Low plasma vitamin B-6 concentrations and modulation of coronary artery disease risk\textsuperscript{1–3}

Simonetta Friso, Domenico Girelli, Nicola Martinelli, Oliviero Olivieri, Valentina Lotto, Claudia Bozzini, Francesca Pizzolo, Giovanni Faccini, Federico Beltrame, and Roberto Corrocher

**ABSTRACT**

**Background:** Low concentrations of pyridoxal-5' phosphate (PLP), the active metabolite of vitamin B-6, are associated with high C-reactive protein (CRP) concentrations. Both low PLP and elevated inflammatory markers, such as high-sensitivity CRP (hs-CRP) and fibrinogen, are related to higher risk of coronary artery disease (CAD).

**Objectives:** The objectives were to evaluate the relation between PLP and acute-phase reactants in affecting CAD risk and to estimate the risk of CAD related to low plasma PLP, either alone or in combination with high concentrations of acute-phase reactants and other classic risk factors for CAD.

**Design:** A case-control study was conducted with 742 participants: 475 with severe multivessel CAD and 267 free from coronary atherosclerosis (CAD-free). We measured plasma PLP, fibrinogen, hs-CRP, and serum lipid concentrations and all major biochemical CAD risk factors, including total homocysteine.

**Results:** A significant, inverse, graded relation was observed between PLP and both hs-CRP and fibrinogen ($P < 0.001$). The prevalence of PLP concentrations in the lower half of the population (<50th percentile: 36.3 nmol/L) was significantly higher among CAD patients than among CAD-free subjects ($P < 0.001$). The odds ratio for CAD risk related to low PLP concentrations after adjustments for the major classic CAD risk factors, including hs-CRP and fibrinogen, was 1.89 (95% CI: 1.18, 3.03; $P = 0.008$). The CAD risk as a result of low PLP was additive when considered in combination with elevated hs-CRP concentrations or with an increased ratio of LDL to HDL.

**Conclusion:** Low plasma PLP concentrations are inversely related to major markers of inflammation and independently associated with increased CAD risk. *Am J Clin Nutr* 2004;79:992–8.

**KEY WORDS** Vitamin B-6, coronary artery disease, inflammation, C-reactive protein, fibrinogen, homocysteine, LDL:HDL ratio

**INTRODUCTION**

Early animal studies showed the occurrence of atherosclerotic lesions in monkeys fed a vitamin B-6–deficient diet (1). Further studies in humans identified an independent association of lower vitamin B-6 concentrations with higher risk of coronary artery disease (CAD) (2–4).

These reports are consistent with several possible mechanisms for a role of vitamin B-6 in the pathogenesis of atherosclerosis. The active metabolite of vitamin B-6, pyridoxal-5'-phosphate (PLP), serves as a coenzyme in a large number of reactions, most of which are related to amino acid metabolism, including that of homocysteine. Because of the role of PLP as a cofactor in the transsulphuration pathway of homocysteine, low plasma PLP concentrations represent a determinant of higher total plasma homocysteine (tHcy), an independent risk factor for occlusive vascular disease (5). Therefore, the role of PLP in atherosclerosis has been mostly addressed as a determinant of tHcy (6).

There are, however, other potential explanations for a role of PLP in atherogenesis. PLP was reported to affect platelet aggregation by way of inhibition of adenosine 5'-diphosphate receptors (7, 8) or down-regulation of the glycoprotein IIb gene expression (9). An impaired activity of lysyl oxidase, another vitamin B-6–dependent enzyme which is involved in the cross-linking of collagen and elastin, could favor arterial wall degeneration even in conditions of mild vitamin deficiency (10). Furthermore, low plasma PLP concentrations could increase the risk of CAD by affecting cholesterol metabolism, as shown by the advanced degree of hypercholesterolemia reported in a vitamin B-6–deficient animal model (11). An alternative possibility is that the relation between low PLP and increased CAD risk is due to the common relations with inflammatory markers.

In patients with rheumatoid arthritis, reduced plasma PLP concentrations were associated with inflammatory status and tumor necrosis factor $\alpha$ production (12). More recently, a cross-sectional survey reported an inverse association between concentrations of vitamin B-6 and plasma fibrinogen (13), another major marker of inflammation. Indeed, a growing body of evidence supports the theory of atherosclerosis as an inflammatory disease (14–16). Systemic markers of inflammation, such as...

