The Journal of Nutrition
**Nutritional Epidemiology**

# Low Plasma Vitamin B-6 Status Affects Metabolism through the Kynurenine Pathway in Cardiovascular Patients with Systemic Inflammation[1–4]

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## Abstract

It is unclear whether reduced plasma pyridoxal 5′-phosphate (PLP) during inflammation reflects an altered distribution or increased requirement of vitamin B-6 that may impair overall vitamin B-6 status in tissues. In plasma from 3035 patients undergoing coronary angiography for suspected coronary heart disease, we investigated if plasma concentrations of any metabolites in the kynurenine pathway, which depend on PLP as cofactor, may serve as metabolic marker(s) of vitamin B-6 status. We also examined the association of vitamin B-6 status with serum or plasma concentrations of several inflammatory markers. Among the kynurenines, only 3-hydroxykynurenine (HK) was inversely related to PLP and showed a positive relation to 4 investigated inflammatory markers. A segmented relationship was observed between PLP and HK, with a steep slope at PLP concentrations $< 18.4$ nmol/L, corresponding to the 5th percentile, and an almost zero slope at higher PLP concentrations. Low PLP and the steep PLP-HK slope were essentially confined to participants with 1 or more inflammatory markers in the upper tertile. Oral supplementation with pyridoxine hydrochloride (40 mg/d) for 1 mo increased plasma PLP 8-fold, reduced the geometric mean (95% CI) of HK from 29.5 to 20.2 nmol/L ($P < 0.001$), and abolished the steep segment of the PLP-HK curve. The steep inverse relationship of plasma PLP with HK at low plasma PLP and the lowering of HK by pyridoxine suggest plasma HK as a metabolic marker of vitamin B-6 status. Thus, low plasma PLP during inflammation may reflect impaired cellular vitamin B-6 status, as indicated by the concurrent increase in plasma HK. J. Nutr. 141: 611–617, 2011.

## Introduction

Low plasma concentrations of the vitamin B-6 form pyridoxal 5′-phosphate (PLP)[10] have been reported in patients with high levels of inflammatory markers (1–7) and with conditions associated with inflammation, including rheumatoid arthritis (2,8), cardiovascular disease (7), and diabetes (9). It has not been settled whether this merely reflects altered distribution of PLP (8,10) or increased PLP requirements leading to low PLP and impaired vitamin B-6 status in tissues (5).

Plasma PLP is the most commonly used vitamin B-6 marker (11–13) and is suggested to reflect stores of vitamin B-6 in liver and other tissues (14). Erythrocyte PLP is strongly related to plasma PLP and has been considered as an indicator of long-term vitamin B-6 status (12), at least in healthy individuals (15). However, in patients with an inflammatory condition like rheumatoid arthritis, erythrocyte PLP seems to be a measure of vitamin stores restricted to the erythrocyte compartment, and

[1] Supported by the Advanced Research Program and Research Council of Norway, the Norwegian Foundation for Health and Rehabilitation, the Norwegian Heart and Lung Patient Organization, the Norwegian Ministry of Health and Care Services, the Western Norway Regional Health Authority, the Department of Heart Disease at Haukeland University Hospital, Locus for Homocysteine and Related Vitamins at the University of Bergen, Locus for Cardiac Research at the University of Bergen, the Foundation to Promote Research Into Functional Vitamin B-12 Deficiency, Bergen, Norway, and Alpharma Inc, Copenhagen, Denmark (for The Western Norway B-Vitamin Intervention Trial). The main sponsors were nonprofit organizations with no participating role in the trial. Alpharma Inc provided the study capsules, generated the randomization sequence, and concealed the randomization code free of charge and rendered a limited grant to finance the initial phase of the trial. However, Alpharma Inc had no role in the design or implementation of the trial, had no access to study data, and did not participate in data analysis or interpretation or in the preparation, review, or approval of the manuscript.

[10] Abbreviations used: AA, anthranilic acid; ALP, alkaline phosphatase; CRP, C-reactive protein; GAM, generalized additive model; HAA, 3-hydroxyanthranilic acid; HK, 3-hydroxykynurenine; IDO, indoleamine (2,3)-dioxygenase; KA, kynurenic acid; KTR, kynurenine:tryptophan ratio; PLP, pyridoxal 5′-phosphate; WBC, white blood cell count; WENBIT, Western Norway B-Vitamin Intervention Trial; XA, xanthurenic acid.

[2] Author disclosures: Ø. Midttun, A. Ulvik, E. Ringdal Pedersen, M. Ebbing, Ø. Bleie, R. M. Nilsen, and H. Schartum-Hansen, no conflicts of interest; P. M. Ueland is a member of the steering board of the nonprofit Foundation to Promote Research Into Functional Vitamin B-12 Deficiency.

