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Population and Prevention Science

Cancer Research

Results from the European Prospective Investigation into Cancer and Nutrition Link Vitamin B6 Catabolism and Lung Cancer Risk

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Abstract

Circulating pyridoxal-5′-phosphate (PLP) has been linked to lung cancer risk. The PAr index, defined as the ratio 4-pyridoxic acid/(pyridoxal + PLP), reflects increased vitamin B6 catabolism during inflammation. PAr has been defined as a marker of lung cancer risk in a prospective cohort study, but analysis of a larger numbers of cases are needed to deepen the significance of this study. Here, we conducted a nested case–control study within the European Prospective Investigation into Cancer and Nutrition (EPIC, $n = 521{,}330$), which included 892 incident lung cancer cases and 1,748 controls matched by center, gender, date of blood collection, and date of birth. The association of PAr with risk of lung cancer was evaluated by using conditional logistic regression. Study participants with elevated PAr experienced higher risk of lung cancer in a dose–response fashion, with a doubling in PAr levels associated with 52% higher odds of lung cancer after adjustment for tobacco smoking, serum cotinine levels, educational attainment, and BMI [OR, 1.52; 95% confidence interval (CI) 1.27–1.81; $P < 0.001$]. Additional adjustment for intake of vegetables and fruits and physical activity did not materially affect risk association. The association of PAr with lung cancer risk was similar in both genders but slightly stronger in former smokers and in participants diagnosed with squamous cell carcinoma. This study provides robust evidence that increased vitamin B6 catabolism is independently associated with a higher risk of future lung cancer.

Significance: This large cohort study firmly establishes an association between an index of vitamin B6 levels with lung cancer risk. *Cancer Res; 78(1); 302–8. ©2017 AACR.*

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Introduction

Vitamin B6 status has been associated with risk of and mortality from several diseases, including cardiovascular disease, cancer, diabetes, and rheumatoid arthritis (1–4). Circulating pyridoxal-5′-phosphate (PLP) is the most commonly used indicator of vitamin B6 status in clinical and epidemiological studies, and has been linked to lung cancer risk (5, 6). In two previous case–control studies, high serum levels of PLP were reported to be associated with approximately 50% reduction in lung cancer risk (highest vs. lowest quartile; refs. 6, 7). Most recently, in a consortium-based analysis of over 5,500 prospective case–control pairs from 20 prospective cohorts, the association between PLP and lung cancer risk was weaker, and primarily constrained to ever smoking men (8).

The distribution and catabolism of PLP in the body can be altered by factors other than intake, such as inflammation, serum albumin, and alkaline phosphatase levels. Therefore, the validity of circulating PLP as an indicator of vitamin B6 status in diseased populations has been questioned (2). Chronic inflammation is a common characteristic of lung cancer, and may also influence the association with vitamin B6 and the risk of lung cancer (3).

Uptake of vitamin B6 in tissues is facilitated by metabolic trapping, that is, PLP is converted to pyridoxal to facilitate cellular uptake and then rephosphorylated to PLP for retention inside the cell. In the liver, pyridoxal is irreversibly converted to the catabolite, 4-pyridoxic acid (PA; refs. 1, 2).

We recently proposed an alternative marker of vitamin B6 status and catabolism, the PA-ratio (PAr), defined as PA/(pyridoxal + PLP; ref. 9). In a multivariate model, PAr was almost exclusively associated with inflammatory markers (9). Several processes associated with inflammation are likely to contribute to a skewing of the concentrations of B6 vitamers toward more PA relative to PL+PLP, that is, an increase in PAr, as illustrated in Fig. 1 (3, 4, 9). PAr was found to be associated with all-cause mortality in two large Norwegian cohorts consisting of patients with suspected or confirmed cardiovascular disease (4). In a relatively small population-based study, we also found PAr being associated with overall cancer risk, with the highest effect estimate for lung cancer (10). To further investigate the role of the PAr index in lung cancer, we conducted a large nested case–control study within the European Prospective Investigation into Cancer and Nutrition (EPIC).