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fibrinogen and, particularly, high-sensitivity C-reactive protein (hs-CRP), were proposed to be solid predictors of CAD risk (17–20).

We reported that low plasma concentrations of PLP are inversely related to C-reactive protein in the population-based Framingham Heart Study cohort (21). This association appeared strong and independent of other biomarkers related to vitamin B-6 metabolism (21). Other researchers reported, in healthy middle-aged adults, an inverse association of plasma PLP with white blood cell count but not C-reactive protein (22).

We designed the present study to investigate a cohort of subjects who were characterized by angiography for severe coronary atherosclerosis and a control group of CAD-free individuals, with the primary aim of evaluating the relation between plasma PLP and major markers of acute-phase reaction in affecting CAD risk.

**SUJECTS AND METHODS**

**Study population**

Criteria for selection of the study population were described in detail elsewhere (23, 24). For the present investigation we excluded subjects with conditions known to influence B-vitamin metabolism and tHcy concentrations, such as current or recent (ie, in the previous 3 mo) use of folate, vitamin B-6 or B-12 supplements, or any multivitamin preparation as well as current or recent use of drugs interfering with tHcy concentrations (ie, anticonvulsants, methotrexate, penicillamine). We also excluded subjects affected by any major systemic acute illness (including myocardial infarction in the previous 3 mo) use of folate, vitamin B-6 or B-12 metabolisms and tHcy concentrations, such as current or recent anticonvulsants, methotrexate, penicillamine. We also excluded subjects with conditions known to influence B-vitamin metabolism and tHcy concentrations, such as current or recent anticonvulsants, methotrexate, penicillamine. We also excluded subjects with conditions known to influence B-vitamin metabolism and tHcy concentrations, such as current or recent anticonvulsants, methotrexate, penicillamine. We also excluded subjects with conditions known to influence B-vitamin metabolism and tHcy concentrations, such as current or recent anticonvulsants, methotrexate, penicillamine.

We examined 742 unrelated adult patients recruited from patients referred to the Institute of Cardiovascular Surgery or to the Department of Clinical and Experimental Medicine in Verona, Italy. Of these patients, 475 had angiographically proven severe CAD, most of them being candidates for coronary artery bypass grafting. The existence of CAD was defined by the presence of at least 1 coronary artery with luminal stenosis > 50%. Two cardiologists, unaware that the patients were participating in the study, evaluated the coronary angiograms. As a control group, we considered 267 subjects with angiographically documented normal coronary arteries (CAD-free), examined for reasons other than potential CAD, mainly valvular heart disease. Furthermore, control subjects had neither history nor clinical or instrumental evidence of atherosclerosis in the other vascular districts. The whole population was from the same geographic area (Northern Italy), with a similar socioeconomic background. At the time of the blood sampling, a complete clinical history that included the assessment of cardiovascular risk factors such as smoking, hypertension, and diabetes was collected for all participants. The study was approved by the Ethics Committee of the University Hospital and University of Verona School of Medicine review boards. Informed consent was obtained from every subject after a full explanation of the study.

**Laboratory testing**

Samples of venous blood were drawn from each subject after an overnight fast. Serum lipids, as well as other CAD risk factors, including fibrinogen and tHcy, were determined immediately after collection, as previously described (23, 24). High-sensitivity C-reactive protein (hs-CRP) was measured by a particle-enhanced nephelometric immunoassay with commercially available methods in a BNII Behring Nephelometer Analyzer (Dade Behring Inc, Newark, DE). Plasma folate and vitamin B-12 concentrations were measured by an automated chemiluminescence method (Chiron Diagnostics, East Walpole, Massachusetts).