[3] This trial was registered at clinicaltrials.gov as NTC00354081.

[4] Supplemental Table 1 is available with the online posting of this paper at jn.nutrition.org.

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Manuscript received September 27, 2010. Initial review completed November 19, 2010. Revision accepted January 12, 2011.
First published online February 10, 2011; doi:10.3945/jn.110.133082.

plasma PLP may be a better indicator of overall vitamin B-6 status (16).

PLP is the active form of vitamin B-6 and functions as cofactor in >100 metabolic reactions (17), including several steps of the kynurenine pathway of tryptophan metabolism (18). The first step in the oxidation of tryptophan to kynurenine is catalyzed by the hepatic enzyme tryptophan dioxygenase or the ubiquitous and inducible indoleamine (2,3)-dioxygenase (IDO) (19). Kynurenine is further metabolized to kynurenic acid (KA) by kynurenine transaminase, or to anthranilic acid (AA) by kynureninase. Both of these enzymes require PLP as coenzyme. Alternatively, kynurenine may be converted to 3-hydroxykynurenine (HK) by the FAD-dependent enzyme kynurenine 3-hydroxylase (20). HK may be further converted to xanthurenic acid (XA) by kynurenine transaminase, or to 3-hydroxyanthranilic acid (HAA) by kynureninase (20).

Because of the involvement of PLP in the kynurenine pathway, circulating kynurenines are candidate metabolic biomarkers of impaired vitamin B-6 status in tissues. This possibility has not yet been explored despite the observations that urinary HK and XA drastically increased after tryptophan load during vitamin B-6 depletion and were normalized following pyridoxine supplementation (21–23).

Metabolic markers of vitamin status have several advantages compared with circulating vitamin concentrations, as documented for methylmalonic acid (vitamin B-12) (24) and homocysteine (folate and vitamin B-12) (25). Metabolic markers reflect vitamin function in tissues, are less affected by short-term vitamin intake and vitamin redistribution, and allow assessment of vitamin status during supplementation. A metabolic marker of vitamin B-6 status, particularly in inflammation, has not been established.

C-reactive protein (CRP) is the most commonly measured inflammatory marker. Its synthesis in the liver is induced by cytokines like IL-1, IL-6 ,and TNF$\alpha$ (26). These cytokines also have effects on the hematopoietic system and mediate leukocytosis. The proinflammatory cytokine IFN$\gamma$ activates IDO (19), thereby increasing the conversion of tryptophan to kynurenine and thus the plasma kynurenine:tryptophan ratio (KTR) (19,27), and also induces the formation of neopterin in macrophages. Thus, KTR and neopterin are both markers of immune activation mediated by IFN$\gamma$ (19). Notably, kynurenine, HK, and HAA may modulate T-cell functions (28).

The primary objective of the present study was to investigate if low plasma PLP was associated with impaired cellular function of vitamin B-6. We first explored plasma concentrations of several kynurenines as potential metabolic markers of vitamin B-6 status and then investigated the association between vitamin B-6 indices and inflammatory status. Finally, the effect of vitamin B-6 supplementation for 1 mo was studied. The investigation was a substudy of the Western Norway B-Vitamin Intervention Trial (WENBIT) and involved 3035 patients undergoing coronary angiography for suspected coronary artery disease.

## Materials and Methods

*Participants.* The study included 3035 adults (>98% Caucasians), which constitutes a subset of the WENBIT who were undergoing coronary angiography for suspected coronary artery disease between 1999 and 2004 at the Haukeland University Hospitals in Bergen and Stavanger University Hospital in Stavanger, Norway (29). Details of the WENBIT study have been published elsewhere (29). In the current study, we used data at baseline for all participants and after 1 mo of follow-up for participants randomly allocated to receiving placebo ($n$ = 763) or 40 mg/d pyridoxine (pyridoxine hydrochloride) ($n$ = 759).

Written informed consent was obtained from all participants. The study protocol was in accordance with the principles of the Declaration of Helsinki and the trial was approved by the Regional Committee for Medical and Health Research Ethics, the Norwegian Medicines Agency, and the Data Inspectorate.

