Plasma Pyridoxal-5-Phosphate Is Inversely Associated with Systemic Markers of Inflammation in a Population of U.S. Adults

Lydia Sakakeeny,¹ Ronenn Roubenoff,³ Martin Obin,² Joao D. Fontes,⁸ Emelia J. Benjamin,⁸ Yoram Bujanover,⁷ Paul F. Jacques,⁶ and Jacob Selhub⁴

Abstract

Low vitamin B-6 status, based on plasma concentrations of pyridoxal-5-phosphate (PLP), has been identified in inflammatory diseases, including cardiovascular disease, rheumatoid arthritis, inflammatory bowel disease, and diabetes. Our objective was to examine the association between plasma PLP and multiple markers of inflammation in a community-based cohort \( \{n = 2229 \text{ participants (55\% women, mean age 61 ± 9 y)} \} \). We created an overall inflammation score (IS) as the sum of standardized values of 13 individual inflammatory markers. Multivariable-adjusted regression analysis was used to assess the associations between the IS and plasma PLP. Geometric mean plasma PLP concentrations were lower in the highest tertile category of IS relative to the lowest \( 61 \text{ vs. 80 nmol/L;} \ P\text{-trend < 0.0001} \). Similarly, the prevalence of PLP insufficiency was significantly higher for participants in the highest compared with the lowest tertiles for IS categories. These relationships persisted after accounting for vitamin B-6 intake. Also, there were significant inverse relationships between plasma PLP and 4 IS based on functionally related markers, including acute phase reactants, cytokines, adhesion molecules, and oxidative stress. In addition, secondary analyses revealed that many of the individual inflammatory markers were inversely associated with plasma PLP after adjusting for plasma C-reactive protein concentration. This study, in combination with past findings, further supports our hypothesis that inflammation is associated with a functional deficiency of vitamin B-6. We discuss 2 possible roles for PLP in the inflammatory process, including tryptophan metabolism and serine hydroxymethyltransferase activity. J. Nutr. 142: 1280–1285, 2012.

Introduction

Low vitamin B-6 status, marked by low concentrations of plasma pyridoxal-5'-phosphate (PLP)¹⁰, has been identified as a risk factor for cardiovascular disease (CVD) morbidity and mortality, including myocardial infarction (1), atherosclerosis (2), and stroke (3). Patients with confirmed CVD often present with lower PLP plasma concentrations compared with healthy controls (1,4,5). Low plasma PLP is also seen in patients with rheumatoid arthritis (6–9), inflammatory bowel disease (10,11), and diabetes (12,13).

Inflammation is generally recognized as a contributing factor to the development of CVD (14). For instance, C-reactive protein (CRP), an acute phase protein synthesized and secreted by the liver in response to proinflammatory cytokines (15), is a powerful indicator of future CVD risk (16). A recent analysis of >2500 participants in the 2003–2004 NHANES found an inverse relationship between vitamin B-6 status and serum CRP concentrations, which remained significant after adjusting for vitamin B-6 intake, supplement use, and homocysteine levels among other covariates (17). This large population study confirmed previous findings of homocysteine-independent inverse associations between plasma PLP and CRP (18–20) and other markers of inflammation (10,19).

The present study was undertaken to further understand the basis of the associations between vitamin B-6 and inflammation...
by examining the relationship between vitamin B-6 status and overall inflammation, functional indicators of inflammation, and individual biomarkers of inflammation.

Participants and Methods

Participants

The design and selection criteria of the Framingham Offspring Study have been described elsewhere (21). Men and women recruited in 1971 have been examined every 3–8 y since. For this cross-sectional analysis of the relations between vitamin B-6 status and inflammatory biomarkers, data were used from the seventh examination, which took place from 1998 to 2001. Participants underwent routine physical examination, medical history, and laboratory assessments. Participants were excluded from the current study if they did not have valid dietary intake information (n = 134), were missing data on inflammatory biomarkers (n = 1014; excluding TNFα, which was measured on a subset of the cohort), or were missing data on other covariates (n = 162). Of the 3539 members of the cohort who participated in the seventh study examination, data on 2229 men and women were available for analysis. The Framingham Heart Study protocol is reviewed annually by the Boston University Medical Center Institutional Review Board and all participants signed written informed consent.

