Plasma pyridoxal-5-phosphate and future risk of myocardial infarction in the European Prospective Investigation into Cancer and Nutrition Potsdam cohort1–3

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ABSTRACT

Background: Retrospective studies indicate that low concentrations of plasma pyridoxal-5-phosphate (PLP) are associated with cardiovascular events; however, few prospective studies of this issue have been conducted.

Objective: We therefore investigated whether PLP concentrations are independently associated with myocardial infarction (MI) in the European Investigation into Cancer and Nutrition (EPIC) Potsdam Study.

Design: After exclusion of prevalent MI or stroke, incident cases of MI were identified among 26 761 participants (aged 35–65 y at baseline). The current analysis is based on a nested case-cohort study consisting of a control group of 810 subjects without MI or stroke at baseline and a case group of 148 subjects who had an MI during a mean follow-up period of 6.0 ± 1.5 y. Cox proportional hazard models were used to evaluate the association between plasma PLP and risk of MI.

Results: In the age- and sex-adjusted analysis, subjects in the highest quintile of PLP had a significantly reduced risk of MI (hazard ratio: 0.50; 95% CI: 0.29, 0.83). Adjustment for either low-grade inflammation or smoking diminished this association. When both low-grade inflammation and smoking were adjusted for, the association was abolished. In addition, adjustment for established risk factors also abolished the association between PLP and risk of MI.

Conclusion: These findings from a prospective German cohort study suggest that PLP is not independently associated with risk of MI. Am J Clin Nutr 2007;86:214–20.

KEY WORDS Cardiovascular disease, epidemiology, vitamin B-6, inflammation, smoking

INTRODUCTION

There is increasing interest in B vitamins as risk factors for cardiovascular disease (CVD) because of the recognition of homocysteine as a potential risk factor for CVD (1), and it has been recognized that subnormal, but not necessarily deficient, concentrations of vitamins can be associated with an increased risk of CVD (2). Of the vitamins of interest, vitamin B-6 has received much attention because of its involvement in homocysteine metabolism, polyunsaturated fatty acid and lipid metabolism, and protein metabolism. Furthermore, vitamin B-6 was shown to inhibit platelet aggregation and endothelial cell proliferation, which suggests that it has an antithrombotic effect (3). Epidemiologic evidence for a potential protective action of vitamin B-6 first emerged from small studies in patients after a myocardial infarction (MI) (4) and in mostly retrospective case-control studies involving patients with coronary, cerebral, or peripheral atherosclerotic disease (5–11). In most of those retrospective studies an association was found between low plasma pyridoxal-5-phosphate (PLP) and CVD. In addition, 2 corresponding prospective studies were published: one involved men participating in the Physicians’ Health Study (12), the other men and women of the Atherosclerosis Risk Factors in the Community (ARIC) study (13). These prospective studies reported divergent results for the association between PLP and CVD. Although in the Physicians’ Health Study the association was no longer significant after adjustment for established CVD risk factors, in the ARIC study multiple adjustment did not alter the results. Regardless of the study design, all studies so far investigated the risk of CVD associated with the plasma PLP concentration as a measure of the nutritional status of vitamin B-6. Subsequently, the interpretation of low plasma PLP became more difficult, because low-grade inflammation was found to be associated with low plasma PLP.

Friso et al (14) showed that in the Framingham cohort chronic inflammation modulated plasma PLP concentrations. Despite similar vitamin B-6 intake, the plasma PLP concentrations were significantly lower in subjects with C-reactive protein (CRP) > 6 mg/L than subjects with CRP concentrations < 6 mg/L. This observation was confirmed in healthy elderly subjects in the United Kingdom (15), patients with rheumatoid arthritis (16),...
inflammatory bowel disease (17), and type 2 diabetes (18). During the past decade the view on chronic inflammation as a cardiovascular disease risk factor changed considerably because of the discovery that chronic low-grade chronic inflammation is present in patients at high risk of CVD or patients with CVD (19, 20). The diagnosis of chronic low-grade inflammation became only possible after the introduction of measurement of highly sensitive CRP (hsCRP) as a sensitive laboratory method (21, 22). These findings question in particular the results of the retrospective studies on low plasma PLP and risk of CVD. So far, neither of the prospective studies and only 2 of the retrospective studies were adjusted for hsCRP (10, 11). Although Prisco et al (10) observed a significant association between low plasma PLP and CVD both before and after adjustment for CRP in patients with coronary artery disease, Dierkes et al (11) reported that the association disappeared completely after adjustment for hsCRP in a study involving women with prior MI or acute coronary syndrome.