*Biochemical analyses.* Plasma samples for measurements of biomarkers were stored at −80°C until analyzed. Plasma concentrations of PLP, tryptophan, kynurenine, KA, AA, HK, XA, HAA, neopterin, and cotinine were measured by HPLC-MS/MS (30) and creatinine by including it and its deuterated internal standard (d3-creatinine) in an established HPLC-MS/MS assay (31), using the ion pairs 114/44.2 and 117/47.2, respectively. KTR was calculated by dividing the plasma concentration of kynurenine (in nmol/L) by the concentration of tryptophan (in $\mu$mol/L). CRP was determined in serum by an ultrasensitive immunoassay applying the Behring nephelometer II system (Latex CRP mono, Behring Diagnostics). White blood cell count (WBC) was determined in EDTA-blood by Abbott Cell Dyn 4000 (Abbott Diagnostics) or by ADVIA 120 (Bayer Diagnostics). Serum total cholesterol was measured at the central laboratory of the Haukeland University Hospital with the use of Technicon Chem 1 (Bayer).

*Statistical analyses.* Data were given as means or medians (5th, 95th percentiles) or geometric means (95% CI). Correlations among age, sex, and plasma indices were assessed by partial Spearman correlation after adjustment (where appropriate) for age, sex, study center, and creatinine.

The effect of pyridoxine supplementation on plasma indices was tested by using mixed linear models with a random effect for volunteer and fixed effects for time point (d 0 or 28), supplement (placebo or pyridoxine), and the time point-supplement interaction. Post hoc analyses with Bonferroni correction for multiple comparisons were then performed to test whether the supplementation and placebo group differed at d 0 (baseline) and whether the placebo group changed from baseline to d 28. Plasma indices were log-transformed before analysis to obtain normal-like data. As such, the $P$-values obtained refer to the differences in log-transformed values or, accordingly, to relative differences in geometric means.

Graphical representations of dose-response relationships between PLP and HK were obtained by generalized additive models (GAM) adjusted for age, sex, study center, smoking (cotinine), creatinine, tryptophan, and BMI. We tested for a non-zero difference in slope of a segmented linear relationship by regressing HK on PLP at baseline, using Davies' test. The PLP-HK relationship at baseline was also fitted by segmented regression using the breakpoint value from Davies' test as the starting estimate for the breakpoint between regression segments. Segmented regression was also performed on subgroups defined by individuals having each inflammatory marker in the lower, middle, or upper tertile. The regression models were adjusted for age, sex, study center, smoking (cotinine), creatinine, and BMI and returned slopes of both segments and the breakpoint with 95% CI. A binomial test was used to investigate if the number of participants with each inflammatory marker in the upper tertile and PLP < 18.4 nmol/L was different from the expected one-third.

Statistical analyses were performed by using SAS version 9.2 (SAS Institute), R version 2.8.1 (The R Foundation for Statistical Computing), and SPSS (version 15) software for Windows. GAM models were computed using the mgcv-package (version 1.4–0) and segmented regression by the segmented-package (version 0.2–6), both in R (version 2.8.1) (32). All $P$-values were 2 sided, and values < 0.05 were considered significant.

## Results

*Participant characteristics.* A total of 79.6% of the participants were male and the mean age was 61.6 y (**Table 1**). At baseline, 83.7% had stable angina pectoris, 14.9% had acute coronary syndrome, and 59.3% had double- or triple-vessel disease.

*Plasma indices and correlations at baseline.* The median plasma concentrations were 38.7 nmol/L for PLP and 28.9

**TABLE 1** Baseline characteristics of the cardiovascular patients studied[1]

| Characteristics | |
|---|---|
| Age, *y* | 61.8 (44.7–77.3) |
| Male sex, *n (%)* | 2414 (79.6) |
| BMI, *kg/m²* | 26.9 (21.5–33.5) |
| Current smoking, *n (%)* | 875 (28.8) |
| Cotinine, *nmol/L* | 1.7 (0.0–1693) |
| Vitamin supplement use, *n (%)* | 1117 (36.8) |
| Disease history, *n (%)* | |
| Myocardial infarction | 1259 (41.5) |
| Percutaneous coronary intervention | 623 (20.5) |
| Coronary artery by-pass grafting | 408 (13.4) |
| Stroke, TIA, or carotid artery stenosis[2] | 188 (6.2) |
| Treatment for hypertension | 1396 (46.0) |
| Lipid lowering statin treatment | 2173 (71.6) |
| Diabetes mellitus | 348 (11.5) |
| Blood indices and inflammatory markers | |
| Total cholesterol, *mmol/L* | 4.9 (3.5–7.1) |
| Creatinine, *μmol/L* | 73.0 (52.8–102.0) |
| PLP, *nmol/L* | 38.7 (18.4–96.4) |
| CRP, *mg/L* | 2.0 (0.4–19.3) |
| WBC, *x10⁹/L* | 7.0 (4.5–11.2) |
| KTR, *nmol/μmol* | 23.6 (15.7–39.4) |
| Neopterin, *nmol/L* | 8.0 (5.2–14.8) |
| Tryptophan and kynurenines | |
| Tryptophan, *μmol/L* | 68.0 (47.3–92.5) |
| Kynurenine, *μmol/L* | 1.63 (1.1–2.6) |
| KA, *nmol/L* | 47.3 (25.1–91.5) |
| AA, *nmol/L* | 13.6 (7.7–26.3) |
| HK, *nmol/L* | 28.9 (15.1–59.0) |
| XA, *nmol/L* | 13.8 (5.9–29.5) |
| HAA, *nmol/L* | 33.8 (15.8–65.7) |

