Cancer Causes Control (2016) 27:1447–1456
DOI 10.1007/s10552-016-0822-6

ORIGINAL PAPER

Serum B_6 vitamers (pyridoxal 5′-phosphate, pyridoxal, and 4-pyridoxic acid) and pancreatic cancer risk: two nested case–control studies in Asian populations

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Received: 14 July 2016 / Accepted: 21 October 2016 / Published online: 9 November 2016

Abstract

Background Vitamin B_6 is an important enzymatic cofactor in pathways relevant for the development of pancreatic cancer. In order to evaluate vitamin B_6 as a preventive factor for pancreatic cancer, a biomarker approach is needed to overcome the limitations inherent in self-reported dietary information.

Methods To determine whether levels of serum B_6 vitamers, including pyridoxal 5′-phosphate (PLP), pyridoxal (PL), 4-pyridoxic acid (PA), and the PA/(PLP + PL) ratio (PAr), were associated with risk of pancreatic cancer, two nested case–control studies of 187 incident pancreatic cancer cases and 258 individually matched controls were conducted within two prospective cohorts of 81,501 participants in Shanghai, China, and Singapore. PLP, PL, and PA were quantified in pre-diagnostic serum samples. Odds ratios and 95% confidence intervals (CIs) were calculated using conditional logistic regression with adjustment for potential confounders.

Results The median (5th–95th percentiles) concentrations of serum PLP among control subjects of the Shanghai and Singapore cohorts were 25.7 (10.0–91.7) nmol/L and 58.1 (20.8–563.0) nmol/L, respectively. In pooled analyses, high serum PLP was associated with a reduced risk of pancreatic cancer (P for trend = 0.048); the adjusted odds ratio for the highest category of PLP (>52.4 nmol/L) was 0.46 (95% CI 0.23, 0.92) compared to vitamin B_6 deficiency (<20 nmol/L). No associations were found for serum PL, PA, or PAr with pancreatic cancer risk.

Conclusions Higher concentrations of PLP may protect against the development of pancreatic cancer. The protective effect may be more apparent in populations with low concentrations of circulating vitamin B_6.

Keywords Biomarker · Case–control studies · Cohort studies · Epidemiology · Pancreatic cancer · Pyridoxal 5′-phosphate · Risk factors · Vitamin B_6

Electronic supplementary material The online version of this article (doi:10.1007/s10552-016-0822-6) contains supplementary material, which is available to authorized users.

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Introduction

Pancreatic cancer is the seventh leading cause of cancer-related death in the world, with an estimated 331,000 deaths due to pancreatic cancer in 2012 [1]. Cigarette smoking and excess body fatness are of the few

established modifiable risk factors for pancreatic cancer [2, 3], and studies are needed to identify novel targets of primary prevention for pancreatic cancer. Consumption of fruits and vegetables has been associated with reduced risk of pancreatic cancer in some epidemiological studies [4, 5]. However, the associations between specific nutrients and pancreatic cancer risk have not been well studied.

Vitamin B_6 is present in a wide variety of foods such as beef liver, tuna, and bananas [6]. We recently reported an inverse association between dietary intake of vitamin B_6 and risk of pancreatic cancer in a prospective cohort of Chinese in Singapore; there was a 48% reduction in risk of developing pancreatic cancer associated with the highest (>1.21 mg/day) versus lowest (<0.96 mg/day) quartile of vitamin B_6 intake [7]. However, similar studies in populations with higher intake (i.e., Europe and USA) did not observe inverse associations [i.e., highest (≥2.22–2.81 mg/day) versus lowest quartiles (<1.77–2.09 mg/day)] [8–10]. It is possible that the etiologically relevant range of intake was not captured in the European and US populations, or the discrepancies could be due to the inherent limitation of measurement error associated with assessing dietary vitamin B_6 intake from food frequency questionnaires. A biomarker approach for vitamin B_6 and its related metabolites in bodily fluid would overcome the limitation of relying on self-reported diet, and it would provide insights on the potential role of the various B_6 vitamers in the development of pancreatic cancer.

Pyridoxal 5′-phosphate (PLP), the metabolically active form of vitamin B_6, is a coenzyme in the synthesis of nucleic acids, amino acids, and cellular antioxidants [11]. PLP accounts for most of the total vitamin B_6 in the circulation and is commonly used as a primary measure of whole-body vitamin B_6 status [12]. Besides PLP, other major forms of vitamin B_6 in the circulation in humans include pyridoxal (PL) and 4-pyridoxic acid (PA) [13]. The ratio of PA to the sum of PLP and PL (PAr) is speculated as a marker of increased vitamin B_6 catabolism during inflammation [14]. Recently, PAr has been shown to be positively associated with several inflammatory markers [14], thus suggesting PAr may be a biomarker for pancreatic cancer risk [15]. Given the inconsistent results on the relationship between circulating PLP concentration and pancreatic cancer risk [16–18], we conducted a comprehensive assessment of the individual B_6 vitamer levels, as well as PAr in relation to pancreatic cancer risk in two prospective cohorts of Asians in order to clarify the potential role of vitamin B_6 in pancreatic cancer development.

Materials and methods

Study subjects

The design of the Shanghai Cohort Study has been described in detail elsewhere [19]. Briefly, 18,244 men aged 45–64 years in Shanghai, China, were enrolled between 1986 and 1989. At the time of recruitment, all participants were interviewed in person by a trained nurse using a structured questionnaire that asked for information on demographics, height, weight, use of tobacco and alcohol, and medical history. In addition, each study participant provided a non-fasting blood samples and a spot urine sample following the interview. All collected biospecimens were kept on ice (at around 4 °C) before they were processed, and aliquots of serum and urine specimens have been stored at −80 °C until laboratory analysis.