There is therefore a need for prospective studies on the association between plasma PLP and cardiovascular disease risk that would take first markers of inflammation into account in the statistical procedure, and, second, investigate the associations of plasma PLP with other cardiovascular disease risk factors. In this analysis, we present data from the European Prospective Investigation into Cancer and Nutrition (EPIC) Potsdam cohort in Germany, with respect to the association of plasma PLP and future risk of MI.

SUBJECTS AND METHODS

Study population

The EPIC Potsdam cohort was recruited between August 1994 and September 1998 as part of the multicenter EPIC study. Details of this cohort as a part of the German study population were published elsewhere (23). Briefly, women aged 35–65 y and men aged 40–65 y were recruited at random from the general population. In total, 27,548 subjects (16,644 women and 10,904 men) underwent the baseline examination, including standardized blood pressure measurements, measurements of weight and height, self-administered questionnaires on diet and lifestyle, computer-guided interviews, and blood sampling. The study was approved by the Ethics Committee of Federal State Brandenburg, Germany, and written informed consent was obtained from all participants.

After the exclusion of subjects with a history of MI and stroke at baseline, we identified 156 participants who had a MI during follow-up until 30 April 2004. For the purpose of biomarker measurement a study in a case-cohort design was established (24, 25). In this study type, a random subcohort of the total baseline cohort is drawn as a reference group. If a subset member develops a disease of interest during follow-up, this person is also treated as a case and contributes to the exposure distribution among the cases (24). For the current analyses, a subcohort comprising 851 subjects free of MI and stroke at baseline was randomly drawn from the EPIC-Potsdam cohort (n = 26,761). Five incident MIs occurred among these subcohort members. For the present analyses we excluded 8 cases and 36 noncases without sufficient plasma or DNA samples, leaving a final case-cohort sample of 148 cases and a subcohort of 815 subjects, including 810 noncases and 5 cases.

Baseline examinations

A total of 30 mL venous blood was collected at baseline from each study participant during examinations for the cohort study at the Potsdam center. The plasma was separated from the blood cells within 2 h, and all samples were frozen at −80 °C until the time of analysis.

Standardized anthropometric measurements were performed by trained personnel (26). The body mass index (BMI; in kg/m²) was calculated. Lifestyle characteristics, including regular physical exercise and smoking history, were documented by trained interviewers during a computer-guided interview. Smoking status was expressed as “current smoker,” “nonsmoker,” and “former smoker.” Physical exercise was defined as the mean time spent on sporting activities during the summer and winter seasons (in h/wk). For the assessment of their medical history, the participants answered questions about past diseases and regular use of medication.

With the use of oscillometric devices (BOSO-Oscillomat; Bosch & Sohn, Jungingen, Germany), presence of hypertension was defined on the basis of the mean values of the second and third blood pressure measurements during baseline examinations as systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg or from the subjects’ reports, disease-specific medication, or verification from medical records. Definition of diabetes was based on subjects’ reports or disease-specific medication or verification from medical records.

At baseline, the dietary habits during the preceding year were assessed by a validated self-administered food-frequency questionnaire (27, 28). The food-frequency questionnaire included questions on frequency and portion size of 146 individual food items and on regular use of vitamin supplements (during the previous year); it was described in detail elsewhere (29).

Definition of MI

Every 2–3 y, the participants were asked to complete a mailed follow-up questionnaire that included sections about self-reported MI. Potential incident MIs were verified from medical records or death certificates, applying the criteria based on the WHO-MONICA (MONItoring CArdiovascular disease) study (Internet: http://www.ktl.fi/publications/monica/manual/part4/iv-1.htm); Thus, cases were defined as incident MIs, according to codes I21.0–I21.9 from the 10th revision of the International Classification of Diseases, occurring between the baseline examination and 30 April 2004.