**TABLE 1**

Clinical and biochemical characteristics of the study cohort

<table>
<thead>
<tr>
<th></th>
<th>CAD-free subjects (n = 267)</th>
<th>CAD patients (n = 475)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (y)</strong></td>
<td>58.02 ± 12.93</td>
<td>60.32 ± 9.39</td>
</tr>
<tr>
<td><strong>M/F [n (%)]</strong></td>
<td>173/94 (64.8/35.2)</td>
<td>395/80 (83.1/16.9)</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>25.12 ± 3.45</td>
<td>26.57 ± 3.38</td>
</tr>
<tr>
<td><strong>Smoking (% of patients)</strong></td>
<td>40.6</td>
<td>69.7</td>
</tr>
<tr>
<td><strong>Hypertension (% of patients)</strong></td>
<td>28.3</td>
<td>57</td>
</tr>
<tr>
<td><strong>Diabetes mellitus (% of patients)</strong></td>
<td>5.3</td>
<td>13.8</td>
</tr>
<tr>
<td><strong>Total cholesterol (mmol/L)</strong></td>
<td>5.50 ± 1.06</td>
<td>5.96 ± 1.15</td>
</tr>
<tr>
<td><strong>LDL cholesterol (mmol/L)</strong></td>
<td>3.53 ± 0.9</td>
<td>4.08 ± 1</td>
</tr>
<tr>
<td><strong>HDL cholesterol (mmol/L)</strong></td>
<td>1.46 ± 0.4</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td><strong>Triacylglycerol (mmol/L)</strong></td>
<td>1.48 ± 0.70</td>
<td>2.02 ± 1.16</td>
</tr>
<tr>
<td><strong>PLP (nmol/L)</strong></td>
<td>33.9 (31.7, 36.2)</td>
<td>31.2 (29.8, 32.7)</td>
</tr>
<tr>
<td><strong>Hcy (µmol/L)</strong></td>
<td>49.8</td>
<td>62.9</td>
</tr>
<tr>
<td><strong>Homocysteine, fasting (µmol/L)</strong></td>
<td>15.0 (14.3, 15.7)</td>
<td>16.5 (15.9, 17.1)</td>
</tr>
<tr>
<td><strong>Folate (nmol/L)</strong></td>
<td>12.9 (12.2, 13.5)</td>
<td>11.5 (11.1, 12.0)</td>
</tr>
<tr>
<td><strong>Vitamin B-12 (pmol/L)</strong></td>
<td>305 (291, 320)</td>
<td>286 (276, 297)</td>
</tr>
<tr>
<td><strong>Fibrinogen (g/L)</strong></td>
<td>3.1 (3.0, 3.2)</td>
<td>3.4 (3.3, 3.5)</td>
</tr>
<tr>
<td><strong>hs-CRP (mg/L)</strong></td>
<td>1.9 (1.6, 2.2)</td>
<td>3.1 (2.8, 3.4)</td>
</tr>
<tr>
<td><strong>Creatinine (µmol/L)</strong></td>
<td>90.1 (88.4, 92.8)</td>
<td>96.3 (93.7, 98.1)</td>
</tr>
</tbody>
</table>

1 CAD, coronary artery disease; PLP, pyridoxal-5'-phosphate; hs-CRP, high-sensitivity C-reactive protein.

2 x ± SD (all such values).

3 P < 0.05 (Student’s t test).

4 P < 0.001 (chi-square test).

5 Geometric x; 95% CI in parentheses (all such values).
centrations of PLP was highly skewed, we analyzed the data divided by quartiles, and we chose the 50th percentile of PLP concentration to define “low” vitamin B-6 status for concentrations below that value (36.3 nmol/L). To evaluate the association between PLP and CAD, odds ratios (ORs) with 95% confidence intervals (CIs) were estimated by univariate logistic regression analysis. To determine the independent predictive value of PLP in the development of CAD, we performed a series of multivariate logistic regression analyses that simultaneously controlled for the subjects’ sex and smoking status as well as for other classic risk factors for CAD, including hs-CRP, fibrinogen, and tHcy. Because the distribution of hs-CRP values was highly skewed and was only partially corrected after logarithmic transformation, the association between hs-CRP and PLP was also analyzed after dividing hs-CRP concentrations into quartiles. As reported also by others (19, 26), the regression analysis based on cutoff points for quartiles produces a more significant and predictive effect in determining CAD risk, for such a hs-CRP test, than a regression analysis based on cutoff points for the division of the study group into thirds (19, 26). Analyses for potential interactions in determining CAD risk were performed with use of a likelihood-ratio test. A test of homogeneity (equal odds) followed by a score test for trend of odds was performed to compare the risk of CAD in groups with different PLP, hs-CRP, or LDL: HDL (Stata Statistical software, release 8.0, 2003; Stata Corporation, College Station, TX). All P values were 2-tailed, and values of P < 0.05 were considered to indicate statistical significance. All CIs were calculated at the 95% level.

RESULTS

The relevant characteristics of the cohort divided into CAD and CAD-free subjects are shown in Table 1. As expected, patients with CAD had more conventional risk factors compared with patients who were in the CAD-free group, including higher concentrations of tHcy, fibrinogen, and hs-CRP.

