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Low pyridoxal 5′-phosphate is associated with increased risk of coronary artery disease

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Abstract

Objective: The purpose of this study was to investigate the association between plasma pyridoxal 5′-phosphate (PLP) status and lipid profiles and to estimate the relation to the risk of coronary artery disease (CAD).

Methods: Patients who were identified by cardiac catheterization as having ≥70% stenosis of one major coronary artery were assigned to the case group (n = 184). The control group (n = 516) was comprised of healthy individuals with normal blood biochemical values. Plasma PLP, homocysteine, high-sensitivity C-reactive protein, lipid profiles (total cholesterol, low-density lipoprotein, high-density lipoprotein, very low-density lipoprotein, and triacylglycerol) were determined.

Results: Subjects with a plasma PLP level <30 nmol/L exhibited a significantly increased risk of CAD compared with subjects with a plasma PLP level ≥30 nmol/L (odds ratio, 1.85; 95% confidence interval, 1.16–2.95) after adjusting for homocysteine and high-sensitivity C-reactive protein. The association between PLP and the risk of CAD remained significant after each lipid profile was additionally adjusted. In addition, the combined presence of low PLP level and an abnormal lipid level increased the risk of CAD to an even greater degree.

Conclusions: A borderline vitamin B6 deficiency (plasma PLP concentration <30 nmol/L) is strongly associated with the risk of CAD. The combined presence of low PLP and abnormal lipid levels increased the risk of CAD even further.

Keywords: Vitamin B6; Pyridoxal-5′-phosphate; Lipid; Borderline hyperlipidemia; Coronary artery disease

Introduction

In the past decade, a great deal of attention has been paid to the relations between non-traditional risk factors and coronary artery disease (CAD). Among these non-traditional risk factors (i.e., homocysteine, C-reactive protein [CRP], and vitamins), low vitamin B-6 status has been demonstrated to be independent of homocysteine as a risk factor for cardiovascular disease [1–6]. Although the exact pathogenesis of vitamin B6 deficiency in cardiovascular disease is unknown, low vitamin B6 may play a role in the derangement of lipid metabolism [7–9].

Labadarios et al. [10] studied 34 patients who had chronic glomerulonephritides with and without the nephrotic syndrome and observed that patients with low plasma levels of pyridoxal 5′-phosphate (PLP; the biologically active form of vitamin B6) exhibited higher serum total cholesterol levels. Harripersad et al. [9] also found that subnormal vitamin B6 intake resulted in an elevation of plasma low-density lipoprotein (LDL) in rats. Serfontein et al. [2] administered multivitamin supplements (containing 10 mg of pyridoxine) to 34 hypercholesterolemic patients, and the total cholesterol (TC) concentration was reduced by

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30.9 mg/dL, with a significant reduction especially observed in the LDL concentration. However, not all of the evidence supports vitamin B6 having an effect on lipid metabolism. Pike et al. [11] reported that 200 mg/d of pyridoxine hydrochloride supplementation did not significantly alter the lipoprotein profile in 11 patients using long-term hemodialysis. A lack of correlation between the levels of lipid parameters and B vitamins was also observed in populations with an elevated risk for atherosclerosis [12].

The biochemical link between vitamin B6 deficiency and lipid metabolism is still poorly understood and highly controversial. The question is whether vitamin B6 is independently related to the risk for CAD or mediates the risk of CAD in connection with high lipid levels. This study investigated the association between plasma PLP status and lipid profiles and analyzed the relation with the risk for CAD.

Materials and methods

Subjects

Case subjects were recruited from the cardiology clinic of Taichung Veterans General Hospital, which is a teaching hospital in the central part of Taiwan. The case group was comprised of patients who were suspected of having CAD, underwent coronary angiography, and were identified by cardiac catheterization as having ≥70% stenosis of one major coronary artery. The use of medications was recorded. To minimize the influence of other cardiovascular risk factors, case subjects with diabetes (defined by a history of antidiabetic drugs use or a fasting plasma glucose concentration >140 mg/dL) or liver or renal diseases (identified by serum creatinine and aspartate aminotransferase analyses) were excluded. Control subjects were recruited from the physical examination unit of Taichung Veterans Hospital who exhibited normal blood biochemical values, including a fasting blood glucose level <110 mg/dL, blood urea nitrogen level <7.9 mmol/L, creatinine level <1.4 mg/dL, alkaline phosphate level <190 U/L, glutamic oxaloacetic transaminase level <35 U/L, and glutamic pyruvate transaminase level <45 U/L. Control subjects had no illness or history of gastrointestinal disorder, cardiovascular disease, hypertension, hyperlipidemia, liver or renal disease, diabetes, cancer, alcoholism or other metabolic disease. In addition, control subjects had normal electrocardiograms. Informed consent was obtained from each subject. This study was approved by the institutional review board of Chung Shan Medical University.