The design of the Singapore Chinese Health Study has been described in detail elsewhere [20]. Briefly, 63,257 Chinese men and women aged 45–74 years in Singapore were enrolled between 1993 and 1998. At the time of recruitment, all participants were interviewed in person using a structured questionnaire including sections of background information, occupational exposure, physical activity, and family history of cancer and provided information on height, weight, use of tobacco and alcohol, dietary supplemental use, and medical history. Information on habitual diet was collected using a validated 165-item food frequency questionnaire [21]. Daily intake of nutrients including vitamin B_6 was calculated using the nutrient content information from the Singapore Food Composition Database [21]. Non-fasting blood samples and spot urine samples were collected from a 3% random sample of cohort members between 1994 and 1999 and extended to all surviving cohort members between 2000 and 2005. By April 2005, blood and/or urine specimens were collected from 32,543 participants, representing a consent rate of 60%. Serum and urine specimens were kept in insulated boxes with ice (4 °C) until processing and stored at −80 °C. For Singapore subjects, blood sample was collected on average 6.5 (range 1.2–11.0) years after the baseline interview. Follow-up I interview ($n = 52,322$) was administered during 1999–2003, and the consent rate reached over 90% among surviving cohort members. Since the status of smoking and diabetes may change over time, the information on smoking and diabetes was derived mainly from follow-up I interview (98%), which was administered on average 8 months before blood draw, supplemented by baseline interview (2%). A validation study of the incident diabetes cases in the Singapore cohort observed that 99% of individuals who reported a history of diabetes were considered valid cases [22]. Another study

analyzed percentage of hemoglobin A1c (HbA1c) (glycated hemoglobin) among individuals who reported no history of diabetes at baseline and follow-up interview and observed 94.4% of those individuals were below the HbA1c threshold for diabetes [23]. Other demographic and lifestyle factors used were derived from the baseline interview only.

Written informed consent was obtained from all participants. The study was approved by the Institutional Review Boards of the Shanghai Cancer Institute, the National University of Singapore, and the University of Pittsburgh.

Case ascertainment and control selection

In the Shanghai cohort, all surviving cohort participants were re-contacted annually and interviewed in person to update the information on selected lifestyle factors and medical history. As of the most recent follow-up in 2015, 3.7% of original cohort participants were lost to the follow-up interview and 3.3% declined the continued follow-up interview. The incident cancer cases and deaths among cohort participants were identified through annual re-contacts of surviving study participants or next of kin for deceased participants, and through record linkage analyses with the databases of the population-based Shanghai Cancer Registry and the Shanghai Municipal Vital Statistics Office. The diagnosis of all incident cancer cases was confirmed via review of medical records. As of 31 December 2009, the cutoff date for the present study, 129 incident cases of pancreatic cancer [International Classification of Disease (ICD)-9 code, 157] were identified among participants of the Shanghai cohort.

In the Singapore cohort, <1% of original cohort members were lost to follow-up due to their migration out of Singapore. The incident cancer cases and deaths among cohort members of the Singapore cohort were identified through routine record linkage with databases of the Singapore National Birth and Death Registry and National Cancer Registry [24]. As of 31 December 2013, 58 incident pancreatic cancer cases (ICD-Oncology code, C25) were identified among participants of the Singapore cohort who had available serum samples.

For each case, two control subjects were randomly selected among all eligible participants who were free of cancer at the time of cancer diagnosis of the index case within the same cohort. To be consistent with the matching criteria used in previous nested case–control studies in the Shanghai cohort, controls were matched to the index case on date of birth (±2 years), date of biospecimen collection (±1 month), and neighborhood of residence at time of enrollment [25]. In the Singapore cohort, cases and controls were matched on age at baseline interview (±3 years), date of baseline interview (±2 years), gender, dialect group (Cantonese, Hokkien), and date of biospecimen collection (±6 months).

Assessment of serum biomarkers

For each subject, 60 μL serum was pulled from the biorepository. Serum PLP, PL, PA, and creatinine were measured by liquid chromatography–tandem mass spectrometers (LC–MS/MS) using the methods described previously [26]. All biochemical analyses were performed at Bevital A/S (www.bevital.no) at Bergen, Norway. Serum specimens of cases and their matched controls were processed, aliquoted, shipped in insulated boxes with dry ice, and assayed together in the same batch. Laboratory technicians were blinded about case–control status of the test samples. For quality control purposes, 14 duplicated samples (2% of testing samples) from a pooled serum sample collected from potential study subjects for the Shanghai Cohort Study but later determined ineligible were included in seven batches (two duplicated samples per batch). The within-batch coefficients of variation (CVs) for PLP, PL, PA, and creatinine were 3.3, 7.5, 6.0, and 3.3%, respectively. The corresponding between-batch CVs were 7.9, 8.1, 7.5, and 4.0%.

Statistical analysis

PAr was calculated by dividing serum concentrations of PA by the sum of PL and PLP. We logarithmically transformed original values of PLP, PL, PA, and PAr to normalize their skewed distributions toward high values. Pairwise correlations between biomarkers of PLP, PL, PA, and PAr were evaluated using Spearman correlation coefficients. The differences in concentrations of PLP, PL, PA, and the value of PAr between different categories of baseline demographic characteristics and lifestyle factors were evaluated using analysis of covariance (ANCOVA).