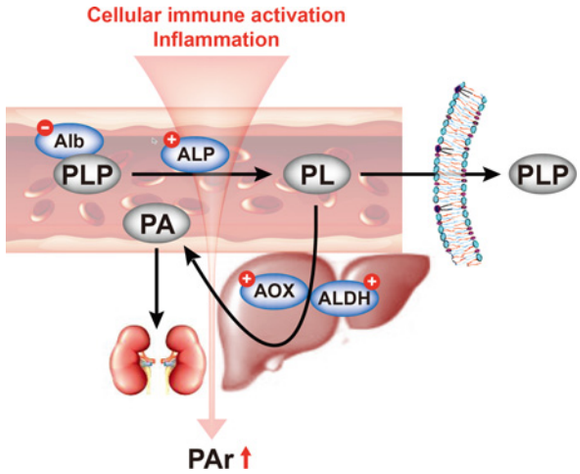

Figure 1.
Mechanisms leading to increase in PAr. Several processes may act in concert to skew the distribution of B6 vitamers in plasma toward more PA relative to PLP + pyridoxal (PL), that is, increased PAr during inflammation. The main carrier of PLP, albumin, is reduced, and increased activity of membrane-bound phosphatases, for example, alkaline phosphatase (ALP) facilitates uptake of PLP into tissue. Oxidative stress leads to upregulation of aldehyde metabolizing enzymes such as aldehyde oxidase (AOX) and aldehyde dehydrogenases (ALDH), which convert PL to PA. Finally, progressive kidney damage resulting from chronic inflammation may increase plasma PA relative to PLP + PL.

Materials and Methods

EPIC cohort

EPIC is a multicenter prospective cohort study designed to investigate the association of diet, lifestyle, and environmental factors with the incidence of cancer and other diseases. Recruitment procedures and data collection have been described in detail elsewhere (11). In brief, 521,330 individuals were recruited from 23 centers in 10 countries between 1992 and 2000, and blood samples were collected from 385,747 participants at recruitment. This study includes individuals diagnosed with lung cancer after blood collection (cases) and matched control participants, from 8 countries: France, Italy, Spain, United Kingdom, the Netherlands, Greece, Germany, and Sweden (6). The study was approved by the Institutional Review Board at the International Agency for Research on Cancer and by the local ethical committees of the respective centers.

Cases ascertainment and control selection

This study was nested within the EPIC study. Details on the selection of cases and controls have been described previously (6, 12). In brief, incident cancer cases were identified through linkage of study databases with identifying information on the participants from population cancer registries (Italy, the Netherlands, Spain, Sweden, and the United Kingdom) or by a combination of methods (France, Germany, and Greece). The combined methods included access to health insurance records, cancer and pathology registries, and active follow-up of study participants or their next of kin. Data on histology were collected from each center where possible. Subjects were followed up from study entry until cancer diagnosis (excluding nonmelanoma skin cancer), death, emigration, or the end of the follow-up period for each study center. The end of follow-up/closure dates of the study period varied between 2002 and 2005.

Lung cancer cases were defined according to the International Classification of Diseases for Oncology, Second Edition. For each incident case, two controls were selected by incidence density sampling and matched by center, gender, date of blood collection (±1 month, relaxed to ±5 months for sets without available controls), and date of birth (±1 year, relaxed to ±5 years for sets without available controls). Overall, 900 cases and 1,828 controls were included. We further excluded subjects with missing PA, pyridoxal, and PLP measurements (6 cases and 23 controls), 2 cases without matched controls, and 57 controls without matched cases. The final dataset was thus composed of 892 cases and 1,748 controls (856 cases had 2 controls each and the other 36 cases had 1 control each).

