 SD increment after log-transformation). Multivariable adjusted associations between Par and risk of overall and lung cancer were visualized by plots using generalized additive models (GAM). To test for potential confounding or mediation by plasma CRP, we additionally adjusted for CRP in the Cox models examining the association of Par with overall cancer risk. Tests for potential interaction with smoking were performed based on first-degree multiplicative models.

Sensitivity analyses were performed to determine the robustness of findings in the primary analysis by exclusion of cases who were diagnosed during the first 2 years of follow-up. We further tested for the possibility of reverse causality

Table 2. HRs and 95% CIs for overall cancer risk by PLP, HK/XA ratio and PAR, respectively, the Hordaland Health Study ($n = 6,539$)

Cancer cases	Gender, age-adjusted		Multivariate-adjusted		
	HR (95% CI)	p trend ¹	HR (95% CI)	p -trend ¹	
PLP (nmol l ⁻¹)					
Quartile 1	293	1.00 (ref.)	0.14	1.00 (ref.)	0.10
Quartile 2	232	0.76 (0.64–0.91)		0.83 (0.68–1.00)	
Quartile 3	194	0.68 (0.56–0.81)		0.69 (0.56–0.84)	
Quartile 4	244	0.82 (0.69–0.98)		0.82 (0.68–0.99)	
Per 2 SD increment ²		0.88 (0.77–1.00)	0.05	0.90 (0.78–1.04)	0.16
HK/XA ratio					
Quartile 1	190	1.00 (ref.)	0.02	1.00 (ref.)	0.04
Quartile 2	217	1.00 (0.82–1.22)		1.03 (0.83–1.28)	
Quartile 3	259	1.10 (0.91–1.33)		1.09 (0.88–1.34)	
Quartile 4	297	1.22 (1.01–1.47)		1.21 (0.98–1.49)	
Per 2 SD increment ²		1.11 (0.97–1.26)	0.14	1.06 (0.92–1.23)	0.44
Par					
Quartile 1	162	1.00 (ref.)	<0.01	1.00 (ref.)	<0.01
Quartile 2	215	1.09 (0.88–1.33)		1.04 (0.83–1.31)	
Quartile 3	262	1.19 (0.97–1.46)		1.22 (0.98–1.52)	
Quartile 4	324	1.34 (1.10–1.64)		1.29 (1.04–1.61)	
Per 2 SD increment ²		1.31 (1.15–1.50)	<0.01	1.31 (1.12–1.52)	<0.01

Abbreviations: HK/XA, 3-hydroxykynurenine/xanthurenic acid; PAR, 4-pyridoxic acid/(pyridoxal + Pyridoxal 5'-phosphate) ratio; PLP, pyridoxal 5'-phosphate.

Multivariate-adjusted models: adjusted for gender, age (46–49 years vs. 70–74 years), BMI (continuous), smoking (never, former, light smokers or heavy smokers), alcohol intake (continuous), intake of vegetables and fruits (continuous) and creatinine (continuous).

¹Tests for a linear trend across quartiles were performed by modelling quartile-specific median values as a continuous variable.

²HRs (95% CIs) are reported per 2 standard deviation (SD) increment in log-transformed data. The SD for ln PAR was 0.45, therefore 2 SD increment in log PAR corresponds to approximately 2.4 times of PAR ratio.

Table 3. Adjusted HRs and 95% CIs for the risk of main specific cancer types in relation to plasma PAR¹

Cancer type	Number of cases	HR (95% CI) per 2SD increment			
		Model 1 ²	p	Model 2 ³	p
Colorectal cancer	158	1.45 (1.02–2.06)	0.04	1.45 (1.00–2.08)	0.05
Prostate cancer	129	1.00 (0.65–1.52)	0.98	0.90 (0.58–1.39)	0.63
Breast cancer	98	1.03 (0.65–1.61)	0.91	1.00 (0.63–1.60)	0.99
Lung cancer	85	2.46 (1.49–4.05)	<0.01	2.37 (1.43–3.92)	<0.01

Abbreviations: PAR, 4-pyridoxic acid/(pyridoxal + Pyridoxal 5'-phosphate) ratio.

¹Plasma PAR were log-transformed

²Adjusted for gender, age (46–49 years vs. 70–74 years), BMI (continuous), smoking (never, former, light smokers or heavy smokers), alcohol intake (continuous), intake of vegetables and fruits (continuous) and creatinine (continuous)

³Additionally adjusted for CRP (continuous).

by plotting geometric mean PAR for the cancer cases by follow-up using GAM regression adjusted for the above-mentioned confounders. All tests were two sided and p value <0.05 was considered statistically significant.