Conditional logistic regression [27] was used to calculate odds ratios (ORs) and their 95% confidence intervals (CIs) of pancreatic cancer associated with higher categories of PLP, PL, PA, and PAr, comparing with the lowest category. In the primary analysis of both cohorts combined, we used <20 nmol/L PLP as the lowest (i.e., reference) category for OR because it has been suggested as a cut point for vitamin B_6 deficiency [28], and divided the remaining total subjects into equal tertiles based on the distribution of PLP among controls of both cohorts. For PL, PA, and PAr, study subjects were divided into quartiles based on the distribution of individual biomarkers among total controls. In cohort-specific analysis, quartiles of PLP, PL, PA, and PAr were derived from their distributions among controls within each cohort. The potential

modifying effect of study location on the biomarker–pancreatic cancer risk association was assessed by including an interaction term between a biomarker and study location (Shanghai versus Singapore) in the regression models. Ordinal values (e.g., 1, 2, 3, and 4) for each of biomarkers were used for testing linear trend in the biomarker–pancreatic cancer risk association.

The multivariable logistic regression models included following reported risk factors for pancreatic cancer as potential confounders: body mass index (BMI) (<18.5, 18.5 to <23, ≥23), level of education (no formal schooling, primary school, secondary school, and above), smoking status (never smokers, former smokers, current smokers), alcohol consumption (number of drinks per day), history of physician-diagnosed diabetes (no, yes), and study site (Shanghai, Singapore). Given the impact of renal clearance on PA [13], we further adjusted for estimated glomerular filtration rate (eGFR) [29] in the analysis for the association between PA, PAr, and pancreatic cancer risk.

To minimize the potential residual confounding of diabetes, we conducted a sensitivity analysis by excluding subjects who reported a history of diabetes. In addition, to reduce the potential effect of disease progression on diminishing circulating B_6 vitamers, we conducted separate analysis after excluding cases diagnosed within 2 years after blood draw and their matched controls.

Statistical analyses were carried out using SAS software version 9.3 (SAS Institute, Cary, NC) All p values reported are two-sided, and those that were <0.05 were considered to be statistically significant.

Results

The mean age at pancreatic cancer diagnosis was 69.0 and 71.7 years in the Shanghai and Singapore cohorts, respectively. The average (range) time between blood draw and cancer diagnosis was 12.5 years (3 months to 23.2 years) for cases of the Shanghai cohort and 6.8 (5 months to 13.0 years) for cases of the Singapore cohort. Patients who developed pancreatic cancer were more likely to smoke cigarettes at baseline in the Shanghai cohort, whereas the distributions of smoking status between cases and controls in the Singapore cohort were comparable (Table 1). Overall circulating mean levels of PLP, PL, and PA were 20–56% lower in controls of the Shanghai cohort than those of the Singapore cohort. Compared with controls, patients who developed pancreatic cancer had lower serum levels of PLP and PL at baseline in the Shanghai cohort and similar levels in the Singapore cohort. No difference in PA and PAr between cases and controls was seen in both cohorts. Serum concentrations of PLP, PL, and PA were highly correlated with each other in the study population (Supplemental Table S1, Online Resource).

Current smokers showed the lowest concentrations of serum PLP, PL, and PA among controls of both cohorts, whereas smoking status was not associated with PAr (Table 2). Alcohol intake was inversely associated with PAr in the Shanghai study controls. In Singapore cohort, controls who reported use of any vitamins or minerals showed a markedly increase in concentrations of PLP, PL, and PA, and PAr compared to nonusers. PAr was higher in diabetic patients than non-diabetics in both cohorts. Lower renal function (i.e., low eGFR) was associated with higher levels of PL, PA, and PAr in both cohorts.

High levels of PLP were associated with reduced risk of pancreatic cancer (Table 3). Compared with PLP <20 nmol/L, subjects with PLP >52.4 nmol/L at baseline had a 59% reduced risk of developing pancreatic cancer. Adjustment for level of education, BMI, cigarette smoking, alcohol intake, and history of diabetes slightly attenuated the association with PLP (Table 3). Circulating PL and PA levels were inversely associated with pancreatic cancer risk (both P trend values ≥0.06), and these associations were further attenuated with the adjustment for potential confounders. No association between PAr and pancreatic cancer risk was observed. In cohort-specific analysis, we used quartile levels of all B_6 vitamers for the risk association analysis because there were very few subjects with PLP <20 nmol/L in the Singapore cohort (Supplemental Table S2, Online Resource). The inverse association between PLP and risk of pancreatic cancer was stronger in the Shanghai cohort ($p = 0.01$) compared with the Singapore cohort ($p = 0.58$) (Supplemental Table S3, Online Resource). There was no evidence for associations between other biomarkers of vitamin B_6 and pancreatic cancer in either cohort. There was no modifying effect of study location on the associations between PLP, PL, PA, or PAr and risk of pancreatic cancer. The p values for interaction between B_6 vitamers or PAr and study site were >0.46.

Excluding cases and controls with a history of diabetes (7 cases and 14 controls), the inverse association between serum PLP and pancreatic cancer risk remained; the multivariable-adjusted ORs (95% CIs) for the 2nd, 3rd, and 4th quartile of PLP were 0.70 (0.41–1.20), 0.72 (0.4–1.29), and 0.45 (0.22–0.93), respectively, compared with <20 nmol/L (P for trend = 0.048). Excluding cases ($n = 13$) whose blood samples were collected within 2 years prior to pancreatic cancer diagnosis and their matched controls ($n = 26$) did not appreciably change the association with PLP. The multivariable-adjusted OR of pancreatic cancer for PLP >52.4 nmol/L relative to PLP <20 nmol/L was 0.45 (0.22–0.94) (P trend = 0.056). No association of PL, PA, or PAr with pancreatic cancer risk was found.