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Table 1. Baseline and clinical characteristics of the study participants

	Cases ($n = 892$)	Controls ($n = 1{,}748$)	P^a
Discrete variables, No. (%)			
Participating countries			0.99
France	24 (2.7)	48 (2.8)	
Italy	139 (15.6)	276 (15.8)	
Spain	130 (14.6)	259 (14.8)	
United Kingdom	175 (19.6)	347 (19.9)	
The Netherlands	121 (13.6)	241 (13.8)	
Greece	90 (10.1)	180 (10.3)	
Germany	157 (17.6)	304 (17.4)	
Sweden	56 (6.3)	93 (5.3)	
Sex			0.96
Men	555 (62.2)	1,086 (62.1)	
Women	337 (37.8)	662 (37.9)	
Smoking status			<0.001
Never	96 (10.8)	676 (38.7)	
Former	257 (28.8)	647 (37.0)	
Current	525 (58.9)	395 (22.6)	
Unknown	14 (1.6)	30 (1.7)	
Education			<0.001
None/primary school	458 (51.4)	752 (43.0)	
Technical/professional school	193 (21.6)	369 (21.1)	
Secondary school	109 (12.2)	238 (13.6)	
Higher education	93 (10.4)	311 (17.8)	
Unknown	39 (4.4)	78 (4.5)	
Continuous variables, median (5th–95th percentile)			
Age at blood draw (y)	59 (43–73)	59 (43–73)	0.82
BMI (kg/m^2)	25.8 (20.2–32.5)	26.4 (20.8–33.4)	<0.001
Cotinine (nmol/L)	930 (0–2354)	2.8 (0–1483)	<0.001
PLP (nmol/L)	31.7 (13.2–87.9)	40.5 (17.0–115.7)	<0.001
Pyridoxal (nmol/L)	14.1 (7.3–37.7)	16.8 (8.9–46.2)	<0.001
PA (nmol/L)	17.9 (9.2–51.1)	19.1 (9.6–62.3)	<0.001
PAr[b]	0.39 (0.20–0.80)	0.34 (0.18–0.70)	<0.001
Clinical characteristics, cases only			
Age at diagnosis, median (range; y)	64 (38–85)		
Years from blood draw to diagnosis, median (range)	5.2 (0.1–12.6)		
Histology, No. (%)			
Small-cell lung carcinoma	108 (12.1)		
Adenocarcinoma	270 (30.3)		
Large cell carcinoma	50 (5.6)		
Squamous cell carcinoma	199 (22.3)		
Other carcinoma	265 (29.7)		

Abbreviations: BMI, body mass index; PA, 4-pyridoxic acid; PLP, pyridoxal-5′-phosphate.
[a]Differences between cases and controls were analyzed by χ^2 tests (categorical variables) and Wilcoxon–Mann–Whitney tests (continuous variables).
[b]PAr, PA/(pyridoxal + PLP).

Biochemical measurement

All biochemical analyses were performed at Bevital A/S (http://www.bevital.no), Bergen, Norway. Serum concentrations of PA, pyridoxal, PLP, and cotinine were determined by LC-MS/MS (13). Cotinine, a nicotine metabolite, was used as a biomarker of recent nicotine exposure. The laboratory staff were blinded to the case–control status of the blood samples. The within-day coefficients of variation (CV) for the assays were 2.3%–4.6% and between-day CVs were 4.8%– 11.1% (13).

Statistical analysis

PAr was $\log_2$ transformed when used as a continuous variable and divided into quintiles when used as a categorical variable. Quintile cutoff points for PAr were defined on the basis of its distribution among the controls. The correlation between PAr and serum cotinine levels was assessed by partial Spearman correlation adjusted for age, sex, country, and body mass index (BMI).

Conditional logistic regression (*proc logistic* in SAS) was used to calculate ORs with 95% confidence intervals (CI) for lung cancer, conditioning on individual case sets. ORs were calculated for each PAr quintile relative to the first quintile, as well as for $\log_2$-transformed PAr as an overall test for association with the risk. The models were adjusted for smoking status (never, former, current, unknown) and serum cotinine concentrations as quartiles defined from the distribution for current smokers. Further adjustments included educational attainment (none/primary school, technical/professional school, secondary school, higher education, and unknown), and BMI (continuous; full model). Sensitivity analyses were performed to assess the consistency of results after exclusion of cancer cases diagnosed during the first 2 years of follow-up to exclude the possibility of reverse causation.

Risk analyses were also conducted in strata by sex, smoking status, years from blood draw to diagnosis, and by histology using unconditional logistic regression adjusted for the matching variables (age in 5-year categories, sex, and country) when appropriate in addition to the variables in the full model. We used χ^2 tests to examine heterogeneity in OR estimates across strata.