Experimental protocol

Subjects' age, gender, smoking and drinking habits, and family history were recorded. Body weight and height were measured; body mass index (kilograms divided by square millimeters) was then calculated. Blood pressure (systolic and diastolic) was measured after a resting period of ≥5 min.

Blood analyses

Fasting venous blood specimens (15 mL) were collected in Vacutainer tubes (Becton Dickinson, Rutherford, NJ, USA) containing ethylenediaminetetraacetic acid as an anticoagulant or without an anticoagulant as required to estimate hematologic and vitamin statuses. Serum or plasma were separated within 30 min after the blood was drawn and then stored frozen (−80°C) until analysis. Plasma homocysteine was measured by using high-performance liquid chromatography according to the method of Araki and Sako [13]. The intra- and interassays of fasting plasma homocysteine coefficient of variation were 1.6% ($n = 3$) and 4.3% ($n = 15$), respectively. Plasma PLP was determined by high-performance liquid chromatography according to the method of Bates et al. [14] and carried out under yellow light to prevent photodestruction. The intra- and interassays of plasma PLP coefficient of variation were 2.0% ($n = 4$) and 3.6% ($n = 4$), respectively. Hematologic entities (i.e., blood urea nitrogen, creatinine, glutamic oxaloacetic transaminase, glutamic pyruvate transaminase, TC, triacylglycerol [TG], LDL, and high-density lipoprotein [HDL]) were measured by using an automated biochemical analyzer. The very low-density lipoprotein (LDL) value was calculated from the value of TG divided by 5 (TG/5). A borderline level of hyperlipidemia was defined as a TC level ≥200 mg/dL, an LDL level ≥130 mg/dL, an HDL level <60 mg/dL, a TC-to-HDL ratio ≥4.4, and/or a TG level ≥150 mg/dL according to the criteria of the National Cholesterol Education Program. Automated high-sensitivity CRP (hs-CRP) measurements were obtained with particle-enhanced immunonephelometry with an image analyzer [15].

Statistical analyses

Data were analyzed with SigmaStat 2.03 (Jandel Scientific, San Rafael, CA, USA). The lowest quartile value of plasma PLP from our control samples was 28.3 nmol/L, which is close to the suggested value for adequate vitamin B6 status (>30 nmol/L) [16]. We therefore used 30 nmol/L to define the borderline level of vitamin B6 status in this study. Differences in subjects' demographic data and the data of hematologic measurements between the case and control groups or between the stratification by PLP value was analyzed by Student's *t* test. For categorical response variables, differences between groups were assessed by Fisher's exact test. Multiple linear regression analyses with the lipid profile elements (TC, LDL, HDL, TC/HDL ratio, or TG) as a dependent variable was used to determine the association between plasma PLP and the lipid profile after adjustment for age, gender, homocysteine, or potential confounders. Adjusted odds ratios with 95% confidence intervals for borderline hyperlipidemia were calculated from a logistic regression model using a cutoff point of of 30 nmol/L

Table 1
Demographic and clinical characteristics of subjects*

Characteristics	Case (n = 184)	Control (n = 516)
Male/female	146/38	293/223
Age (y)	67.9 ± 11.0[a]	52.1 ± 11.8[b]
Body mass index (kg/m^2)	25.3 ± 3.3[a]	23.9 ± 3.3[b]
Blood pressure (mmHg)		
Systolic	131.2 ± 19.4[a]	120.5 ± 18.7[b]
Diastolic	73.4 ± 12.9[a]	75.8 ± 11.3[b]
Cholesterol (mg/dL)		
Total	190.3 ± 43.7	186.2 ± 31.4
VLDL	30.8 ± 19.1[a]	24.8 ± 15.8[b]
LDL	123.5 ± 39.2[a]	101.2 ± 30.2[b]
HDL	41.7 ± 10.6[a]	60.2 ± 15.3[b]
TC/HDL ratio	4.8 ± 1.4[a]	3.3 ± 0.9[b]
Triacylglycerol (mg/dL)	153.9 ± 95.7[a]	123.9 ± 79.2[b]
hs-CRP (mg/dL)	1.1 ± 2.7[a]	0.2 ± 0.3[b]
Serum creatinine (mg/dL)	1.4 ± 1.5	1.0 ± 0.2
Plasma homocysteine (μmol/L)	13.0 ± 6.3[a]	9.8 ± 3.9[b]
Plasma PLP (nmol/L)	40.4 ± 41.6[a]	59.2 ± 47.0[b]
Smoking (n, %)	40 (21.7%)	111 (21.5%)
Drinking (n, %)	19 (10.3%)	93 (18.0%)