Table 1 Baseline demographic characteristics and lifestyle factors of pancreatic cancer cases and control subjects, the Shanghai Cohort Study (Shanghai) and the Singapore Chinese Health Study (Singapore)

Characteristic	Shanghai cohort			Singapore cohort		
	Controls	Cases	p^a	Controls	Cases	p^a
n	258	129		104	58	
Age at interview, mean (SD), years	56.4 (5.5)	56.5 (5.5)	0.74	57.1 (7.2)	57.9 (7.5)	0.51
Age at blood draw, mean (SD), years	56.4 (5.5)	56.5 (5.5)	0.74	64.0 (7.1)	64.9 (7.6)	0.47
BMI, mean (SD), kg/m^2	21.9 (2.8)	22.5 (3.0)	0.08	23.1 (3.2)	23.2 (3.6)	0.79
Female (%)	0	0		39.4	39.7	0.98
Education level (%)			0.36			0.42
No formal schooling	5.0	2.3		20.2	12.1	
Primary school	28.7	26.4		43.3	48.3	
Secondary school or above	66.3	71.3		36.5	39.7	
Smoking status (%)			0.003			0.87
Never	43.8	27.1		60.6	58.6	
Former	6.2	4.7		22.1	20.7	
Current	50.0	68.2		17.3	20.7	
Alcohol intake, drinks/week (%)			0.74			0.61
0	56.6	54.3		82.7	87.9	
<7	11.2	14.0		10.6	8.6	
≥7	32.2	31.8		6.7	3.5	
Diabetes (%)			0.52			0.88
No	98.5	99.2		90.4	89.7	
Yes	1.55	0.78		9.6	10.3	
Weekly use of any vitamins or minerals (%)		–			0.69	
No	–	–		89.4	91.4	
Yes	–	–		10.6	8.62	
Serum biomarker concentrations (median, 5th–95th)						
PLP (nmol/L)	25.7 (10.0–91.7)	21.7 (8.9–60.0)	0.01	58.1 (20.8–563.0)	50.6 (23.8–465.0)	0.29
PL (nmol/L)	15.0 (15.0–55.0)	14.0 (6.8–34.8)	0.03	20.0 (8.4–2680.0)	21.1 (8.2–3960.0)	0.84
PA (nmol/L)	10.9 (4.6–64.2)	9.6 (4.0–28.4)	0.11	21.6 (9.9–1727.0)	22.8 (9.1–2887.0)	0.82
PAr	0.28 (0.11–0.62)	0.30 (0.12–0.55)	0.68	0.32 (0.14–0.85)	0.31 (0.17–1.02)	0.86
eGFR (mL/min/1.73 m^2)	92.7 (65.7–106.5)	93.3 (70.6–106.3)	0.33	77.2 (47.6–99.8)	76.8 (44.2–106.9)	0.82

eGFR estimated glomerular filtration rate (<60, moderate to severe renal function loss; 60–89, mild renal function loss; ≥90, normal renal function), *PLP* pyridoxal 5′-phosphate, *PL* pyridoxal (PL), *PA* 4-pyridoxic acid, *PAr* PA/(PLP + PL) ratio

[a] Two-sided *p* values were based on t test for normally distributed continuous variables, Mann–Whitney *U* test for non-normally distributed continuous variables, or Chi-square test for categorical variable

Discussion

The present study demonstrated that higher concentrations of PLP in serum collected many years before cancer diagnosis were associated with reduced risk of developing pancreatic cancer in two prospective cohorts of Chinese populations. Compared with vitamin B_6-deficient individuals, participants with PLP at the highest quartile (>52.4 nmol/L) had a 54% reduced risk of pancreatic cancer. These results supported an inverse association between dietary intake of vitamin B_6 and pancreatic cancer risk that we reported previously in the Singapore cohort [7] and suggested that vitamin B_6 may play a protective role in the development of pancreatic cancer. The present study did not demonstrate an association for serum levels of PL, PA, and PAr with risk of pancreatic cancer.

Table 2 Geometric means of serum pyridoxal 5′-phosphate (PLP), pyridoxal (PL), 4-pyridoxic acid (PA), and PA/(PLP + PA) ratio (PAr) in relation to demographic characteristics and lifestyle factors among control subjects, the Shanghai Cohort Study and the Singapore Chinese Health Study