Nonlinear association between PAr and lung cancer risk was investigated using restricted cubic splines (14, 15) fitted to conditional logistic regression model using the SAS macro

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Table 2. ORs and 95% CIs for lung cancer risk by PAr

		OR (95% CI)		
	Cases/controls	Unadjusted[a]	Adjusted for tobacco exposure[b]	Additionally adjusted for other risk factors[c]
PAr quintile (range)				
1 (≤0.25)	125/349	1 (ref.)	1 (ref.)	1 (ref.)
2 (0.25–0.31)	125/350	1.03 (0.77–1.37)	0.81 (0.57–1.13)	0.83 (0.59–1.17)
3 (0.31–0.38)	163/350	1.37 (1.04–1.81)	1.24 (0.89–1.73)	1.25 (0.89–1.74)
4 (0.38–0.48)	198/350	1.69 (1.28–2.22)	1.37 (0.99–1.90)	1.35 (0.97–1.87)
5 (>0.48)	279/349	2.52 (1.91–3.33)	1.81 (1.30–2.52)	1.80 (1.29–2.51)
P trend		<0.001	<0.001	<0.001
PAr Continuous[d]	892/1,748	1.80 (1.56–2.09)	1.54 (1.29–1.83)	1.52 (1.27–1.81)
P		<0.001	<0.001	<0.001

Abbreviation: PAr, 4-pyridoxic acid /(pyridoxal + PLP).

[a]Assessed by conditional logistic regression, conditioning on individual case set.

[b]Assessed by conditional logistic regression, conditioning on individual case set, and adjusted for smoking status (never/former/current/missing) and serum cotinine (quartiles defined by the distribution for current smokers).

[c]Further adjusted for educational attainment (none/primary school, technical/professional school, secondary school, higher education, and unknown) and BMI (continuous).

[d]Assessed by using the base 2 logarithm of PAr.

"lgtphcurv9" (16). For tests for nonlinearity we used the likelihood ratio test, comparing the model with only the linear term to the model with the linear and the cubic spline terms (16). The test for a possible nonlinear relationship was nonsignificant, therefore, only the linear spline analysis is presented.

All statistical analyses were performed using SAS (Version 9.4; SAS Institute, Inc.). Two-sided statistical tests with $P < 0.05$ were considered as statistically significant.

Results

Baseline and clinical characteristics of the study participants are shown in Table 1. Of the individually matched cases and controls, 62% were men. The median age at blood draw was 59 years (5th–95th percentile: 43–73), and the median time from blood draw to diagnosis of lung cancer was 5.2 years. Compared with the controls, the cases had a lower BMI, a higher proportion of current smokers and lower education levels. The case group also had higher PAr and higher concentrations of cotinine, but lower concentrations of pyridoxal, PLP and PA. PAr was positively correlated with serum cotinine levels (Spearman $\rho = 0.11$, $P < 0.001$). PAr at baseline was highest in current smokers (median: 0.38; 5th–95th percentile: 0.19–0.77), followed by former smokers (0.35; 95% CI, 0.18–0.72) and never smokers (0.34; 95% CI, 0.18–0.66; $P < 0.001$).

PAr was positively associated with lung cancer risk (Table 2, highest vs. lowest quintile OR, 1.81; 95% CI, 1.30–2.52; $P_{trend} < 0.001$) after controlling for tobacco exposure. When analyzing PAr as a continuous $\log_2$-transformed variable, a doubling in PAr was associated with 1.54-fold risk of lung cancer (OR per $\log_2$ unit, 1.54; 95% CI, 1.29–1.83; $P < 0.001$). Further adjustment for educational attainment and BMI did not alter the findings (Table 2), but a slight attenuation for the overall OR estimate was seen when additionally adjusting for intake of vegetables and fruits and physical activity (OR, 1.47; 95% CI, 1.23–1.76 for $\log_2$-transformed PAr). Spline regression showed that the observed association was essentially linear (Fig. 2). The risk estimates were not reduced when cancer cases diagnosed during the first 2 years of follow-up ($n = 135$) were excluded (OR, 1.60; 95% CI, 1.31–1.94 for $\log_2$-transformed PAr).