Plasma PLP values were significantly lower in the CAD group than in the CAD-free group (χ values: 31.2 nmol/L compared with 33.9 nmol/L, respectively, P < 0.05) (Table 1), and the prevalence of subjects with low PLP concentrations was higher among patients with CAD than among CAD-free individuals (62.9% among CAD subjects compared with 49.8% in CAD-free individuals, P < 0.001). Plasma PLP was inversely related to fasting tHcy concentrations (r = −0.082; P = 0.026). Concentrations of plasma folate and vitamin B-12 were also inversely related to fasting tHcy concentrations and, as expected, with a stronger correlation than that for PLP (r = −0.386 and −0.26, respectively, P < 0.001).

Plasma PLP concentrations were inversely related to both inflammation markers hs-CRP (r = −0.154; P < 0.001) and fibrinogen (r = −0.218; P < 0.001). As illustrated in Figure 1A, an inverse correlation was observed also between PLP and hs-CRP quartiles. An analogous inverse correlation was found for PLP and fibrinogen quartiles (Figure 1B). The P value of Cuzick test for trend was statistically significant for both variables (P < 0.001) (Figure 1, A and B). The percentage of subjects with low vitamin B-6 (defined for PLP values <50th percentile; 36.3 nmol/L) also increased across increasing hs-CRP and fibrinogen quartiles (P < 0.0001 and P < 0.01, respectively, for the comparison of the correspondent lowest with highest quartile) (data

Statistical analysis

The statistical computations were performed with SPSS statistical software (version 10.0; SPSS Inc, Chicago). Distributions of continuous variables were expressed as mean ± SD. Logarithmic transformation was performed on all the skewed variables to normalize their distribution. Therefore, geometric means (antilogarithms of the transformed means) are presented for tHcy, folate, PLP, vitamin B-12, creatinine, hs-CRP, and fibrinogen. Statistical significance for differences in quantitative variables was tested by Student’s unpaired t test or by analysis of variance with use of Tukey procedure for post hoc multivariate comparison of the means. Qualitative data were analyzed with use of a chi-square test. Because the distribution of plasma concentrations of PLP was highly skewed, we analyzed the data divided by quartiles, and we chose the 50th percentile of PLP concentration to define “low” vitamin B-6 status for concentrations below that value (36.3 nmol/L). To evaluate the association between PLP and CAD, odds ratios (ORs) with 95% confidence intervals (CIs) were estimated by univariate logistic regression analysis. To determine the independent predictive value of PLP in the development of CAD, we performed a series of multivariate logistic regression analyses that simultaneously controlled for the subjects’ sex and smoking status as well as for other classic risk factors for CAD, including hs-CRP, fibrinogen, and tHcy. Because the distribution of hs-CRP values was highly skewed and was only partially corrected after logarithmic transformation, the association between hs-CRP and PLP was also analyzed after dividing hs-CRP concentrations into quartiles. As reported also by others (19, 26), the regression analysis based on cutoff points for quartiles produces a more significant and predictive effect in determining CAD risk, for such a hs-CRP test, than a regression analysis based on cutoff points for the division of the study group into thirds (19, 26). Analyses for potential interactions in determining CAD risk were performed with use of a likelihood-ratio test. A test of homogeneity (equal odds) followed by a score test for trend of odds was performed to compare the risk of CAD in groups with different PLP, hs-CRP, or LDL: HDL (Stata Statistical software, release 8.0, 2003; Stata Corporation, College Station, TX). All P values were 2-tailed, and values of P < 0.05 were considered to indicate statistical significance. All CIs were calculated at the 95% level.

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not shown). As shown in Table 2, the unadjusted OR for CAD risk with respect to low plasma PLP was 1.71 (95% CI: 1.26, 2.32; \( P < 0.001 \)). In further multiple logistic regression models adjusted for the main classic coronary heart disease risk factors, the estimate of risk of low plasma PLP remained statistically significant (OR = 1.61; 95% CI: 1.05, 2.45; \( P = 0.027 \)) (Table 2). Lower PLP concentrations was confirmed to be independently associated with higher risk of CAD even after inclusion in the model of hs-CRP and fibrinogen (OR = 1.73; 95% CI: 1.11, 2.72; \( P = 0.016 \)), and variables related to homocysteine metabolism, such as tHcy, vitamin B-12, and folate (OR = 1.89; 95% CI: 1.18, 3.03; \( P = 0.008 \)) (Table 2).