Plasma PLP

Vitamin B-6 status was assessed by plasma PLP concentrations. Fasting blood samples were collected at the seventh examination. Plasma PLP was assayed by the tyrosine decarboxylase apoenzyme method (22).

Inflammatory biomarkers

Single measurements of plasma CRP were made using a high-sensitivity assay while the following inflammatory biomarkers were measured in duplicate from fasting blood samples taken during the seventh examination cycle (1998–2001) using commercially available enzyme-linked immunosorbent assays (11): plasma cluster of differentiation 40 ligand (CD40L), plasma P-selectin, plasma osteoprotegerin, plasma TNFα, plasma TNF receptor 2 (TNFR-2), serum soluble intercellular adhesion molecule-1 (ICAM-1), serum IL-6, serum monocyte chemotactic protein-1 (MCP-1), serum myeloperoxidase, plasma lysosomal phospholipase A2 (LPL-A2) mass and activity, and urinary isoprostanes indexed to urinary creatinine. Plasma fibroinogen was measured in duplicate using the clot-time method of Claus (23) with Diagnostica Stago reagents.

Using the aforementioned markers of inflammation, we developed 2 types of scores to represent inflammation: a score representative of overall inflammation and scores based on markers that are thought to be functionally interrelated (24). The individual marker values were first standardized as Z-scores and then summed to compute the different inflammation indices, divided into tertile categories, were used as independent variables. Covariates included: age; sex; BMI; plasma homocysteine, folic acid, and vitamin B-12; serum creatinine and total cholesterol; vitamin B-6, protein, and energy intakes; multivitamin supplement and NSAID use; and current smoking status.

Primary analyses.

To assess associations between biomarkers of inflammation and plasma PLP concentrations, we used multivariable regression analysis to calculate geometric mean plasma PLP (95% CI) across tertile categories of inflammatory biomarkers. In our multivariable regression analysis, plasma PLP, normalized by natural log transformation, was used as a continuous dependent variable and the inflammation indices, divided into tertile categories, were used as independent variables. Covariates included: age; sex; BMI; plasma homocysteine, folic acid, and vitamin B-12; serum creatinine and total cholesterol; vitamin B-6, protein, and energy intakes; multivitamin supplement and NSAID use; and current smoking status.

Additionally, to examine the interrelationship among vitamin B-6 intake, inflammatory status, and plasma PLP concentrations, we constructed a graph presenting the geometric means of plasma PLP adjusted for the abovementioned covariates and stratified by tertile categories of inflammatory status as indicated by IS and tertile categories of vitamin B-6 intake from diet and supplements.

Secondary analyses.

In a secondary analysis, we examined the prevalence (95% CI) of inadequate PLP [plasma PLP < 20 nmol/L (8)] across tertile categories of the overall IS. We also assessed associations between individual biomarkers of inflammation and plasma PLP concentrations using multivariable regression analysis, where plasma PLP was used as a continuous dependent variable and the individual biomarkers of inflammation, divided into tertile categories, were used as independent variables. Each biomarker was entered into the models one at a time. Where noted, natural log-transformed CRP was entered into the model as a continuous covariate to determine whether the relationship between plasma PLP concentrations and individual biomarkers persisted after accounting for CRP. Other covariates included: age; sex; BMI; plasma homocysteine, folic acid, and vitamin B-12; serum creatinine and total cholesterol; vitamin B-6, protein, and energy intakes; multivitamin supplement and NSAID use; and current smoking status.

Results

Characteristics of the population. There was no association between sex or age and plasma PLP (Table 1). However, BMI

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was lower with increasing plasma PLP tertile categories. Similarly, the percentage of cigarette smokers and the prevalence of both diabetes and CVD were lower with increasing plasma PLP tertile categories. Homocysteine and 2 indicators of inflammation (CRP and IS) were also lower with increasing tertile categories of plasma PLP. On the other hand, the percentage of participants taking multivitamin supplements was higher with increasing tertile categories of plasma PLP. Additionally, the intakes of energy, protein, and vitamin B-6 were higher with increasing tertile categories of plasma PLP as were plasma folate and vitamin B-12 in these unadjusted analyses.