	Shanghai cohort					Singapore cohort				
	n	PLP (nmol/L)	PL (nmol/L)	PA (nmol/L)	PAr	*n*	PLP (nmol/L)	PL (nmol/L)	PA (nmol/L)	PAr
	258	26.4	16.7	11.9	0.26	104	69.2	36.3	39.1	0.32
Smoking status										
Never	113	32.3	18.8	14.4	0.28	63	81.8	45.3	48.0	0.32
Former	16	25.0	18.5	11.6	0.26	23	63.9	36.8	38.4	0.34
Current	129	22.3	14.8	10.0	0.26	18	42.6	16.4	19.6	0.32
p value		<0.0001	0.02	0.002	0.51		0.005	0.03	0.04	0.90
Among current smokers										
Cigarettes/day										
≤12	58	24.3	16.9	10.8	0.26	8	41.9	12.2	18.2	0.34
13–22	63	21.3	13.3	9.5	0.26	8	42.2	21.8	21.9	0.32
≥23	8	17.8	13.3	9.2	0.30	2	47.3	16.5	17.2	0.26
P trend		0.09	0.03	0.27	0.36		0.77	0.23	0.84	0.65
Age at blood draw, year[a]										
45–<55	106	28.5	16.5	10.9	0.24	11	64.8	23.1	22.8	0.26
55–<60	67	27.0	17.2	11.7	0.26	21	63.0	29.1	27.0	0.26
60–<65	85	23.6	16.4	13.3	0.32	22	68.1	32.8	35.8	0.32
≥65	0	–	–	–	–	50	73.5	45.9	53.6	0.40
P trend		0.046	0.97	0.09	<.0001		0.49	0.14	0.03	<0.001
BMI[a,b] (kg/m^2)										
<18.5	23	23.2	14.8	10.3	0.26	7	49.0	15.2	21.2	0.32
18.5–<23.0	147	25.3	16.5	11.7	0.28	43	69.7	38.9	40.7	0.34
≥23.0[†]	88	29.3	17.5	12.7	0.26	54	71.9	38.4	41.1	0.32
P trend		0.053	0.27	0.24	0.84		0.41	0.39	0.48	0.83
Level of education[a]										
No formal schooling	13	20.3	12.9	9.4	0.28	21	72.4	44.4	44.8	0.32
Primary school	74	23.8	16.1	11.1	0.28	45	61.2	26.5	30.6	0.32
≥Secondary	171	28.2	17.3	12.4	0.26	38	78.0	47.1	48.6	0.34
P trend		0.02	0.13	0.14	0.75		0.57	0.65	0.64	0.61
Alcohol intake, drinks/week[a]										
0	146	25.9	15.7	12.6	0.30	86	70.3	37.4	41.1	0.34
<7	29	28.9	18.5	11.4	0.24	11	62.8	30.2	29.7	0.28
≥7	83	26.5	18.0	10.9	0.24	7	66.0	32.7	33.3	0.32
P trend		0.77	0.12	0.18	0.001		0.73	0.73	0.56	0.52
Weekly use of any vitamins or minerals[a]										
No	–	–	–	–	–	93	63.0	30.1	32.3	0.32
Yes	–	–	–	–	–	11	152.2	173.3	196.9	0.52
p value		–	–	–	–		0.001	<0.001	<0.001	0.005
Diabetes[a]										
No	254	26.2	16.5	11.6	0.26	94	74.5	40.5	41.5	0.32
Yes	4	41.4	28.6	40.4	0.58	10	34.4	12.7	22.6	0.48
p value		0.16	0.10	0.001	0.003		0.006	0.04	0.23	0.03
eGFR[a,c]										
<60	4	36.1	21.9	21.5	0.36	15	82.7	49.1	90.3	0.46
60–89	102	27.3	17.9	14.2	0.30	60	69.0	39.6	42.7	0.36

Table 2 continued

	Shanghai cohort					Singapore cohort				
	n	PLP (nmol/L)	PL (nmol/L)	PA (nmol/L)	PAr	*n*	PLP (nmol/L)	PL (nmol/L)	PA (nmol/L)	PAr
≥90	152	25.6	15.8	10.4	0.24	29	63.5	25.8	21.2	0.24
P trend		0.22	0.03	<0.001	<0.001		0.24	0.03	0.002	<0.001

eGFR estimated glomerular filtration rate (<60, moderate to severe renal function loss; 60–89, mild renal function loss; ≥90, normal renal function), *PLP* pyridoxal 5′-phosphate, *PL* pyridoxal (PL), *PA* 4-pyridoxic acid, *PAr* PA/(PLP + PL) ratio

[a] Geometric means adjusted for smoking

[b] The group with a BMI ≥27.5 kg/m^2 was collapsed with the group with a BMI 23–<27.5, because there were only six control subjects from the Shanghai cohort and twelve control subjects from the Singapore cohort with a BMI ≥27.5 kg/m^2

[c] Due to the high correlations of PLP and PL with PA, and PA has a high renal clearance. Geometric means of PLP and PL were further adjusted for PA

In the cohort-specific analysis, the inverse association between PLP and pancreatic cancer risk was found in the Shanghai cohort but not in the Singapore cohort. The lack of association in the Singapore cohort was primarily due to the small sample size and relatively higher level of PLP. Overall only 5.4% of the Singapore study controls who did not report use of any vitamins or minerals showed PLP <20 nmol/L; thus, cohort-specific quartile cutoff values were used in the analysis, resulting in a median concentration of 31.7 nmol/L PLP of the lowest quartile. This is not surprising for a relatively weak inverse association between PLP and pancreatic cancer risk in the Singapore cohort study in which a high level of PLP as a reference group was observed. It is interesting to note that the overall incidence rate of pancreatic cancer is approximately 38% higher in Singapore than in Shanghai, China (4.86 vs 6.78 per 100 000 men) based on the GLOBOCAN 2012 estimates [1]. It is possible that the observed association between serum PLP and risk of pancreatic cancer was underestimated given the higher incidence rate of pancreatic cancer in Singapore, with relatively higher concentrations of serum PLP, compared with Shanghai populations.