To investigate whether the presence of low vitamin B-6 concentrations conferred an additional risk to that associated with other well-established risk factors for CAD, we performed further analyses. Preliminary analyses by likelihood-ratio tests excluded any significant interaction between PLP and hs-CRP, classified either on the basis of the 50th percentile (chi-square = 0.03, \( P = 0.8605 \)) or into quartiles (chi-square = 0.13, \( P = 0.7190 \)). Then, the estimate of risk of CAD compared with CAD-free was calculated by multiple logistic regression model adjusted for all the major risk factors for CAD, using, for both PLP and hs-CRP, the values corresponding to the 50th percentile as a cutoff (ie, below or above 36.3 nmol/L for PLP and 1.77 mg/L for hs-CRP, respectively). Low PLP concentrations were associated to an increased risk of CAD both in the high hs-CRP group (OR = 2.37; 95% CI, 1.22, 4.58; \( P < 0.05 \)) and in the low hs-CRP group (OR = 2.08; 95% CI, 1.01, 4.29; \( P < 0.05 \)).

In a further analysis, to determine whether there was a difference in the estimate of risk of CAD according to increasing hs-CRP quartiles and either high or low PLP concentrations, the study participants were stratified into 8 groups according to high or low plasma PLP concentrations and hs-CRP divided into quartiles. The estimate of risk of CAD progressively increased across the 8 subgroups according to increasing hs-CRP concentrations and lower PLP concentrations (test of homogeneity: chi-square = 32.55, \( P < 0.0001 \); score test for trend of odds: chi-square = 27.62; \( P < 0.0001 \)). As shown in Table 3, the risk of CAD was the highest among those subjects within the top hs-CRP quartile and with reduced plasma concentrations of PLP compared with subjects with the lowest hs-CRP and high PLP concentrations (OR = 4.61; 95% CI, 2.43, 8.73).

We, therefore, applied similar models to explore whether the presence of low PLP added a significantly higher risk of CAD to that conferred by a classic CAD risk factor such as a high LDL cholesterol to HDL cholesterol ratio. Likewise, the study subjects were divided into 8 groups according to LDL:HDL quartiles and either high or low PLP concentrations. The estimate of risk of CAD progressively increased across the 8 subgroups according to increasing LDL cholesterol to HDL cholesterol ratio and lower PLP concentrations (test of homogeneity: chi-square = 82.3, \( P < 0.0001 \); score test for trend of odds: chi-square = 74.71; \( P < 0.0001 \)). As shown in Table 4, the risk of CAD was highest among those subjects within the highest quartile of LDL: HDL who also presented low concentrations of plasma PLP (OR = 11.37; 95% CI, 4.92, 26.29).

**DISCUSSION**

The present study in subjects characterized by angiography for coronary atherosclerosis was prompted from the hypothesis that inflammation is the common link between low plasma concentrations of vitamin B-6 and CAD.

The current understanding is that inflammation plays an essential role at all stages of the atherosclerotic process (16). Acute-phase reactants are proven to be strong and independent risk factors for CAD (19, 20). In the present study we observed an inverse relation between concentrations of PLP and 2 major

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**Table 2**

Odds ratios (OR) for coronary artery disease (CAD) according to low vitamin B-6 concentrations

<table>
<thead>
<tr>
<th>PLP Status</th>
<th>Unadjusted OR (95% CI)</th>
<th>( P^2 )</th>
<th>Model 1 OR (95% CI)</th>
<th>( P^3 )</th>
<th>Model 2 OR (95% CI)</th>
<th>( P^4 )</th>
<th>Model 3 OR (95% CI)</th>
<th>( P^5 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B-6</td>
<td>1.71 (1.26, 2.32)</td>
<td>&lt;0.001</td>
<td>1.61 (1.05, 2.45)</td>
<td>0.027</td>
<td>1.73 (1.11, 2.72)</td>
<td>0.016</td>
<td>1.89 (1.18, 3.03)</td>
<td>0.008</td>
</tr>
</tbody>
</table>

\(^1\) Low plasma vitamin B-6 concentrations refer to concentrations below the value corresponding to the 50th percentile (36.3 nmol/L). PLP, pyridoxal-5’-phosphate.

\(^2\) Two-tailed values.