Results

After a median follow-up time of 11.9 years, 963 cancer cases (501 men and 462 women) were identified. Compared with non-cases, incident cancer cases were more often men, older, former or current smokers, had lower intake of vegetables and

fruits, and had higher values of all laboratory measures in plasma/serum at baseline except PLP, which was lower in cases than in non-cases (Table 1). Pearson correlation coefficients adjusted for age and gender showed a negative correlation between PLP and HK/XA (Pearson $r = -0.33$, $p < 0.01$). The change across increasing quartiles of PAR was due to both gradually increasing concentrations of PA and decreasing concentrations of PLP, but little change in PL (data not shown).

Plasma PLP was inversely associated with cancer risk (p -trend = 0.05 after adjustment for gender and age), but this

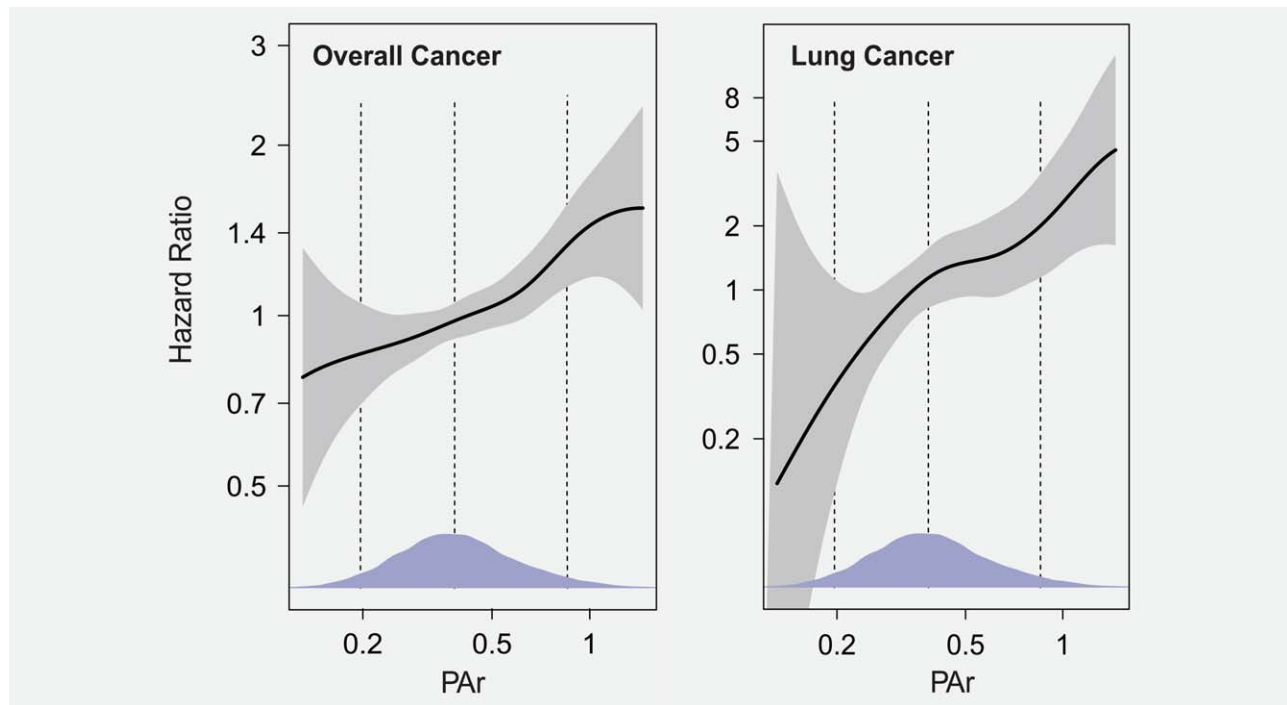


Figure 1. Dose–response relationships of plasma PAR with risk of overall cancer and lung cancer by generalized additive regression. Models were adjusted for gender, age (46–49 years vs. 70–74 years), BMI (continuous), smoking (never, former, light smokers or heavy smokers), alcohol intake (continuous), intake of vegetables and fruits (continuous) and creatinine (continuous). The black solid lines show HRs and the shaded areas show 95% CIs. Density plots show the distribution of PAR (log scale), and vertical lines denote the 5th, 50th, and 95th percentiles. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

association was not statistically significant after multiple adjustment. HR for HK/XA comparing highest vs. lowest quartiles was 1.22 (95% CI: 1.01–1.47) in a model adjusted for age and gender, but the estimate and also HR per 2SD were attenuated after multivariate adjustment (Table 2). Plasma PAR was associated with a significantly elevated risk of incident cancer (Table 2). After adjustment for gender, age, BMI, smoking, alcohol intake, intake of vegetables and fruits and creatinine, the HR for the highest vs. lowest quartile was 1.29 (95% CI: 1.04–1.61; p -trend < 0.01). Analysis of PAR as a continuous variable showed similar results (HR (95% CI) = 1.31 (1.12–1.52) per 2 SD increment). Also, we conducted a secondary analysis on PAR and overall cancer risk by additional adjustment for plasma CRP in the Cox models and observed slightly attenuated but significant results (data not shown).