**Relationship between inflammation and plasma PLP concentrations.** In multivariable-adjusted regression analysis, we found that geometric mean plasma PLP concentrations were significantly inversely associated with tertile category of the IS, including IS, IS-acute phase reactants, IS-cytokines, and IS-oxidative stress (Table 2). For IS, the mean plasma PLP concentrations of individuals in the highest tertile category of inflammation were nearly 25% less than the mean plasma PLP concentrations for those in the lowest tertile category.

In our secondary analyses, we observed a significant trend toward increasing vitamin B-6 inadequacy (plasma PLP < 20 nmol/L) with increasing tertile categories of inflammation (Supplemental Fig. 1). The prevalence of low plasma PLP nearly doubled with increasing categories of inflammation. We also found lower mean plasma PLP concentrations in the higher tertile categories of individual inflammatory biomarkers, including ICAM (Table 2), CRP, fibrinogen, IL-6, lipoprotein phospholipase A2 (LPLP-A2) activity, TNFR-2, and TNFα (Supplemental Table 1). These relationships remained significant after additional adjustment for CRP (Supplemental Table 2). Of note, there was no inverse relationship between plasma folate and vitamin B-12, 2 other B-vitamins involved in homocysteine metabolism and overall inflammation (results not shown).

The analyses were rerun excluding participants with prevalent CVD and diabetes (n = 401) and these exclusions did not substantively affect observations (Supplemental Table 3).

**Relationships among inflammation, vitamin B-6 intake, and plasma PLP concentration.** Those with the highest intake of vitamin B-6 had increased concentrations of plasma PLP (Fig. 1). Yet, regardless of vitamin B-6 intake, those with the greatest degree of inflammation had significantly lower plasma PLP concentrations than their low inflammation counterparts. Additionally, there was no significant interaction between inflammation and vitamin B-6 intake when comparing participants with the highest tertile compared with the lower 2 tertile categories of vitamin B-6 intake category.

### Discussion

In this comprehensive study of the relations between vitamin B-6 status and inflammation, we have shown that overall inflammation is inversely associated with plasma PLP concentrations and that the prevalence of vitamin B-6 inadequacy increases with inflammation. The unique feature of this study is the demonstration of the inverse correlation of plasma PLP with multiple markers of inflammation, including an overall IS and scores representing functional groups. Such results expand on previous findings that showed inverse relationships between

### Table 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1 (35, 36)</th>
<th>2 (69, 71)</th>
<th>3 (177, 181)</th>
<th>P-trend&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants, n</td>
<td>743</td>
<td>743</td>
<td>743</td>
<td></td>
</tr>
<tr>
<td>Male, %</td>
<td>42</td>
<td>48</td>
<td>44</td>
<td>0.35</td>
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<tr>
<td>Age, y</td>
<td>61 (61, 62)</td>
<td>61 (61, 62)</td>
<td>61 (60, 62)</td>
<td>0.87</td>
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<tr>
<td>BMI, kg/m²</td>
<td>29 (29, 30)</td>
<td>28 (27, 28)</td>
<td>27 (27, 28)</td>
<td>&lt;0.001</td>
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<tr>
<td>Multivitamin use, % yes</td>
<td>21</td>
<td>52</td>
<td>82</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NSAID use, % yes</td>
<td>10</td>
<td>11</td>
<td>11</td>
<td>0.56</td>
</tr>
<tr>
<td>Cigarette use, % yes</td>
<td>21</td>
<td>10</td>
<td>8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes, % yes</td>
<td>10</td>
<td>7</td>
<td>6</td>
<td>0.001</td>
</tr>
<tr>
<td>CVD, % yes</td>
<td>14</td>
<td>13</td>
<td>11</td>
<td>0.1</td>
</tr>
<tr>
<td>Energy intake, kcal/d</td>
<td>1770 (1720, 1810)</td>
<td>1820 (1780, 1870)</td>
<td>1870 (1830, 1910)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Protein intake, g/d</td>
<td>76.3 (74.2, 78.4)</td>
<td>77.9 (75.8, 80.0)</td>
<td>82.5 (80.4, 84.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vitamin B-6 intake, g/d</td>
<td>2.7 (0.9, 4.5)</td>
<td>5.2 (3.4, 6.9)</td>
<td>18.8 (16.8, 20.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma cholesterol, mmol/L</td>
<td>199 (196, 201)</td>
<td>201 (198, 203)</td>
<td>201 (198, 203)</td>
<td>0.21</td>
</tr>
<tr>
<td>Plasma homocysteine, μmol/L</td>
<td>9.3 (9.0, 9.6)</td>
<td>8.3 (8.0, 8.6)</td>
<td>8.0 (7.7, 8.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma folate, nmol/L</td>
<td>12.3 (11.8, 12.7)</td>
<td>16.4 (15.8, 17.0)</td>
<td>21.1 (20.3, 21.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma vitamin B-12, pmol/L</td>
<td>340 (331, 349)</td>
<td>407 (397, 418)</td>
<td>473 (461, 486)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum creatinine, μmol/L</td>
<td>94.6 (91.9, 96.4)</td>
<td>94.8 (92.8, 97.2)</td>
<td>95.5 (92.8, 97.2)</td>
<td>0.60</td>
</tr>
<tr>
<td>Plasma C-reactive protein, mg/L</td>
<td>3.1 (2.9, 3.4)</td>
<td>2.1 (1.9, 2.3)</td>
<td>1.8 (1.6, 1.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IS&lt;sup&gt;3&lt;/sup&gt;</td>
<td>1.7 (1.3, 2.1)</td>
<td>−0.2 (−0.6, 0.2)</td>
<td>−1.1 (−1.5, −0.7)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