Stratified risk analyses (Fig. 3) showed that risk estimates were similar for men and women ($P_{heterogeneity} = 0.70$). There were some indications that the risk association of PAr was stronger in former smokers ($P_{heterogeneity} = 0.07$), for cancers histologically classified as squamous cell carcinoma (SCC; $P_{heterogeneity} = 0.27$), and for cases with lung cancer diagnosis ≥2 years since blood draw ($P_{heterogeneity} = 0.06$, <2 years vs. ≥2 years).

Discussion

Principal findings

On the basis of a large European study population, we observed that study participants with elevated PAr, an indication of increased vitamin B6 catabolism, had a significantly increased risk of developing lung cancer. The association

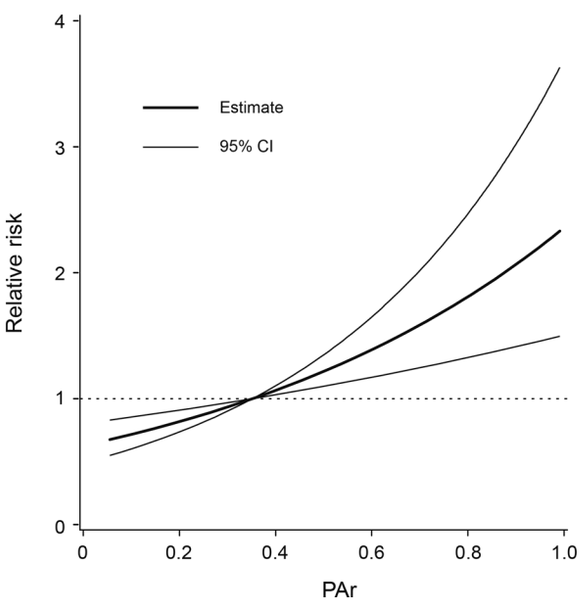

Figure 2.
Association between PAr and lung cancer in a spline regression model. The model was controlled for matching factors (age, sex, and country) and with additional adjustment for smoking status, serum cotinine, educational attainment, and BMI. The median PAr level was used as the reference. The thick solid line indicates ORs and thin lines 95% CI boundaries.

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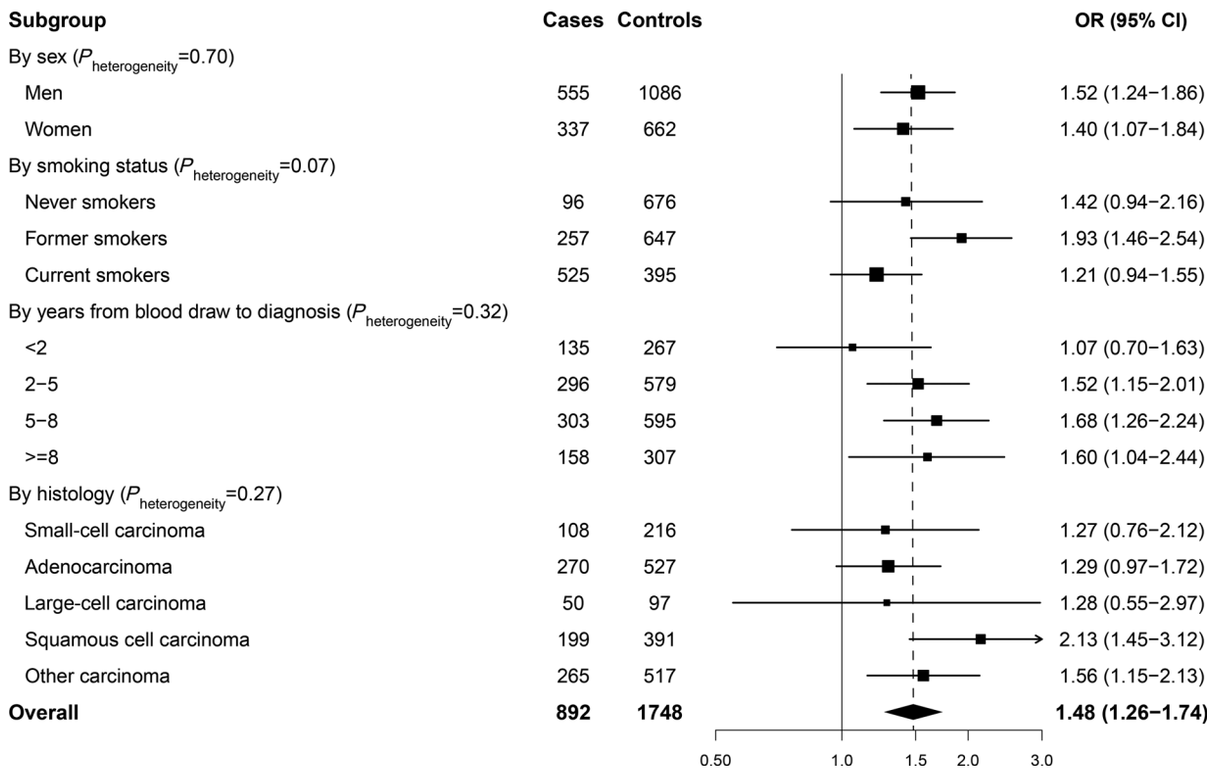