Blood measurements

Blood samples of selected study subjects were dispatched to the Institute of Clinical Chemistry on dry ice to be analyzed for PLP, folate, and creatinine. Plasma folate was measured with the use of a commercial test assay (Roche Diagnostics, Mannheim, Germany). PLP was measured by HPLC with fluorescence detection with the use of a commercial test kit (Immundiagnostik GmbH, Bensheim, Germany). The normal range of this assay is >18.2 nmol/L. Creatinine was measured enzymatically on a random access autoanalyzer (Roche Diagnostics). Plasma homocysteine was measured by HPLC with fluorescence detection (30). Blood lipids and hsCRP were measured at the German Institute of Human Nutrition Potsdam-Rehbrücke. Total and HDL cholesterol and hsCRP were measured with the use of standard methods with reagents from Horiba ABX (Shefford,
United Kingdom). The intraassay CVs were 0.9% (total cholesterol), 1.2% (HDL cholesterol), and 2.6% (hsCRP), and the interassay CVs were 4.7%, 5.2%, and 7.9%, respectively.

Statistics

The statistical analysis was performed with the use of SAS software package, release 9.1 (SAS Institute, Cary, NC). All tests were 2-sided, \( P < 0.05 \) as the significance threshold. Associations of PLP with the plasma concentrations of hsCRP, HDL cholesterol, creatinine, folate, and cobalamin and also with dietary variables such as intakes of vitamin B-6 and folate were studied by calculating Spearman \( \rho \) coefficients. The effect of smoking on PLP was tested with the use of the general linear model with 3 smoking categories. Cox proportional hazard regression analysis was used for the examination of the relation between PLP and risk of MI. Age was the underlying time variable in the counting process, with entry defined as the subjects’ age at the time of recruitment and exit defined as age at the diagnosis of MI or censoring. As suggested by Prentice (24), the Cox models were modified to account for the case-cohort design. With the Prentice method, participants within the subcohort are given a weight of 1 at all times, whereas cases outside the subcohort have a weight of 1 at time of event and have weight of 0 at all other times (24). Hazard rate ratios were calculated for quintiles of PLP. Quintiles of PLP plasma concentrations are based on distribution of the subcohort. The lowest quintile was used as the reference category. The association of PLP with risk of MI was first calculated after adjustment for age and for sex. As a control for the known association of hsCRP on PLP, hsCRP was first introduced into the age- and sex-adjusted model. Because smoking also showed a significant association with PLP, a model containing age, sex, and smoking in 3 categories (never smoked, former smoker, current smoker) was constructed. In addition, a model of age, sex, and the ratio of total cholesterol to HDL cholesterol (total cholesterol:HDL cholesterol) was constructed. The effect of multiple risk factors on the association of PLP with risk of MI was then tested in a model consisting of age and sex, hsCRP, smoking, and total cholesterol:HDL cholesterol. To this model, other known risk factors for ischemic heart disease were then added, including calculated alcohol intake, regular exercise (<2 h/wk, \( >2 \) h/wk), educational level (university degree, no university degree), and in addition BMI, hypertension, and diabetes mellitus. Tests for trends across increasing quintiles of PLP concentration were conducted by using the quintile number as a continuous variable in Cox proportional hazard models.

To evaluate putative interactions between PLP and important confounders, multiplicative interaction terms of PLP and sex, PLP and smoking, and PLP and hsCRP were included and tested in the fully adjusted Cox regression model. Furthermore, we investigated whether the effect of PLP on incidence of MI was changing over time, applying the test for proportionality assumption of the Cox model. The proportionality assumption was not rejected in our analyses, suggesting that the influence of PLP on incidence of MI did not change over time.

RESULTS

Subject characteristics

The current analysis is based on a nested case-cohort study consisting of 815 subjects in a subcohort of the EPIC Potsdam study and 148 subjects who had an MI during a mean follow-up of 6.0 \( \pm \) 1.5 y. One hundred eleven cases were nonfatal and the remaining 37 were fatal.