1 Values are mean (95% CI), geometric mean (95% CI) or percent. CD40L, CD40 ligand; CVD, cardiovascular disease; ICAM-1, intracellular adhesion molecule-1; IS, inflammation score; LPLP-A2, lipoprotein phospholipase A2; NSAID, nonsteroidal antiinflammatory drug; PLP, pyridoxal-5-phosphate; TNFR-2, TNF receptor-2.
2 P-value of the regression coefficient for the independent variable
3 Summed Z-scores of: CRP, fibrinogen, IL-6, TNFa, TNFR-2, osteoprotegerin, P-selectin, CD40L, ICAM-1, MCP-1, myeloperoxidase, LPLP-A2 mass, LPLP-A2 activity, and isoprostanes.
plasma PLP and inflammatory diseases (6,10,31) and/or individual biomarkers of inflammation, including CRP (17,19,32), IL-6 (19), a1-antichymotripsin (33), thus revealing a nearly universal relationship between vitamin B-6 and numerous aspects of inflammation. These relationships persisted after accounting for relevant covariates, including homocysteine and vitamin B-6 intake. Additionally, there was no inverse relationship between plasma folate and vitamin B-12 and overall inflammation, indicating the inverse association between vitamin B-6 and inflammation did not extend to other B vitamins.

The mechanisms that underlie the associations between plasma PLP and markers of inflammation remain to be determined. An attractive hypothesis is that the low plasma PLP is a reflection of mobilization of this coenzyme into inflammatory sites. Because of the cross-sectional nature of the analyses presented in this paper, we cannot determine causality in the relationship between PLP and inflammation. However, Figure 1 illustrates that even among participants with the highest intake of vitamin B-6, there is a significant trend of decreasing plasma PLP with higher tertile categories of inflammation. This finding suggests that inflammation alters plasma PLP status. Further support for the interpretation that the inflammation-PLP association is secondary to the mobilization of PLP to inflammatory sites derives from a number of studies that showed that vitamin B-6 supplementation was without effect on inflammatory markers despite the improvement in plasma PLP levels (31,34,35).

Furthermore, some data suggest that inflammation-associated changes in PLP resemble those of CRP, because they reflect an acute phase response. In inflammatory bowel disease, low PLP was seen only in patients with active disease, whereas those in remission had normal plasma PLP levels without any apparent increase in vitamin B-6 intake (10). Similarly, Bates et al. (33) found that low plasma PLP among elderly during winter months was not coincident with lower vitamin B-6 intakes, but rather the concentrations were inversely associated with makers of the acute phase response and possibly the occurrence of winter illnesses.