The inverse association between serum PLP and pancreatic cancer in our study is consistent with some previous studies but not others. There were three previous studies that evaluated associations between circulating PLP and risk of pancreatic cancer. The first study was a nested case–control study among current smokers that included 126 cases of pancreatic cancer and 247 matched control subjects within the Alpha-Tocopherol, Beta-Carotene (ATBC) Cancer Prevention Study in Finland (Supplemental Table S4, Online Resource). The ATBC study reported an inverse association between serum PLP, determined by the enzymatic method (i.e., tyrosine decarboxylase apoenzyme method) [30], and pancreatic cancer risk; OR was 0.48 for the highest (>39.46 nmol/L) versus lowest tertile (<26.34 nmol/L) of PLP (*P* for trend = 0.02) [18]. The second study was also a nested case–control study of pancreatic cancer pooled from four US cohorts including the Nurses' Health Study, the Health Professionals Follow-up Study, the Physicians' Health Study, and the Women's Health Initiative involving 208 cases and 623 controls [16]. That study reported a slightly reduced risk of pancreatic cancer associated with highest quartile of PLP (OR = 0.87, 95% CI = 0.55–1.37). It is worth noting that a radioenzymatic assay was used to quantify plasma PLP that yielded an average of 12.7 nmol/L PLP [16], which was well below 20 nmol/L as the cutoff value for vitamin B_6 deficiency [28]. This level was 65–80% lower than the median plasma PLP (39.0–60.2 nmol/L) that was recently measured using the LC–MS/MS method in plasma samples from these same four cohorts, which were part of the Lung Cancer Cohort Consortium project (per communication, Øivind Midttun, 2015). The LC–MS/MS quantification of these samples was conducted by the same laboratory as our study samples in the present study. The third nested case–control study was conducted within the European Prospective Investigation into Cancer and Nutrition cohort and also found an inverse association between plasma PLP, determined by the same LC–MS/MS method, and pancreatic cancer risk in women (OR = 0.4, 95% CI = 0.2–0.8 for 5th versus 1st quintile of PLP) but not in men (OR = 1.1, 95% CI = 0.6–2.3) [17]. The inconsistent results from the previous studies could be attributed to different levels of exposure to vitamin B_6 across different study populations and different methods used in PLP quantification.

Vitamin B_6 may play a role in protecting DNA against oxidative damage and subsequent mutations and therefore reduce the potential to develop cancer. As a cofactor for cystathionine β-synthase and cystathionine γ-lyase, PLP is involved in the production of the important cellular antioxidant glutathione. In addition to its cofactor role,

Table 3 Associations between serum concentrations of pyridoxal 5′-phosphate (PLP), pyridoxal (PL), and 4-pyridoxic acid (PA), and PA/(PL + PLP) ratio (PAr) and pancreatic cancer risk in pooled analysis of both cohorts

Biomarkers	Controls	Cases	OR (95% CI)[a]	OR (95% CI)[b]
PLP (nmol/L)				
<20.0	89	58	1.00	1.00
20.0–29.0	89	42	0.67 (0.4–1.13)	0.68 (0.4–1.15)
29.1–52.4	93	53	0.69 (0.4–1.18)	0.74 (0.42–1.31)
>52.4	91	34	0.41 (0.21–0.78)	0.46 (0.23–0.92)
P trend			0.01	0.048
PL (nmol/L)				
<11.8	92	56	1.00	1.00
11.8–16.6	89	58	1.02 (0.63–1.67)	1.18 (0.71–1.97)
16.7–24.0	92	29	0.47 (0.27–0.84)	0.51 (0.28–0.92)
>24.0	89	44	0.74 (0.43–1.27)	0.82 (0.46–1.44)
P trend			0.06	0.14
PA (nmol/L)				
<8.8	92	52	1.00	1.00
8.8–13.0	89	56	1.03 (0.63–1.66)	1.19 (0.71–1.97)
13.1–20.4	91	34	0.55 (0.3–0.99)	0.61 (0.33–1.13)
>20.4	90	45	0.69 (0.38–1.25)	0.94 (0.49–1.84)
P trend			0.09	0.44
PAr				
<0.21	91	48	1.00	1.00
0.21–0.29	90	39	0.79 (0.47–1.35)	0.76 (0.44–1.31)
0.30–0.39	91	53	1.08 (0.67–1.74)	1.22 (0.74–2.04)
>0.39	90	47	0.95 (0.58–1.56)	1.07 (0.63–1.81)
P trend			0.85	0.48

PLP pyridoxal 5′-phosphate, *PL* pyridoxal (PL), *PA* 4-pyridoxic acid, *PAr* PA/(PL + PLP) ratio

[a] Unadjusted odds ratios

[b] Odds ratios were derived from conditional logistic regression models that adjusted for smoking status (never, former, and current smokers), number of alcoholic drinkers per week (continuous), level of education (no formal schooling, primary school, and secondary school or above), history of diabetes (no, yes), BMI (<18.5, 18.5–<23.0, ≥23.0 kg/m^2), and study site (Shanghai, Singapore). The models including PA and PAr were further adjusted for estimated glomerular filtration rate

vitamin B_6 may serve and act as a scavenger of reactive oxidative species [31]. In men, serum vitamin B_6 was inversely associated with urinary 8-hydroxy-deoxyguanosine, a marker of DNA oxidative damage [32]. It has recently been shown that PLP deficiency resulted in formation of advanced glycation end products (AGEs), a major contributor of cellular oxidative stress, and subsequent chromosome aberrations in HeLa cells [33]. Interestingly, higher serum concentrations of soluble receptors for AGEs that neutralizes and blocks the effect of AGEs were associated with reduced risk of multiple cancers including pancreatic cancer [34, 35]. Future studies are warranted to study the biological pathways underlying the potential protective effect of PLP against pancreatic cancer development.

