Low Circulating Vitamin B<sub>6</sub> Is Associated With Elevation of the Inflammation Marker C-Reactive Protein Independently of Plasma Homocysteine Levels

Simonetta Friso, MD; Paul F. Jacques, ScD; Peter W.F. Wilson, MD; Irwin H. Rosenberg, MD; Jacob Selhub, PhD

**Background**—Lower vitamin B<sub>6</sub> concentrations are reported to confer an increased and independent risk for cardiovascular disease (CVD). The mechanism underlying this relationship, however, remains to be defined. Other diseases, such as rheumatoid arthritis, are associated with reduced vitamin B<sub>6</sub> levels. Despite a clear distinction in pathophysiology, inflammatory reaction may be the major link between these diseases. We hypothesized a relationship between pyridoxal 5′-phosphate (PLP), the active form of vitamin B<sub>6</sub>, and the marker of inflammation C-reactive protein (CRP). We also evaluated whether total plasma homocysteine (tHcy), a well-defined risk factor for CVD and a major determinant of plasma PLP levels, had a possible role as a mediator of this hypothesized relationship.

**Methods and Results**—Data from 891 participants from the population-based Framingham Heart Study cohort were analyzed. Subjects were divided into 2 groups according to normal or elevated CRP values: group 1, CRP <6 mg/L; group 2, CRP ≥6 mg/L. Plasma PLP levels were substantially lower in group 2 than in group 1 (mean values in group 2, 36.5 mmol/L versus 55.8 mmol/L in group 1, P<0.001). In a multiple logistic regression model adjusted for tHcy, the association of PLP with CRP remained highly significant (P=0.003).

**Conclusions**—Low plasma PLP is associated with higher CRP levels independently of tHcy. This observation may reflect a vitamin B<sub>6</sub> utilization in the presence of an underlying inflammatory process and represent a possible mechanism to explain the decreased vitamin B<sub>6</sub> levels in CVD. (Circulation. 2001;103:2788-2791.)

**Key Words:** atherosclerosis ■ risk factors ■ homocysteine ■ vitamins ■ inflammation
cytokines regulating its plasma levels (eg, interleukin-6), have been used to predict the risk of cardiovascular events.\(^14\)

Furthermore, it was recently demonstrated that CRP induces adhesion molecule expression in human endothelial cells, supporting the hypothesis of a direct role for CRP in promoting an inflammatory component in the atherosclerotic process.\(^15\) In patients with RA, low plasma PLP levels have been found to be associated also with erythrocyte sedimentation rate and levels of the inflammatory cytokine tumor necrosis factor-\(\alpha\).\(^3\) More evidence for an inverse correlation between PLP and indices of acute-phase reaction, such as plasma \(\alpha_1\)-antichymotrypsin, copper, and blood leukocyte count, was also recently described in a sample of elderly subjects.\(^16\)

To evaluate the relationship between plasma PLP, the inflammation marker CRP, and tHcy, we analyzed data available from the 20th examination of the Framingham Heart Study, a well-characterized, population-based cohort.

### Methods

#### Study Population

The study sample consisted of the survivors from the original Framingham Heart Study cohort, an epidemiological study established in Framingham, Mass, during the period 1948 through 1950.\(^17\) The original cohort consisted of 5209 subjects of both sexes 30 to 62 years old. The surviving members of this cohort have been examined every 2 years, and in 1988 and 1989, 1402 survivors participated in the 20th examination. Institutional review boards approved the study, and all patients gave informed consent. Characteristics of the study population have been described in detail elsewhere.\(^18\)

Additional covariates assessed for the present analyses were age, sex, cigarette smoking, diabetes, history of coronary artery disease, history of CVD, history of stroke events, and history of hypertension. Detailed operational definitions for all these covariables are provided elsewhere.\(^19,20\)

#### Laboratory Testing

Samples of venous blood were drawn from each subject to determine the concentration of CRP, tHcy, folate, vitamin \(B_6\), and PLP. tHcy was determined by high-performance liquid chromatography with fluorometric detection.\(^21\) Plasma folate was measured by a microbial assay (\textit{Lactobacillus casei}) in a 96-well plate.\(^22\) Plasma PLP was assayed by the tyrosine decarboxylase apoenzyme method.\(^23\) Plasma vitamin \(B_6\) was measured with a (Magic) radioimmunoassay kit from Ciba-Corning. Serum CRP was determined by an immunoturbidimetric assay (SPQ antibody reagent set II, DiaSorin). Creatinine levels were measured in nonfasting plasma by the Jaffé method, adapted for autoanalyzers. Dietary vitamin \(B_6\) intake was estimated from diet records by use of a semiquantitative food-frequency questionnaire.\(^24\)