The targets for PLP involvement during inflammation remain to be determined. However, the fact that associations with PLP include a large number of inflammatory markers, as presented in this study, strongly implies that its interaction with these targets precede the formation of these markers. Whether the association of PLP is obligatory to the formation of these inflammatory markers or merely coincidental is of potential importance.

A potential site of PLP involvement is the degradation of tryptophan. The kynurenine pathway, which is responsible for over 95% of tryptophan degradation, utilizes many PLP-dependent enzymes (36). Indoleamine 2,3-dioxygenase (37), one of the enzymes that catalyzes the initial rate-limiting step in the conversion of tryptophan to kynurenine, is upregulated by inflammatory stimuli (38). Kynurenine is further degraded by PLP-dependent enzymes to a number of metabolites (36). It is quite possible that the induction of indoleamine 2,3-dioxygenase in response to inflammatory stimuli will result in the mobilization of plasma PLP for use in the degradation of kynurenine formed by the action of this enzyme. Thus, the relations between plasma PLP and inflammation may be a passive consequence of its pivotal role in tryptophan metabolism.

A more active potential role for PLP in the inflammatory process is as a cofactor of serine hydroxymethyltransferase, an enzyme essential for 1-carbon metabolism (39). Serine hydroxymethyltransferase activity is increased in lymphocytes stimulated to proliferate and the addition of 4-deoxypyridoxine, an inhibitor of certain PLP-dependent enzymes, to lymphocyte cultures has been shown to inhibit mitogen-stimulated serine hydroxymethyltransferase activity and cause a decrease in cell proliferation as well as the production of the cytokines IL-1b, IL-2 and the expression of the cytokine receptor, IL-2 receptor (40). This observation suggests that PLP is required for serine hydroxymethyltransferase activity and thus plays a role in serine hydroxymethyltransferase-stimulated inflammation.

**Strengths and limitations.** Strengths of the present study include the community-based cohort design with systematic assessment of biomarkers and vitamin intake and levels, the strict laboratory quality control used to measure the outcome and exposure variables as well as clinical covariates, and the large cohort of men and women data available for analysis. Limitations include the observational nature of these associations, preventing any inference regarding a causal connection. The inclusion of participants without consideration for illness has strengths and limitations. Although their inclusion enhances the pool of individuals with inflammation, it is also possible that in primary analyses where the presence of chronic diseases was not controlled for, their inclusion introduces confounding. In secondary analyses where participants with diabetes and known CVD were excluded, the inverse association between vitamin B-6 status and inflammation remained significant. The multiple statistical tests conducted raise the possibility of false-positive
findings, particularly for the individual biomarkers. However, given the strength of the findings, the majority of the associations remain significant even after accounting for multiple testing. Finally, the generalizability of these findings to other populations is unclear. First, participants of the seventh examination of the Framingham Offspring Study had higher median intakes of vitamin B-6 [3.1 mg/d] from foods and supplements compared with 1.6 mg/d among supplement users who participated in the 1999-2000 NHANES [41], a population representative of the U.S. population. The implications of the findings presented in this paper may be far greater among a population whose vitamin B-6 intakes are substantially lower, because increased inflammation may result in severe vitamin B-6 inadequacy. Second, the participants were largely white and of European ancestry, so the generalizability to other ethnicities is limited.

In conclusion, because of the strong inverse relationship between inflammation and plasma PLP in this large community-based population and the prevalence of people with inflammatory conditions, as well as those with inflammatory/immune related conditions, further studies on the mechanism of the relationship between PLP and inflammation are clearly warranted. This phenomenon would be better examined in experimental models in which one can either induce inflammation and examine the impact on vitamin B-6 status or modify vitamin B-6 status and examine the impact on inflammation.

Acknowledgments

P.F.J., J.S., and Y.B. designed the research; L.S. performed statistical analyses; E.J.B. and J.D.F. provided essential materials and critical revisions of the manuscript; L.S., R.R., M.O., P.F.J., and J.S. wrote the paper; and L.S., P.F.J., and J.S. had responsibility for final content. All authors read and approved the final manuscript.

Literature Cited