HDL, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein cholesterol; PLP, pyridoxal-5′-phosphate; TC, total cholesterol; VLDL, very low-density lipoprotein

* Values are means ± standard deviation. Values with different superscript letters are significantly different between two groups ($P < 0.05$).

for plasma PLP. We further used the logistic regression model to calculate the adjusted odds ratio for CAD. Results were considered statistically significant at $P < 0.05$. Values presented in the text are means ± standard deviation.

Results

Table 1 lists the demographic data and health characteristics of the subjects. Subjects in the case group had significantly higher values for age, body mass index, systolic blood pressure, VLDL, LDL, TC/HDL ratio, TG, hs-CRP, and plasma homocysteine and lower diastolic blood pressure, HDL, and plasma PLP concentrations than did subjects in the control group. After stratification by the value of plasma PLP concentration (Table 2), subjects with a plasma PLP level <30 nmol/L had significantly higher LDL, TC/HDL ratio, homocysteine values and a lower HDL value than did subjects with a plasma PLP concentration ≥30 nmol/L in the pooled group.

The association between plasma PLP and lipid profiles is presented in Table 3. Plasma PLP had a significant positive association with HDL but a negative association with the TC/HDL ratio in the case and pooled groups after adjusting for major CAD risk factors. However, plasma PLP had no significant associations with any lipid profiles in the control group.

To understand the association between plasma PLP and lipid profiles and the risk for CAD, we then calculated CAD risk factors using the logistic regression model. Subjects with a plasma PLP level <30 nmol/L exhibited a significantly increased risk of CAD than did subjects with a plasma PLP level ≥30 nmol/L after adjusting for potential confounders (odds ratio 1.85, 95% confidence interval 1.16–2.95). When each lipid profile (TC level ≥200 mg/dL, LDL level ≥130 mg/dL, HDL level <60 mg/dL, TC/HDL ratio ≥4.4, or TG level ≥150 mg/dL) was additionally adjusted, PLP remained a significant risk factor for CAD.

The association of lipid and PLP to the risk of CAD was then simultaneously considered (Table 4). The combined presence of low PLP level and an abnormal lipid level enhanced the risk of CAD and the magnitude was substantially greater.

Discussion

The role vitamin B6 plays in lipid metabolism is intriguing but highly controversial. Serfontein et al. [17] reported that vitamin B6 was predominately related through a de-

Table 2
Lipid profiles and plasma homocysteine concentrations after stratification by plasma PLP status*

Characteristics	Case (n = 184)		Control (n = 516)		Pooled (n = 700)	
	PLP <30 nmol/L (n = 104)	PLP ≥30 nmol/L (n = 80)	PLP <30 nmol/L (n = 149)	PLP ≥30 nmol/L (n = 367)	PLP <30 nmol/L (n = 253)	PLP ≥30 nmol/L (n = 447)
Cholesterol (mg/dL)						
Total	191.1 ± 43.8	189.2 ± 43.8	183.5 ± 33.9	187.4 ± 30.3	186.6 ± 38.4	187.7 ± 33.1
VLDL	30.0 ± 18.9	31.9 ± 19.6	24.7 ± 16.1[†]	24.8 ± 15.7[‡]	26.8 ± 17.5	26.1 ± 16.7
LDL	127.3 ± 37.8	118.5 ± 40.7	98.9 ± 33.0[†]	102.1 ± 29.0[‡]	110.6 ± 37.7[a]	105.1 ± 32.0[b]
HDL	40.6 ± 9.8	43.2 ± 11.5	59.9 ± 17.0[†]	60.4 ± 14.5[‡]	52.0 ± 17.3[a]	57.3 ± 15.5[b]
TC/HDL ratio	4.9 ± 1.6	4.6 ± 1.2	3.3 ± 1.0†	3.2 ± 0.8‡	4.0 ± 1.5[a]	3.5 ± 1.0[b]
Triacylglycerol (mg/dL)	149.4 ± 94.4	159.7 ± 97.8	123.3 ± 80.7†	124.1 ± 78.6‡	134.0 ± 87.4	130.5 ± 83.4
Plasma homocysteine (μmol/L)	13.2 ± 6.0	12.8 ± 6.7	10.1 ± 3.2[†]	9.7 ± 4.2[‡]	11.4 ± 4.8[a]	10.2 ± 4.9[b]

HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; PLP, pyridoxal-5′-phosphate; TC, total cholesterol; VLDL, very low-density lipoprotein

* Values are means ± standard deviations. Values with different superscript letters are significantly different within the group ($P < 0.05$).