PLP is the primary form of circulating vitamin B_6 and accounts for 70–90% of the total circulating B_6 vitamers (i.e., sum of PLP, PL, and PA) [36]. PL is the transport form of PLP across cellular membranes, while PA is the vitamin B_6 catabolite excreted through urine [13]. Our study evaluated serum concentrations of PL and PA and pancreatic cancer risk and did not find any associations. Therefore, PLP may be more relevant in pancreatic carcinogenesis compared with PL and PA. No previous study has evaluated the association between PAr and pancreatic cancer risk. In the current study, we found no association between PAr, a marker of vitamin B_6 catabolism [14], and pancreatic cancer risk. In summary, overall vitamin B_6 status (e.g., PLP) rather than vitamin B_6 catabolism (e.g., PAr) may be more relevant in pancreatic carcinogenesis.

The strengths of our study are the prospective study design and using a LC–MS/MS-based method with high accuracy and precision to quantify the B_6 vitamers. In addition, compared with the higher levels of PLP in the Singapore cohort and the US and European populations, the Shanghai cohort provided a unique study population to examine the PLP–pancreatic cancer association at the lower end of the exposure spectrum. Moreover, the relatively long time interval between blood collection and pancreatic cancer diagnosis (on average 12.5 years in the Shanghai cases and 6.8 years in the Singapore cases) would diminish the potential impact of disease progression or subclinical symptoms on the circulating PLP concentration. Our study was limited by having a small sample size, especially in the Singapore cohort. Studies with a larger sample size in other study populations are warranted to validate our findings. Participants from the Singapore cohort were older, had lower level of education, and had higher levels of B_6 vitamers compared with those from the Shanghai cohort. We included a variable of study site (Shanghai, Singapore) in the pooled analysis for the associations between B_6 vitamers and pancreatic cancer risk to account for the cohort difference in baseline characteristics. In the Singapore cohort, the baseline questionnaire was administered an average of 6.5 years prior to blood draw. However, for the major potential confounders (i.e., smoking and diabetes), we used information from the follow-up I interview, which was collected an average of 8 months prior to blood draw. No significant change was observed between baseline and follow-up I BMI, and thus, we used baseline BMI in the statistical analysis. Although potential misclassification is still a minor concern, it is unlikely to be differential in cases and control subjects, and thus, the underlying associations between B_6 vitamers and

pancreatic cancer risk could be stronger than what was observed.

In conclusion, our study suggested that sufficient PLP in serum was associated with a 54% reduced risk of pancreatic cancer in a pooled analysis of two prospective cohorts of Asians. These results suggest that a diet high in vitamin B_6 may be protective against the development of pancreatic cancer, especially in populations with relatively low levels of in vivo vitamin B_6. Although vitamin B_6 deficiency in developed countries is rare, certain groups are at higher risk of marginal vitamin B_6 status including the elderly, pregnant women, individuals taking certain drugs, and chronic alcohol abusers [13]. In addition to replication in other observational studies, further studies are needed to investigate the potential mechanisms by which PLP exerts its role against the development of pancreatic cancer.

Acknowledgments We thank Xue-Li Wang of the Shanghai Cancer Institute for assistance with data collection and management and the staff of the Shanghai Cancer Registry for their assistance in verifying cancer diagnoses in study participants. We thank Siew-Hong Low of the National University of Singapore for supervising the fieldwork in the Singapore Chinese Health Study. We also thank The Singapore Cancer Registry for the identification of cancer and mortality outcomes via database linkages. Finally, we acknowledge the founding Principal Investigator of the Singapore Chinese Health Study, Mimi C. Yu.

Funding The work was supported by the National Institutes of Health (NCI R01 CA144034 and UM1 CA182876).

Compliance with ethical standards

Conflict of interest None declared.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