Selected baseline characteristics according to PLP quintiles are presented in Table 1. Subjects with higher PLP concentrations were less likely to be men, to be current smokers, or to have a history of hypertension or diabetes at baseline than were subjects with low PLP concentrations. The plasma PLP at baseline correlated significantly with vitamin B-6 intake \( (r = 0.15, n = 958, P < 0.001) \), hsCRP \( (r = -0.25, n = 958, P < 0.001) \), homocysteine \( (r = -0.11, n = 958, P < 0.001) \), both folate intake \( (r = 0.17, n = 958, P < 0.001) \) and plasma folate \( (r = 0.31, n = 958, P < 0.001) \), and HDL cholesterol \( (r = 0.10, n = 958, P < 0.01) \). The association between PLP and hsCRP remained unchanged when 4 subjects with CRP \( >20 \) mg/L (indicating acute inflammation) were excluded. Smoking exhibited a significant effect on plasma PLP, with lower concentrations in current smokers compared with former smokers or never smokers (Table 2).

PLP and risk of MI

In the Cox regression model adjusted for age and sex, higher quintiles of plasma PLP were significantly associated with a lower risk of MI (Table 3). The relative risk for subjects in the fifth quintile for plasma PLP was 0.50 (95% CI: 0.29, 0.83), compared with subjects with plasma PLP in the lowest quintile. This association was substantially weakened after adjustment for either hsCRP or smoking or for total cholesterol:HDL cholesterol. When the model was adjusted for the combination of these risk factors, the association of PLP and risk of MI was completely abolished.

Further adjustment for other factors known to be associated to coronary risk, such as alcohol intake, physical activity, educational level, hypertension, diabetes, or BMI did not change this result substantially (Table 3). Further adjustment for homocysteine did not change this result substantially (data not shown). When the Cox analysis was performed for PLP tertiles or quartiles, the associations between PLP and risk of MI became weaker. In fact, the association between tertiles or quartiles of PLP and risk of MI disappeared already after adjustment for hsCRP alone (data not shown). We did not observe any statistically significant interaction between PLP and sex, PLP and smoking, or PLP and hsCRP (data not shown).

PLP compared with other risk factors

In the fully adjusted model, established risk factors such as total cholesterol:HDL cholesterol, hypertension at baseline, smoking, and hsCRP still predicted MI. The hazard ratios were 1.66 (95% CI: 1.42, 1.96) for one unit increase of the total cholesterol:HDL cholesterol, 2.02 (95% CI: 1.31, 3.11) for hypertension at baseline, 3.03 (95% CI: 2.03, 4.53) for smoking, and 1.08 (95% CI: 1.01, 1.15) for one unit increase of hsCRP.

DISCUSSION

The present work is the first European prospective study on the association between plasma PLP and cardiovascular disease risk, after the publication of numerous retrospective studies and 2 American studies with nested case-control design (12, 13). The principal finding of the present nested case-cohort study is that the plasma PLP was not associated with future MI in a cohort of
German middle-aged men and women healthy at the time of entry into the study and after adjustment for known cardiovascular disease risk factors. This disagrees with the findings reported by other groups in studies with retrospective design (5, 8, 10) and with studies that also followed a nested case-control design (13), but it is in accord with earlier, prospective studies (6, 7, 11), including our own, and with one of the prospective studies (12).

The main reason to doubt the existence of a causal association between PLP and CVD was the increasing evidence of an effect of the inflammatory response on plasma PLP. This was first introduced into the study and after adjustment for known cardiovascular disease risk factors. This disagrees with the findings reported by other groups in studies with retrospective design (5, 8, 10) and with studies that also followed a nested case-control design (13), but it is in accord with earlier, prospective studies (6, 7, 11), including our own, and with one of the prospective studies (12).

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We also observed an association between HDL-cholesterol and plasma PLP concentrations, especially in men. To our knowledge, no studies in the general population reported this association earlier. This is therefore an interesting observation that deserves future investigations.

The idea that high plasma PLP concentrations may be cardioprotective is indeed supported by some evidence derived from biochemical studies. PLP is a coenzyme of numerous biological pathways in the carbohydrate, amino acid, protein, and lipid metabolism. This offers a great number of mechanisms that may contribute to an antiatherogenic action of PLP. Among these, PLP is a coenzyme of d6-desaturase, involved in the metabolism of polyunsaturated fatty acids (34, 35), and a coenzyme of cystathionine-β-synthase involved in methionine, homocysteine, and cysteine metabolism (36). Furthermore, it can occupy glycoprotein IIb or IIIa, a major receptor on platelets responsible for platelet aggregation (3), and it was shown that PLP can inhibit proliferation of endothelial cells (37).