Figure 3.

Forest plot showing stratified risk estimates (ORs) of lung cancer for PAr. ORs were assessed by unconditional logistic regression by including the base 2 logarithm of PAr (ORs indicate relative risks of a doubling in serum levels) and, where relevant, adjusted for smoking status (never/former/current/ unknown), serum cotinine (quartiles defined by the distribution for current smokers), educational attainment (none/primary school, technical/professional school, secondary school, higher education, and unknown) and BMI (continuous), as well as age (in 5-year categories), sex, and country. The black boxes represent ORs and the horizontal lines indicate the 95% CIs. $P_{heterogeneity}$ indicates results of the χ^2 test assessing the null hypothesis of risk estimates being identical. The diamond represents the overall OR and 95% CIs.

between PAr and lung cancer risk was similar in both genders but appeared stronger in former smokers and for case participants with SCC.

PAr and lung cancer risk

The present study demonstrates that PAr is a fairly strong risk marker of lung cancer risk within the EPIC cohort, including 892 cases and 1748 matched controls after controlling for several potential confounders, including serum cotinine levels. This observation is in agreement with our previously reported results from a Norwegian population-based study demonstrating that PAr is associated with risk of cancer, with the strongest effect estimate for lung cancer (10). The large sample size of the current EPIC study allowed for subgroup analyses, which suggested that the risk associations were strongest for former smokers, and for SCC. These effect estimates have notable similarities with published stratified analyses within the EPIC cohort, demonstrating associations of lung cancer risk with another inflammatory biomarker, the kynurenine/tryptophan ratio (KTR), which increases during cellular immune activation (12).

Characteristics of PAr as a biomarker

The associations of both KTR (12) and PAr with lung cancer risk are consistent with the current interpretation of PAr as a marker of increased vitamin B6 catabolism during inflammation (3, 9). PAr seems to reflect both cellular immune activation (as measured by KTR and neopterin) and inflammatory processes associated with C-reactive protein (9). In the previous study, PAr was little influenced by renal function, smoking, and vitamin B6 intake, and had higher within-subject reproducibility (with an intraclass correlation coefficient of 0.75) compared with the individual B6 vitamers (9). These features demonstrate the advantages of using biomarkers based on metabolite ratios, a strategy that attenuates the influence of common confounders that affect the individual components, thereby increasing performance characteristics.

Lung cancer and inflammation

The association of PAr with lung cancer risk observed in this and a previous study (10) is in line with the consistent observations in epidemiologic studies of a positive association of proinflammatory cytokines and inflammatory biomarkers with risk of subsequent lung cancer (17–20). A stronger association with lung cancer compared with other cancer variants, as shown previously (10), is also in agreement with a generally stronger activation of systemic inflammation in lung cancer compared with other cancer types (21).