\(^3\) Multiple logistic regression model adjusted for the major risk factors for CAD (ie, sex, age, smoking, hypertension, diabetes, total cholesterol, triacylglycerols, BMI, and serum creatinine).

\(^4\) Multiple logistic regression model adjusted for the abovementioned risk factors plus high-sensitivity C-reactive protein and fibrinogen.

\(^5\) Multiple logistic regression model adjusted as in model 2, including also total homocysteine, vitamin B-12, and folate.

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**Table 3**

Odds ratios for coronary artery disease (CAD) according to either high or low plasma pyridoxal-5’-phosphate (PLP) concentrations and high-sensitivity C-reactive protein (hs-CRP) quartiles

<table>
<thead>
<tr>
<th>hs-CRP Quartiles</th>
<th>&lt;0.81 mg/L (n = 122)</th>
<th>0.81–1.77 mg/L (n = 165)</th>
<th>1.77–4.18 mg/L (n = 194)</th>
<th>&gt;4.18 mg/L (n = 239)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma PLP (nmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥36.3 (n = 300)</td>
<td>1(^\dagger)</td>
<td>1.83 (0.95, 3.51)</td>
<td>2.21 (1.14, 4.27)</td>
<td>2.87 (1.45, 5.69)</td>
</tr>
<tr>
<td>&lt;36.3 (n = 420)</td>
<td>1.94 (0.94, 4.02)</td>
<td>2.71 (1.41, 5.22)</td>
<td>3.47 (1.86, 6.48)</td>
<td>4.61 (2.43, 8.73)</td>
</tr>
</tbody>
</table>

\(^1\) Low and high plasma PLP refers to PLP concentrations below or above the 50th percentile (36.3 nmol/L), respectively; 95% CI in parentheses. Test of homogeneity: chi-square = 32.55, \( P < 0.0001 \); score test for trend of odds: chi-square = 27.62; \( P < 0.0001 \).

\(^\dagger\) Reference group (represents the group at lowest risk) = adequate PLP concentrations and hs-CRP concentrations within the lowest quartile.
TABLE 4
Odds ratios for coronary artery disease (CAD) according to either high or low plasma pyridoxal-5′-phosphate (PLP) concentrations and LDL:HDL ratio quartiles

<table>
<thead>
<tr>
<th>Plasma PLP (nmol/L)</th>
<th>LDL:HDL quartiles</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;1.97 (n = 89)</td>
<td>1.89 (1.18, 3.03)</td>
</tr>
<tr>
<td></td>
<td>1.97–2.51 (n = 107)</td>
<td>2.51 (1.45, 3.39)</td>
</tr>
<tr>
<td></td>
<td>2.51–3.23 (n = 127)</td>
<td>7.08 (3.63, 16.78)</td>
</tr>
<tr>
<td>≥36.3 (n = 256)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;36.3 (n = 342)</td>
<td>2.26 (0.94, 5.44)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.30 (1.47, 7.41)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.71 (1.70, 8.07)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11.37 (4.92, 26.29)</td>
<td></td>
</tr>
</tbody>
</table>

1 Low and high plasma PLP concentrations corresponded to PLP concentrations below or above the 50th percentile (36.3 nmol/L), respectively; 95% CI in parentheses. Test of homogeneity: chi-square = 82.3, P < 0.0001; score test for trend of odds: chi-square = 74.71; P < 0.0001.

2 Reference group (represents the group at lowest risk) = adequate PLP concentrations and LDL:HDL ratio within the lowest quartile.

markers of inflammation, hs-CRP and fibrinogen. These results in a group of CAD subjects substantiate and extend our previous observations in a population-based study (21). Furthermore, the high-sensitivity method used to measure C-reactive protein yielded a graded relation across the spectrum of inflammation and suggests that the link between PLP and inflammatory response is not only present in typical models of inflammatory disease such as rheumatoid arthritis (12) and inflammatory bowel disease (27) but also in a low-grade inflammatory status such as atherosclerosis (14, 16).

Our data show also that the association between low plasma PLP concentrations and increased CAD risk was independent of the major classic risk factors for atherosclerosis, including tHcy, a biochemical marker of vitamin B-6 status as well as an independent risk factor for CAD (5). The strength of this independent relation was confirmed even after adjustments for several other conditions known to be associated with low concentrations of plasma PLP, including aging (28), smoking status (29), and impaired renal function (30).