† Values with different symbols are significantly different from the case group in the same stratification of plasma PLP value ($P < 0.05$).

‡ Values with different symbols are significantly different from the case group in the same stratification of plasma PLP value ($P < 0.05$).

Table 3
Association of plasma pyridoxal 5′-phosphate concentration with lipids by using multiple linear regression analysis with lipid profile as a dependent variable

	Case (n = 184)		Control (n = 516)		Pooled (n = 700)	
	β*	P	β	P	β	P
Total cholesterol (mg/dL)						
Model 1†	−0.007	0.927	0.016	0.588	0.010	0.735
Model 2‡	−0.012	0.868	0.013	0.686	0.007	0.806
LDL (mg/dL)						
Model 1	−0.089	0.202	0.010	0.716	−0.038	0.167
Model 2	−0.071	0.292	0.005	0.881	−0.038	0.186
HDL (mg/dL)						
Model 1	0.043	0.023	−0.009	0.505	0.028	0.023
Model 2	0.043	0.022	−0.010	0.503	0.025	0.044
TC/HDL ratio						
Model 1	−0.004	0.077	0.000	0.683	−0.003	0.004
Model 2	−0.005	0.047	0.000	0.811	−0.003	0.006
Triacylglycerol (mg/dL)						
Model 1	0.184	0.259	0.072	0.334	0.057	0.413
Model 2	0.131	0.413	0.086	0.284	0.071	0.325

HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; TC, total cholesterol
* Regression coefficient.
† Adjusted for age and gender.
‡ As in model 1 and also adjusted for plasma homocysteine, body mass index, creatinine, systolic blood pressure, and high-sensitivity C-reactive protein.

pression of the LDL component so as to lower the plasma cholesterol concentration. A greater degree of hypercholesterolemia was observed in a vitamin B6–deficient animal model [18]. A subnormal intake of vitamin B6 could thus contribute to an elevated LDL level [9]. In our study, plasma PLP had a significant association only with HDL and the TC/HDL ratio. Our patients with CAD and healthy subjects had sufficient dietary vitamin B6 intake (data not

Table 4
Multivariate adjusted odds ratios for coronary artery disease when plasma PLP concentration and borderline hyperlipidemia were simultaneously considered

	Factors adjusted*			Additional factors adjusted†		
	OR	95% CI	P	OR	95% CI	P
PLP <30 nmol/L, TC ≥200 mg/dL	2.64	1.43–4.90	<0.01	1.52	0.74–3.10	0.25
PLP <30 nmol/L, TC <200 mg/dL	2.39	1.42–4.00	<0.01	1.98	1.12–3.49	0.02
PLP ≥30 nmol/L, TC ≥200 mg/dL	1.23	0.67–2.25	0.51	0.94	0.46–1.90	0.85
PLP ≥30 nmol/L, TC <200 mg/dL	1.00			1.00		
PLP <30 nmol/L, LDL ≥130 mg/dL	6.85	3.46–13.56	<0.01	4.69	2.16–10.19	<0.01
PLP <30 nmol/L, LDL <130 mg/dL	2.52	1.50–4.22	<0.01	1.90	1.08–3.36	0.03
PLP ≥30 nmol/L, LDL ≥130 mg/dL	4.06	2.12–7.79	<0.01	2.74	1.29–5.82	0.01
PLP ≥30 nmol/L, LDL <130 mg/dL	1.00			1.00		
PLP <30 nmol/L, HDL <60 mg/dL	22.09	9.00–54.26	<0.01	14.94	5.73–38.98	<0.01
PLP <30 nmol/L, HDL ≥60 mg/dL	0.82	0.17–3.93	0.81	0.98	0.06–14.97	0.99
PLP ≥30 nmol/L, HDL <60 mg/dL	8.40	3.43–20.57	<0.01	5.14	2.03–13.05	<0.01
PLP ≥30 nmol/L, HDL ≥60 mg/dL	1.00			1.00		
PLP <30 nmol/L, TC/HDL ≥4.4	15.04	7.40–30.58	<0.01	7.89	3.72–16.74	<0.01
PLP <30 nmol/L, TC/HDL <4.4	2.64	1.48–4.70	<0.01	2.24	1.20–4.21	0.01
PLP ≥30 nmol/L, TC/HDL ≥4.4	12.85	6.26–26.36	<0.01	7.69	3.49–16.93	<0.01
PLP ≥30 nmol/L, TC/HDL <4.4	1.00			1.00		
PLP <30 nmol/L, TG ≥150 mg/dL	5.63	2.83–11.22	<0.01	3.37	1.56–7.30	<0.01
PLP <30 nmol/L, TG <150 mg/dL	2.36	1.35–4.14	<0.01	1.92	1.02–3.59	0.04
PLP ≥30 nmol/L, TG ≥150 mg/dL	2.48	1.34–4.61	<0.01	2.24	1.11–4.50	0.02
PLP ≥30 nmol/L, TG <150 mg/dL	1.00			1.00		