The association of lung cancer risk with several inflammatory cytokines was particularly strong in former smokers and for SCC (20), and in these respects resembles the risk predicted by PAr. Smoking, in particular, causes pulmonary inflammation thereby promoting lung cancer development (22), and among the non–

small cell carcinomas, SCC is the most common cancer types in smokers (23).

Possible mechanisms

Mechanisms behind cancer development during inflammation are complex, and often involve nuclear factor-κB (NF-κB)-IL-6-STAT3 signaling (24). Mechanisms specifically related to vitamin B6 metabolism or function are related to increased oxidative and aldehyde stress resulting in upregulation of aldehyde scavenging enzymes, which includes enzymes that catalyze the conversion of pyridoxal to PA. Concomitantly, recruitment of PLP to inflammatory pathways may lead to increased removal of PLP from plasma and distribution into affected organs for utility in diverse metabolic pathways (4, 25). One such pathway is the kynurenine pathway, which is PLP-dependent at several steps, produces a number of metabolite intermediates with immunomodulating effects (3), and also provides important cofactors for stress relief under oxidative and anoxic conditions (26).

Strengths and limitations

Important strengths of our study include the multicenter design, large sample size, and analysis of all serum samples in the same laboratory. The study participants were from 8 European countries with extensive geographic diversity, with a consequent large variation in the exposure to risk factors and confounders across populations. Another considerable strength is the prospective, nested case–control design, which minimizes selection bias. Notably, stratified analysis showed that the association between PAr and lung cancer risk was, if anything, stronger for those who received a cancer diagnosis >2 years after blood draw, suggesting that reverse causation is unlikely to explain the results. In addition, several potential confounders, including objectively measured recent nicotine exposure were adjusted for. However, regression dilution bias (27) may exist because B6 vitamers were measured on a single blood sample, which may on the other hand underestimate the associations.

Conclusions

Elevated PAr reflecting increased vitamin B6 catabolism linked to cellular inflammation, aldehyde, and oxidative stress, was associated with an increased risk of lung cancer in EPIC. The present study is in line with our previous finding in a Norwegian general population cohort (10). The present work adds to a growing body of evidence that inflammatory modes as reflected by increased PAr are involved in a spectrum of inflammatory related diseases ranging from subtypes of cancer to cardiovascular disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Conception and design: H. Zuo, P.M. Ueland, G.S. Tell, H. Boeing, M.M. Bergmann, H.B. Bueno-de-Mesquita, P.H. Peeters, M.-J. Sanchez, E. Ardanaz, P. Brennan, M. Johansson, A. Ulvik

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References

1. Galluzzi L, Vacchelli E, Michels J, Garcia P, Kepp O, Senovilla L, et al. Effects of vitamin B6 metabolism on oncogenesis, tumor progression and therapeutic responses. Oncogene 2013;32:4995–5004.

2. Ueland PM, Ulvik A, Rios-Avila L, Midttun O, Gregory JF. Direct and Functional biomarkers of vitamin B6 status. Annu Rev Nutr 2015; 35:33–70.