References

1. Ferlay J, Soerjomataram I, Dikshit R et al (2015) Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 136:E359–E386
2. Lynch SM, Vrieling A, Lubin JH et al (2009) Cigarette smoking and pancreatic cancer: a pooled analysis from the pancreatic cancer cohort consortium. Am J Epidemiol 170:403–413
3. Bracci PM (2012) Obesity and pancreatic cancer: overview of epidemiologic evidence and biologic mechanisms. Mol Carcinog 51:53–63
4. Jansen RJ, Robinson DP, Stolzenberg-Solomon RZ et al (2011) Fruit and vegetable consumption is inversely associated with having pancreatic cancer. Cancer Causes Control 22:1613–1625
5. Chan JM, Wang F, Holly EA (2005) Vegetable and fruit intake and pancreatic cancer in a population-based case-control study in the San Francisco bay area. Cancer Epidemiol Biomarkers Prev 14:2093–2097
6. US Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory (2016) USDA National Nutrient Database for Standard Reference, Release 28 (Slightly revised). Version Current: May 2016. http://www.ars.usda.gov/sp2UserFiles/Place/80400525/Data/SR/SR28/sr28_doc.pdf
7. Huang JY, Butler LM, Wang R, Jin A, Koh WP, Yuan JM (2016) Dietary intake of one-carbon metabolism-related nutrients and pancreatic cancer risk: the Singapore Chinese Health Study. Cancer Epidemiol Biomarkers Prev 25:417–424
8. Larsson SC, Giovannucci E, Wolk A (2007) Methionine and vitamin B6 intake and risk of pancreatic cancer: a prospective study of Swedish women and men. Gastroenterology 132:113–118
9. Stolzenberg-Solomon RZ, Pietinen P, Barrett MJ, Taylor PR, Virtamo J, Albanes D (2001) Dietary and other methyl-group availability factors and pancreatic cancer risk in a cohort of male smokers. Am J Epidemiol 153:680–687
10. Gong Z, Holly EA, Bracci PM (2009) Intake of folate, vitamins B6, B12 and methionine and risk of pancreatic cancer in a large population-based case-control study. Cancer Causes Control 20:1317–1325
11. Choi SW, Friso S (2012) Vitamins B6 and cancer. Subcell Biochem 56:247–264
12. Albersen M, Bosma M, Luykx JJ et al (2014) Vitamin B-6 vitamers in human plasma and cerebrospinal fluid. Am J Clin Nutr 100:587–592
13. Ueland PM, Ulvik A, Rios-Avila L, Midttun O, Gregory JF (2015) Direct and functional biomarkers of vitamin B6 status. Annu Rev Nutr 35:33–70
14. Ulvik A, Midttun O, Pedersen ER, Eussen SJ, Nygard O, Ueland PM (2014) Evidence for increased catabolism of vitamin B-6 during systemic inflammation. Am J Clin Nutr 100:250–255
15. Greer JB, Whitcomb DC (2009) Inflammation and pancreatic cancer: an evidence-based review. Curr Opin Pharmacol 9:411–418
16. Schernhammer E, Wolpin B, Rifai N et al (2007) Plasma folate, vitamin B6, vitamin B12, and homocysteine and pancreatic cancer risk in four large cohorts. Cancer Res 67:5553–5560
17. Chuang SC, Stolzenberg-Solomon R, Ueland PM et al (2011) A U-shaped relationship between plasma folate and pancreatic cancer risk in the European Prospective Investigation into Cancer and Nutrition. Eur J Cancer 47:1808–1816
18. Stolzenberg-Solomon RZ, Albanes D, Nieto FJ et al (1999) Pancreatic cancer risk and nutrition-related methyl-group availability indicators in male smokers. J Natl Cancer Inst 91:535–541
19. Yuan JM, Ross RK, Wang XL, Gao YT, Henderson BE, Yu MC (1996) Morbidity and mortality in relation to cigarette smoking in Shanghai, China. A prospective male cohort study. JAMA 275:1646–1650
20. Yuan JM, Stram DO, Arakawa K, Lee HP, Yu MC (2003) Dietary cryptoxanthin and reduced risk of lung cancer: the Singapore Chinese Health Study. Cancer Epidemiol Biomarkers Prev 12:890–898
21. Hankin JH, Stram DO, Arakawa K et al (2001) Singapore Chinese Health Study: development, validation, and calibration of the quantitative food frequency questionnaire. Nutr Cancer 39:187–195
22. Odegaard AO, Pereira MA, Koh WP, Arakawa K, Lee HP, Yu MC (2008) Coffee, tea, and incident type 2 diabetes: the Singapore Chinese Health Study. Am J Clin Nutr 88:979–985
23. Odegaard AO, Koh WP, Arakawa K, Yu MC, Pereira MA (2010) Soft drink and juice consumption and risk of physician-diagnosed incident type 2 diabetes: the Singapore Chinese Health Study. Am J Epidemiol 171:701–708
24. Parkin DM, Whelan SL, Ferlay J, Teppo L, Thomas DB (eds) (2002) Cancer incidence in five continents vol. VIII. IARC

Publication No. 155. https://www.iarc.fr/en/publications/pdfs-online/epi/sp155/CI5V8.pdf. Accessed Nov 2016
25. Yuan JM, Koh WP, Murphy SE et al (2009) Urinary levels of tobacco-specific nitrosamine metabolites in relation to lung cancer development in two prospective cohorts of cigarette smokers. Cancer Res 69:2990–2995
26. Midttun O, Kvalheim G, Ueland PM (2013) High-throughput, low-volume, multianalyte quantification of plasma metabolites related to one-carbon metabolism using HPLC-MS/MS. Anal Bioanal Chem 405:2009–2017
27. Breslow N, Day N (1980) Statistical methods in cancer research, vol 1: the analysis of case–control studies. IARC: IARC Scientific Pub No. 32, Lyon
28. Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate OBV, and Choline (1998) Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline. Washington (DC)
29. Levey AS, Stevens LA, Schmid CH et al (2009) A new equation to estimate glomerular filtration rate. Ann Intern Med 150:604–612
30. Shin-Buehring Y, Rasshofer R, Endres W (1981) A new enzymatic method for pyridoxal 5′-phosphate determination. J Inherit Metab Disord 4:123–124
31. Kannan K, Jain SK (2004) Effect of vitamin B6 on oxygen radicals, mitochondrial membrane potential, and lipid peroxidation in H2O2-treated U937 monocytes. Free Radic Biol Med 36:423–428
32. Kuwahara K, Nanri A, Pham NM et al (2013) Serum vitamin B6, folate, and homocysteine concentrations and oxidative DNA damage in Japanese men and women. Nutrition 29:1219–1223
33. Marzio A, Merigliano C, Gatti M, Verni F (2014) Sugar and chromosome stability: clastogenic effects of sugars in vitamin B6-deficient cells. PLoS Genet 10:e1004199
34. Jiao L, Weinstein SJ, Albanes D et al (2011) Evidence that serum levels of the soluble receptor for advanced glycation end products are inversely associated with pancreatic cancer risk: a prospective study. Cancer Res 71:3582–3589
35. Jiao L, Taylor PR, Weinstein SJ et al (2011) Advanced glycation end products, soluble receptor for advanced glycation end products, and risk of colorectal cancer. Cancer Epidemiol Biomarkers Prev 20:1430–1438
36. Leklem JE (1990) Vitamin B-6: a status report. J Nutr 120(Suppl 11):1503–1507