#### Statistical Analysis

The statistical analyses were confined to a subset of 891 subjects for whom a complete set of CRP, vitamin, and tHcy values was available. To evaluate the relationships among plasma PLP, tHcy, and CRP, we divided the population into 2 groups according to CRP values: group 1, CRP <6 mg/L, ie, within the range of normality (n=834, 93.6%); group 2, CRP \(\geq\)6 mg/L, ie, increased levels (n=57, 6.4%). Distributions of continuous variables were expressed as mean\(\pm\)SD. Logarithmic transformation was performed on all skewed variables to normalize their distributions. Therefore, geometric means (antilogarithms of the transformed means) are presented for tHcy, folate, PLP, vitamin \(B_6\), creatinine, albumin, and dietary vitamin \(B_6\) intake. Ninety-five percent CIs for the geometric means and these intervals are displayed as the antilogarithm of the transformed data. Adjust-

### Results

The relevant characteristics of the study sample divided according to CRP values, ie, group 1, CRP <6 mg/L and group 2, CRP \(\geq\)6 mg/L, are shown in Table 1. As indicated, the 2 groups did not differ with respect to age, sex, plasma folate, vitamin \(B_6\), dietary vitamin \(B_6\) intake, and creatinine levels. Neither were they significantly different in terms of clinical characteristics, such as prevalence of hypertension (group 1, 58.6%; group 2, 67.4%, \(P=0.191\)), diabetes (group 1, 14.3%; group 2, 13.7%, \(P>0.2\)), CVD (group 1, 33.8%; group 2, 36.8%, \(P>0.2\)), and coronary heart disease (group 1, 20.4%; group 2, 17.5%, \(P>0.2\)). There was a higher prevalence of stroke occurrence in group 2 (12.3%) than in group 1 (6.2%); however, the difference was not statistically significant (\(P=0.08\)). Nonsmokers were 88.9% of the population sample, and the cumulative percentage of subjects smoking \(<10\) cigarettes/d was 91.6% (data not shown). Plasma PLP was significantly lower in the group with abnormal CRP values than in the other group (\(P<0.001\)). Serum albumin was significantly lower in group 2 than in group 1 (\(P<0.001\)). Nevertheless, the difference in albumin concentration between the 2 groups ranged within the reference intervals and did not match the greater difference in PLP concentration. The 2 groups did also differ in tHcy levels, but the difference did not reach statistical significance (\(P=0.063\)). To evaluate a possible role for tHcy in the relationship between PLP and CRP values, we performed a general linear model analysis.

### Table 1. Characteristics of the Framingham Heart Study Cohort According to CRP Levels

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group 1, CRP &lt;6 mg/L (n=834, 93.6%)</th>
<th>Group 2, CRP (\geq)6 mg/L (n=57, 6.4%)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>76±6 (n=834)</td>
<td>77±6 (n=57)</td>
<td>0.107</td>
</tr>
<tr>
<td>Sex, % male</td>
<td>41.1</td>
<td>49.1</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>Plasma folate, nmol/L*</td>
<td>10.1 (9.5–10.7)</td>
<td>9.3 (7.4–11.7)</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>Plasma PLP, nmol/L</td>
<td>55.8 (52.6–59.2)</td>
<td>36.5 (29.2–45.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vitamin (B_6), pmol/L†</td>
<td>276 (265–287)</td>
<td>274 (235–320)</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>Plasma tHcy, (\mu)mol/L</td>
<td>11.9 (11.6–12.2)</td>
<td>13.1 (11.9–14.5)</td>
<td>0.063</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>42.2 (42.0–42.5)</td>
<td>40.5 (39.6–41.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Creatinine, (\mu)mol/L</td>
<td>83.1 (81.6–84.6)</td>
<td>85.3 (79.6–91.5)</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>Vitamin (B_6) intake, mg/d‡</td>
<td>2.40 (2.26–2.55)</td>
<td>2.08 (1.62–2.67)</td>
<td>&gt;0.2</td>
</tr>
</tbody>
</table>

*Values are expressed as mean\(\pm\)SD or as indicated. Folate, PLP, vitamin \(B_6\), creatinine, and vitamin \(B_6\) intake data are presented as geometric means (antilogarithms of the transformed means), and 95% CIs are reported in parentheses with 2-tailed \(P\) values.