Most notable were the data showing that the higher risk of CAD related to lower PLP concentrations persisted significant even when hs-CRP and fibrinogen were included in the multiple logistic regression models. These data, however, confirm previous observations of a role of PLP in CAD (4, 31) yet appear strongly in favor of the possibility that the mechanisms that underlie the relation between low PLP and CAD involve a set of events in which vitamin B-6 deficiency is given a peculiar function in the cascade of phenomena leading to CAD. It is worth mentioning that the magnitude of the association between low PLP and CAD, although statistically significant, was rather mild (OR = 1.89; 95% CI: 1.18, 3.03; P = 0.008). This finding is not surprising if one considers that atherosclerosis is a complex disease, in which there is an interplay of several genetic, nutritional, and lifestyle-related factors. Remarkably, the current results show also that the combined presence of low PLP concentrations in addition to other major markers of risk of CAD, such as higher hs-CRP and elevated LDL:HDL, enhanced the risk of the disease even more significantly and in a graded manner. Indeed, these findings could have important implications in the ability of creating new models for the assessment of CAD risk in which the importance of nutritional status is especially highlighted.

The higher risk of CAD was detected among subjects with PLP concentrations below 36.3 nmol/L, a value that corresponded to the 50th percentile of the distribution of PLP concentrations in this population and that can be defined as a moderate deficiency of the vitamin. In previous studies, the higher risk of CAD associated with reduced vitamin B-6 referred to PLP concentrations that define a clear vitamin B-6 deficiency (4). Yet the results of the present study provide evidence that even a mild impairment in vitamin B-6 status confers higher risk of CAD. Such vitamin status is apparently not a rare occurrence in population-based studies (31) and could, therefore, deserve further investigation for public health issues.

Mechanistic studies are required to clarify the molecular basis of the role of PLP in the cascade of metabolic events related to CAD. Low concentrations of PLP have been related to alteration of immunologic function, including impaired T lymphocyte and macrophage differentiation and interleukin-2 production (32, 33). Abundant data now show that immune mechanisms are involved in atherogenesis (34). Although much remains still to be learned on whether an imbalanced immune system function could be acknowledged as the ultimate trigger behind inflammation in atherogenesis (35), several findings have proven the effect of immune signaling system in modulating inflammatory response for the progression of atheroma (36, 37). The inverse relation between PLP and markers of inflammation together with the finding of an association of low PLP with higher CAD risk could be explained with a role of PLP in affecting the early steps of atherogenesis, in which recruitment of monocytes, T cells, macrophages, and other cells acting for the immune function regulation promote the cytokine-induced mechanisms for initiating the inflammatory response (34, 35). Indeed, PLP is a coenzyme necessary for a large number of metabolic reactions, and an impaired PLP status is related likewise to inflammation (12), immune function (32, 33), and thrombosis (7, 8), all pivotal mechanisms throughout all stages of atherosclerotic process.

Further studies are needed to determine whether vitamin B-6 is merely a marker rather than a cause of the disease. Whether low vitamin B-6 status has a causal relation with CAD can only be established through the demonstration that the increase of plasma concentrations of this vitamin reduces the risk of CAD. There is, however, no definite evidence in this regard. In a large prospective study, higher dietary intake of vitamin B-6 was associated with decreased risk of CAD (38), and it is well established that high doses of vitamin B-6 reduce the risk of both arterial and vein thrombosis in subjects with homocystinuria (39), even despite persistently high homocysteine concentrations (40). One study, furthermore, showed that among patients treated with vitamin B-6 there was a considerable reduction of myocardial infarction events compared with untreated subjects (41). Other studies have shown that treatment with vitamin B-6 produces a significant reduction of plasma thrombotic markers both in homocystinuric subjects (42) and in subjects treated for mild hyperhomocysteinemia (43), giving a further rationale to a key role of vitamin...
B-6 even in the atherothrombotic complications of CAD (44). The results of several ongoing trials on the effect of supplementation with folic acid and other B vitamins, including vitamin B-6, in reducing the risk of CAD associated to hyperhomocysteinemia could certainly contribute to resolve this issue (45–49).

These observations emphasize the importance of PLP as a significant risk factor for CAD and, in addition, underline the importance of considering vitamin B-6 status in the assessment of the risk of CAD, thus opening new insights for the potential identification of innovative as well as easily feasible therapeutic strategies.

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