Epidemiologic studies on vitamin B-6 intake, however, are also inconsistent about whether a higher intake of vitamin B-6 is antiatherogenic. In the Nurses’ Health Study, a higher intake of vitamin B-6 was associated with a reduced risk of MI (38). However, the effect was mostly due to the use of supplements and not related with future strokes (39). In the ARIC study, the association earlier. This is therefore an interesting observation for trend

<table>
<thead>
<tr>
<th>Cases (n)</th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
<th>Q5</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age- and sex-adjusted</td>
<td>42</td>
<td>33</td>
<td>19</td>
<td>29</td>
<td>25</td>
<td>0.004</td>
</tr>
<tr>
<td>Age, sex, hsCRP</td>
<td>1.0</td>
<td>0.83 (0.52, 1.30)</td>
<td>0.44 (0.25, 0.77)</td>
<td>0.62 (0.38, 1.00)</td>
<td>0.50 (0.29, 0.83)</td>
<td>0.004</td>
</tr>
<tr>
<td>Age, sex, smoking</td>
<td>1.0</td>
<td>0.80 (0.50, 1.28)</td>
<td>0.48 (0.28, 0.84)</td>
<td>0.70 (0.43, 1.13)</td>
<td>0.57 (0.34, 0.96)</td>
<td>0.03</td>
</tr>
<tr>
<td>Age, sex, choHDL-C</td>
<td>1.0</td>
<td>1.04 (0.65, 1.65)</td>
<td>0.59 (0.34, 1.03)</td>
<td>0.82 (0.50, 1.32)</td>
<td>0.64 (0.38, 1.07)</td>
<td>0.05</td>
</tr>
<tr>
<td>Age, sex, iHcy</td>
<td>1.0</td>
<td>0.98 (0.62, 1.54)</td>
<td>0.55 (0.31, 0.96)</td>
<td>0.74 (0.47, 1.17)</td>
<td>0.64 (0.37, 1.10)</td>
<td>0.05</td>
</tr>
<tr>
<td>Combined models Age, sex, hsCRP, smoking, choHDL-C</td>
<td>1.0</td>
<td>1.15 (0.71, 1.87)</td>
<td>0.74 (0.43, 1.31)</td>
<td>1.05 (0.66, 1.68)</td>
<td>0.88 (0.51, 1.54)</td>
<td>0.62</td>
</tr>
<tr>
<td>Age, sex, + above + alcohol intake, physical activity, and education</td>
<td>1.0</td>
<td>1.16 (0.71, 1.89)</td>
<td>0.76 (0.42, 1.37)</td>
<td>1.08 (0.65, 1.78)</td>
<td>0.89 (0.49, 1.62)</td>
<td>0.71</td>
</tr>
<tr>
<td>Age, sex, + above + hypertension at baseline, diabetes at baseline, BMI</td>
<td>1.0</td>
<td>1.22 (0.73, 2.05)</td>
<td>0.79 (0.44, 1.41)</td>
<td>1.29 (0.76, 2.19)</td>
<td>1.04 (0.57, 1.89)</td>
<td>0.82</td>
</tr>
</tbody>
</table>

1 Quintiles are based on distribution of PLP in control subjects. Smoking was defined in 3 categories with current smokers (n = 235), former smokers (n = 68), and never smokers (n = 655). Physical activity was defined in 2 categories of regular exercise of <2 h/wk or ≥2 h/wk. Education was defined in 2 categories of university degree or no university degree. hsCRP, highly sensitive C-reactive protein, choHDL-C, total cholesterol-to-HDL-cholesterol ratio.
epidemiologic studies other means of vitamin B-6 status (both measurements of plasma PLP and whole-blood PLP, or plasma or urinary pyridoxic acid), and major confounders should be used if possible to clarify the association between vitamin B-6 and CVD.

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The authors’ responsibilities were as follows—JD and CW: wrote the first draft of the manuscript, conducted the statistical analysis, and contributed equally; JD, SW, CL, MM, and JS: performed the laboratory measurements; HH: planned and conducted the EPIC-Potsdam Study; KK-G: contributed to the study design, acquisition, and interpretation of data in the EPIC-Potsdam Study. All authors critically revised the manuscript for important intellectual content. None of the authors had a conflict of interest in relation to this study.

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