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3. Ueland PM, McCann A, Midttun O, Ulvik A. Inflammation, vitamin B6 and related pathways. Mol Aspects Med 2017;53:10–27.
4. Ulvik A, Pedersen ER, Svingen GF, McCann A, Midttun O, Nygard O, et al. Vitamin B-6 catabolism and long-term mortality risk in patients with coronary artery disease. Am J Clin Nutr 2016;103:1417–25.
5. Hartman TJ, Woodson K, Stolzenberg-Solomon R, Virtamo J, Selhub J, Barrett MJ, et al. Association of the B-vitamins pyridoxal 5′-phosphate (B(6)), B(12), and folate with lung cancer risk in older men. Am J Epidemiol 2001;153:688–94.
6. Johansson M, Relton C, Ueland PM, Vollset SE, Midttun O, Nygard O, et al. Serum B vitamin levels and risk of lung cancer. JAMA 2010;303:2377–85.
7. Takata Y, Cai Q, Beeghly-Fadiel A, Li H, Shrubsole MJ, Ji BT, et al. Dietary B vitamin and methionine intakes and lung cancer risk among female never smokers in China. Cancer Causes Control 2012;23:1965–75.
8. Fanidi A, Muller D, Yuan J-M, Stevens VL, Weinstein SJ, Albanes D, et al. Circulating folate, vitamin B6, and methionine in relation to lung cancer risk in the lung cancer cohort consortium (LC3) J Natl Cancer Inst 2018:110.
9. Ulvik A, Midttun O, Pedersen ER, Eussen SJ, Nygard O, Ueland PM. Evidence for increased catabolism of vitamin B-6 during systemic inflammation. Am J Clin Nutr 2014;100:250–5.
10. Zuo H, Ueland PM, Eussen SJ, Tell GS, Vollset SE, Nygard O, et al. Markers of vitamin B6 status and metabolism as predictors of incident cancer: the Hordaland Health Study. Int J Cancer 2015;136:2932–9.
11. Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr 2002;5:1113–24.
12. Chuang SC, Fanidi A, Ueland PM, Relton C, Midttun O, Vollset SE, et al. Circulating biomarkers of tryptophan and the kynurenine pathway and lung cancer risk. Cancer Epidemiol Biomarkers Prev 2014;23:461–8.
13. Midttun O, Hustad S, Ueland PM. Quantitative profiling of biomarkers related to B-vitamin status, tryptophan metabolism and inflammation in human plasma by liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom 2009;23:1371–9.
14. Durrleman S, Simon R. Flexible regression models with cubic splines. Stat Med 1989;8:551–61.
15. Heinzl H, Kaider A. Gaining more flexibility in Cox proportional hazards regression models with cubic spline functions. Comput Methods Programs Biomed 1997;54:201–8.
16. Li R, Hertzmark E, Louie M, Chen L, Spiegelman D. The SAS LGTPHCURV9 Macro. 2011. https://www.hsph.harvard.edu/donna-spiegelman/software/lgtphcurv9/
17. Zhou B, Liu J, Wang Z-M, Xi T. C-reactive protein, interleukin 6 and lung cancer risk: a meta-analysis. PLoS ONE 2012;7:e43075.
18. Pine SR, Mechanic LE, Enewold L, Chaturvedi AK, Katki HA, Zheng YL, et al. Increased levels of circulating interleukin 6, interleukin 8, C-reactive protein, and risk of lung cancer. J Natl Cancer Inst 2011;103:1112–22.
19. Shiels MS, Pfeiffer RM, Hildesheim A, Engels EA, Kemp TJ, Park JH, et al. Circulating inflammation markers and prospective risk for lung cancer. J Natl Cancer Inst 2013;105:1871–80.
20. Brenner DR, Fanidi A, Grankvist K, Muller DC, Brennan P, Manjer J, et al. Inflammatory cytokines and lung cancer risk in 3 prospective studies. Am J Epidemiol 2017;185:86–95.
21. Proctor MJ, Talwar D, Balmar SM, O'Reilly DS, Foulis AK, Horgan PG, et al. The relationship between the presence and site of cancer, an inflammation-based prognostic score and biochemical parameters. Initial results of the Glasgow Inflammation Outcome Study. Br J Cancer 2010;103:870–6.
22. Smith CJ, Perfetti TA, King JA. Perspectives on pulmonary inflammation and lung cancer risk in cigarette smokers. Inhal Toxicol 2006;18:667–77.
23. Jedrychowski W, Becher H, Wahrendorf J, Basa-Cierpialek Z, Gomola K. Effect of tobacco smoking on various histological types of lung cancer. Lung Cancer 1992;118:276–82.
24. Elinav E, Nowarski R, Thaiss CA, Hu B, Jin C, Flavell RA. Inflammation-induced cancer: crosstalk between tumours, immune cells and microorganisms. Nat Rev Cancer 2013;13:759–71.
25. Paul L, Ueland PM, Selhub J. Mechanistic perspective on the relationship between pyridoxal 5′-phosphate and inflammation. Nutr Rev 2013;71:239–44.
26. Moffett JR, Namboodiri MAA. Tryptophan and the immune response. Immunol Cell Biol 2003;81:247–65.
27. Hutcheon JA, Chiolero A, Hanley JA. Random measurement error and regression dilution bias. BMJ 2010;340:c2289.

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