†Vitamin \(B_6\) levels were available for 888 subjects (group 1, n=831; group 2, n=57).

‡Vitamin \(B_6\) intake was available for 714 subjects (group 1, n=675; group 2, n=39).
As shown in Table 2, the strong association of PLP with CRP remained highly significant even after adjustment for tHcy (P<0.001). Conversely, after adjustment for PLP concentrations, the relationship between CRP and tHcy became clearly not statistically significant (P=0.571).

**Discussion**

These data demonstrate a strong association between decreased plasma PLP and increased levels of CRP, a major systemic marker of inflammation. Moreover, the association of PLP with CRP remained highly significant even after adjustment for tHcy, which is known to be an important metabolic indicator of vitamin B6 status as well as a cardiovascular risk factor. Furthermore, the low vitamin B6 levels were not attributable to impaired dietary intake, because it was similar in the 2 groups.

In a recent study, we also found a negative correlation between PLP and erythrocyte sedimentation rate, disease activity status, disease-related pain, joint swelling, and stiffness in patients with RA (E.-P. Chiang, PhD, et al, unpublished data, 2000). These results were consistent with data from similar studies.3 Furthermore, we demonstrated that the low PLP levels were not due to diminished vitamin B6 intake, nor were they associated with increased urinary excretion of 4-pyridoxic acid, an end product of vitamin B6 catabolism.

Despite the lack of a pathophysiological explanation for an association between PLP and markers of acute-phase status, a plausible interpretation of our data is that PLP is acting as a coenzyme for the inflammation-related functions. Because vitamin B6 is integrally involved in the synthesis of nucleic acids and consequently in mRNA and protein synthesis, the production of cytokines and other polypeptide mediators during the inflammatory response might require an increased utilization of this coenzyme. This model is consistent with the observation that vitamin B6 deficiency is associated with impairment in differentiation and maturation of monocyte-derived macrophages and T lymphocytes, inflammatory cells whose activation leads to the release of several enzymes and cytokines.25 Vitamin B6 deficiency was also reported to alter the regulation of interleukin-2 production.26

The present study, moreover, confirms observations from others of a lack of association of increased CRP and tHcy levels,27,28 suggesting that the relationship between tHcy and atherosclerosis cannot be explained through a link with CRP per se, whereas both are independent risk factors for CVD. Various conditions such as renal failure, smoking, and age are known to be associated with reduced levels of PLP. In addition, PLP, the predominant form of plasma vitamin B6, is primarily bound to albumin, whose diminished levels may result in lower values of circulating PLP. Adjustment for albumin, creatinine, age, sex, and smoking, however, did not affect the observed association.

Indeed, additional studies are necessary to clarify whether inflammation-associated decreases in circulating PLP play a role in the cascade of metabolic events related to certain diseases. PLP is one of the most important coenzymes in maintaining the balance between protein synthesis and degradation. Therefore, it is likely that low levels of PLP may reflect a higher utilization of the coenzyme in an underlying inflammatory process, rather than a defective intake or excessive vitamin B6 catabolism. The low circulating PLP seen as a possible indicator of an inflammatory status as well as a major determinant of tHcy levels may further our understanding of the mechanisms by which this metabolite acts as a risk factor for CVD.

**References**


**TABLE 2. Relationships Among Plasma tHcy, PLP, and CRP in the Framingham Heart Study Cohort**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1, CRP &lt;6 mg/L (n=834, 93.6%)</th>
<th>Group 2, CRP ≥6 mg/L (n=57, 6.4%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma PLP, nmol/L, mean (95% CI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multivariate model</td>
<td>55.8 (52.6–59.2)</td>
<td>36.5 (29.2–45.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adjusted for tHcy</td>
<td>55.5 (52.5–56.6)</td>
<td>39.5 (32.0–48.7)</td>
<td>0.003</td>
</tr>
<tr>
<td>Plasma tHcy, μmol/L, mean (95% CI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multivariate model</td>
<td>11.9 (11.6–12.2)</td>
<td>13.1 (11.9–14.5)</td>
<td>0.063</td>
</tr>
<tr>
<td>Adjusted for PLP</td>
<td>11.9 (11.7–12.2)</td>
<td>12.3 (11.2–13.5)</td>
<td>&gt;0.2</td>
</tr>
</tbody>
</table>

Values are expressed as geometric means (antilogarithms of the transformed means), and 95% CIs are reported in parentheses with 2-tailed P values.


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