Associations between PAR and the risk of main specific cancer types are shown in Table 3. The strongest association was found for lung cancer ($n = 85$) (HR (95% CI) = 2.46 (1.49–4.05) per 2 SD increment). Within the lung cancer group, we compared risk associations of PAR with PLP, HK/XA and the inflammatory markers (CRP, neopterin and KTR). Neither PLP nor HK/XA was significantly associated with lung cancer (data not shown), but a positive association was found for CRP (HR (95% CI) = 2.06 (1.20–3.53) per 2 SD increment). Similar to the analysis on overall cancer risk, analysis on PAR and lung cancer risk by additional adjustment for plasma CRP in the Cox models yielded slightly attenuated but

still significant results. The positive dose-response relations of plasma PAR with risk of overall cancer and lung cancer are shown in Figure 1, demonstrating close to linear associations through the whole distribution of PAR on a logarithmic scale.

Sensitivity analyses in which the first 2 years of follow-up were excluded showed essentially no change in risk estimates (data not shown). In addition, analysis by generalized additive models showed that the concentration of PAR was not associated with time from baseline to diagnosis (follow up) in cancer patients (Fig. 2).

No statistically significant interaction was observed between PAR and smoking status for risk of overall cancer (p interaction = 0.82) and lung cancer (p interaction = 0.14).

Discussion

Principal findings

In this community-based cohort study, we observed that high plasma PA/(PL+PLP) ratio (PAR) was associated with an increased risk of incident cancer. By comparison, the active B6 vitamers PLP and our recently proposed indicator of functional vitamin B6 status, HK/XA, were not significantly associated with overall cancer risk after multiple adjustment. The association of PAR with cancer risk was particularly strong for lung cancer.

Associations of PLP and HK/XA with cancer risk

HK/XA was negatively associated with PLP, and the magnitude of the correlation was similar to that previously reported.¹¹

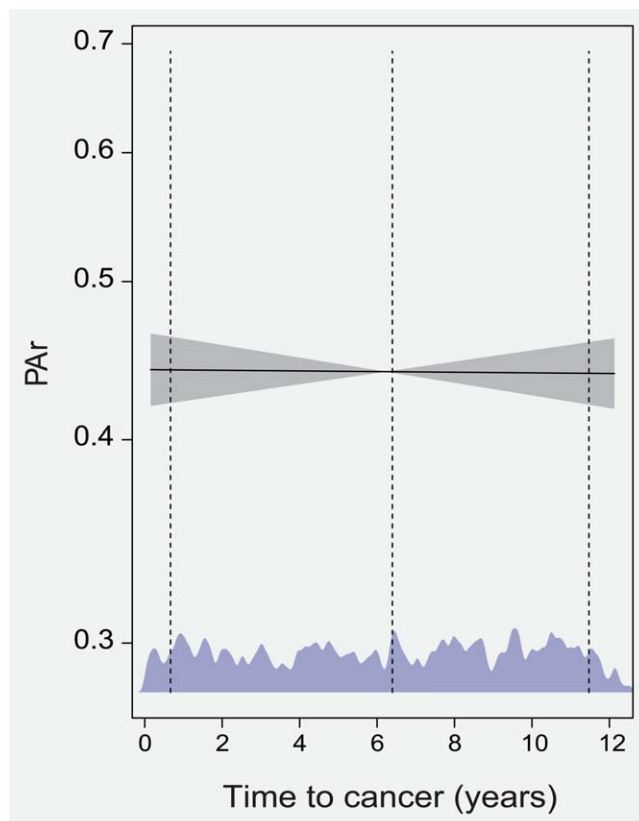


Figure 2. Plasma PAR level versus follow-up time among participants with incident cancer by generalized additive regression. Models were adjusted for age, gender, BMI, smoking, alcohol intake, intake of vegetables and fruits and creatinine. The solid lines show geometric mean of PAR and the shaded area shows 95% CIs for the slope of the PAR-association. Density plot shows the distribution of time to cancer incidence, and vertical lines denote the 5th, 50th and 95th percentiles. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Plasma PLP demonstrated a borderline negative association with cancer risk after adjustment for gender and age, which is essentially consistent with previous findings.^{1,6} After multiple adjustment, this association, and also the positive association of the functional vitamin B6 status, HK/XA, with cancer risk, were no longer significant. This demonstrates agreement between the two indicators of vitamin B6 status, but also that associations of vitamin B6 with cancer are explained through other confounding variables in the study cohort.