CI, confidence interval; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; OR, odds ratio; PLP, pyridoxal 5′-phosphate; TC, total cholesterol; TG, triacylglycerol
* Adjusted for age and gender.
† Also adjusted for body mass index, creatinine, plasma homocysteine, systolic blood pressure, and high-sensitivity C-reactive protein.

shown) and the plasma PLP concentration was not severely deficient, which might be the reason that we did not see the reported relations between plasma PLP and TC or LDL. The lack of an association between lipid levels and vitamin B6 has also been observed in subjects with an elevated risk of atherosclerosis [12].

Unsurprisingly, lipid profiles except for TC were significantly associated with the risk of CAD, and the association was independent of the PLP level. Despite the relation between lipids and the risk of CAD, a lower plasma PLP level was strongly associated with a greater risk of CAD after adjustment for the potential confounders, lipid profiles, homocysteine, and hs-CRP in the present study. Studies have also indicated that low plasma PLP status was an independent risk factor for CAD [19–21]. Although the underlying mechanism of the CAD risk with low plasma PLP remains unclear, vitamin B6 may alter platelet function [22,23] or antithrombin III activity [24]. We further simultaneously considered low PLP concentrations and abnormal lipid levels. In agreement with the findings of Friso et al. [21], the combined presence of a PLP level <30 nmol/L and abnormal lipid profiles, especially for TC/HDL ratios ≥ 4.4 or an HDL level <60 mg/dL, enhanced the risk of CAD and the magnitude is even substantially increased. Subjects with a low vitamin B-6 status and abnormal lipid levels should be more carefully monitored for CAD risk.

Currently, vitamin B6 deficiency has been redefined as a plasma PLP concentration <20 nmol/L [25] instead of 30 nmol/L, a value first defined by Leklem [16]. However, in this study we used a value of 30 nmol/L of PLP, which corresponded to the lowest quartile value of plasma PLP in our control samples (28.3 nmol/L), to indicate borderline vitamin B6 deficiency. A significantly higher CAD risk was detected among our subjects with a plasma PLP level <30 nmol/L and the subjects from the study of Friso et al. [21] who had a PLP level <36.3 nmol/L. It appears evident that even a borderline vitamin B6 deficiency (<30 nmol/L) can thus contribute to a higher risk of CAD. Further study is needed to define the precise cutoff value of plasma PLP when the risk of CAD is being assessed.

The limitation of this study was the selection criteria of the CAD patients. In most clinical settings of Taiwan, patients with CAD were identified as having $\geq 70\%$ stenosis of at least one coronary artery based on the results of cardiac catheterization. However, subjects with 50–70% stenosis of at least one coronary artery could be classified as having mild to moderate CAD [26]. The selection criteria may therefore add some bias to the results of the study.

In conclusion, the present data show that a borderline vitamin B6 deficiency (plasma PLP concentration <30 nmol/L) is strongly associated with the risk of CAD independently of homocysteine, inflammation (hs-CRP), and lipid profiles. In addition, the combined presence of low PLP and abnormal lipid levels results in an even further enhanced risk of CAD. It is necessary to take vitamin B-6 and lipids into account when the risk of CAD is assessed, and a better understanding of the role vitamin B6 plays in the risk of CAD should lead to more feasible CAD therapeutic strategies in the future.

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