Possible mechanisms for the association between PAR and cancer risk

It is now well established that chronic inflammation is causally related to cancer development.^{26–28} Recent epidemiological and experimental work has shown that chronic inflammatory processes contribute to several steps of tumorigenesis.²⁸ Mechanisms involved in cancer-related inflammation may include genotoxicity/genetic instability, aberrant tissue repair, proliferative responses, invasion and metastasis,

and subversion of adaptive immunity.^{16,27} Chronic inflammation is capable of inducing oxidative and nitrosative stress, which leads to DNA mutations in cells.²⁹

Previous evidence has shown lower plasma concentrations of PLP during inflammation and in inflammatory diseases.^{4,13,14} The inverse association may reflect altered distribution of PLP from circulation to tissues affected by, or otherwise involved in inflammation.^{5,14} Suggested mechanisms include an increased recruitment of PLP cofactor for enzymes of the kynurenine pathway of tryptophan degradation, synthesis and catabolism of the immunomodulatory sphingolipids, ceramide and sphingosine 1-phosphate, and for serine hydroxymethylase to support immune cell proliferation.^{4,13} Consequently, it is possible that the previously reported association of low plasma PLP and cancer risk^{1,6–8} is related to these processes. We recently suggested that increased vitamin B6 catabolism during inflammation may be an additional cause of low plasma PLP.¹² Inflammation is accompanied by oxidative and aldehyde stress.²⁹ In response, several enzymes, including aldehyde oxidase and aldehyde hydrogenase in liver and/or extrahepatic tissues are upregulated and may be involved in the spurious oxidation of PL to PA, leading to an increase in PAR.¹² In addition, PAR may capture altered vitamin B6 distribution during the inflammatory process.

PAR by cancer sites

We found a particularly strong association between PAR and the risk of lung cancer, whereas the associations with non-lung cancer sites were weak. This is consistent with previous observations showing that lung cancer is associated with a greater activation of systemic inflammation than other cancer types.³⁰ Notably, the activity of aldehyde dehydrogenase, one of the two enzymes catalyzing PL,³¹ was found to be associated with lung adenocarcinoma stem cells.³² Among the other cancer sites examined, PAR was a stronger risk marker for colorectal than for prostate and breast cancer, reflecting again the degree of inflammatory involvement for these cancer types.³⁰

Causation and interaction with smoking

Reverse causation in the context of the present study means that the carcinogenic process may affect the plasma PAR level. If this was the case one would expect a stronger association of PAR with cancer risk, and higher PAR levels for those with a short time interval between PAR measurement and cancer diagnosis. However, two observations do not support this: First, sensitivity analyses by excluding the first 2 years of follow-up did essentially not affect the risk estimates. Second, the GAM plot showed no association of PAR with time from PAR measurement to cancer diagnosis.

The association between cancer risk and PAR could be explained by an increase in PAR caused by factors involved in carcinogenesis, such as inflammation and related processes including infection, smoking and environmental irritants.²⁸ In other words, inflammation may be the underlying cause of cancer whereas circulating PLP and PAR are only inflammatory

indicators, and have no direct role in the development of cancer. This explains why B-vitamin supplements have failed to decrease cancer risk in the randomized placebo-controlled trials,^{9,10} and support the idea of PAR as a marker of increased vitamin B6 catabolism during inflammation and immune activation.¹²

In addition, smoking is the leading risk factor for lung cancer and is associated with elevated concentrations of inflammatory markers.³³ However, the association of PAR with lung cancer risk was similar across smoking categories. This shows that PAR and smoking are essentially mutually independent risk predictors, and suggests that PAR is an indicator of other aspects of the inflammatory response than those elicited by smoking. In support of these arguments we previously reported a weak association between PAR and smoking.¹²

Strengths and limitations

Strengths mainly lie in the prospective design, and long and complete follow-up. Our study included a comprehensive panel of vitamin B6 markers and their relation to cancer risk. However, the study has some limitations. First, the findings are based on a relatively small number of cases, especially when analyzing risk for specific cancer types. The results should therefore be verified in larger cohorts. Second, a rela-

tively small proportion (3.2%) of our study population had vitamin B6 deficiency defined as plasma PLP < 20 nmol L⁻¹.³⁴ Thus, studies in other populations with a larger variability in vitamin B6 status are needed for generalizability. Third, our results could also be affected by other dietary factors, although intake of vegetables and fruits were considered. However, PAR was, unlike PL, PA and PLP, not related to intake of dietary supplements.¹²

Conclusions

While we found little evidence to support vitamin B6 status as an independent predictor of incident cancer, we are, to the best of our knowledge, the first to present evidence that increased metabolism and disposal of vitamin B6, as captured by plasma PAR, is associated with increased cancer risk. Because of the limited number of specific cancer types, future studies that include a larger number of cases are warranted to confirm these findings, and to further elucidate the role of vitamin B6 catabolism in carcinogenesis.

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