[1] Values are medians (5th–95th percentile), $n$ = 3035, or $n$ (%).
[2] TIA, transient ischemic attack.

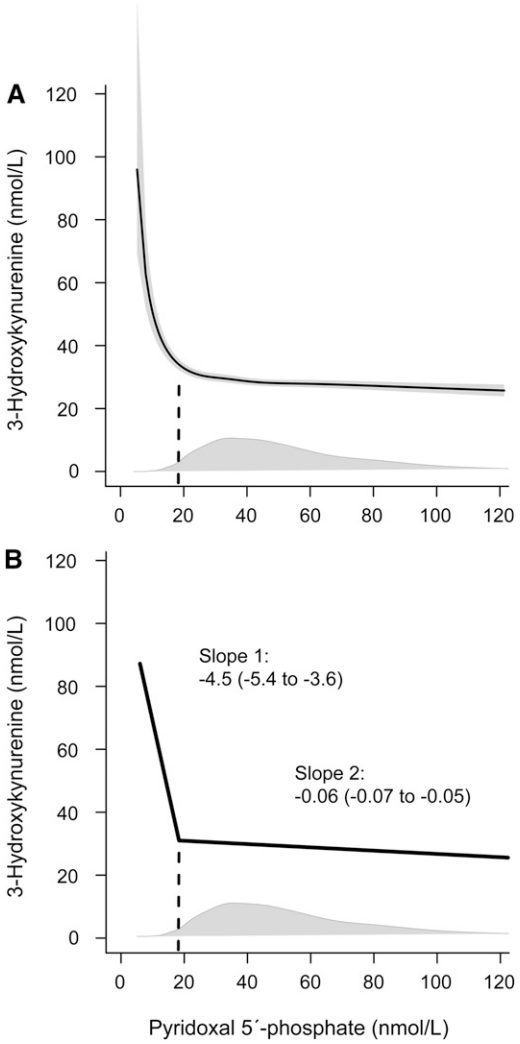


**FIGURE 1** GAM (*A*) and segmented regression model (*B*) relating plasma concentrations of PLP and 3-HK in 3035 cardiovascular patients at baseline. The distribution of plasma PLP is shown at the bottom of each panel. The shaded area around the GAM curve shows the 95% CI around the central estimate.

nmol/L for HK. The median concentrations of other kynurenines (KA, AA, XA, and HAA) were also in the nanomolar range (13.6–47.3 nmol/L), whereas micromolar concentrations were found for median kynurenine (1.63) and tryptophan (68.0) (Table 1).

Median plasma concentrations of the inflammatory markers were 2.0 mg/L for CRP, $7.0 \times 10^9$/L for WBC, 23.6 nmol/μmol for KTR, and 8.0 nmol/L for neopterin. CRP was >10.0 mg/L in 10.7% of the participants and showed a moderate correlation with WBC ($r$ = 0.36; $P < 0.001$) and weak correlations with KTR ($r$ = 0.15; $P < 0.001$) and neopterin ($r$ = 0.23;$P < 0.001$). KTR and neopterin were strongly correlated ($r$ = 0.46; $P <$ 0.001) (**Supplemental Table 1**).

Among the kynurenines, only HK was inversely correlated with PLP ($r = -0.20$; $P < 0.001$) and showed a positive correlation to all inflammatory markers ($r$ = 0.11–0.43; $P <$ 0.001); the strength of the associations decreased in the order KTR, neopterin, CRP, and WBC (Supplemental Table 1).

Plasma PLP was inversely correlated with all inflammatory markers ($-0.15 < r < -0.28$; $P < 0.001$) and the strength of the correlations decreased in the order CRP, WBC, KTR, and neopterin (Supplemental Table 1).

*The PLP–HK relation.* We used GAM to obtain dose-response curves between PLP and the kynurenines. A distinctly nonlinear PLP-HK relation was found with an abrupt change in slope at a PLP concentration of ~20 nmol/L. By applying segmented regression for the biphasic relationship using data from all patients, we obtained a breakpoint of 18.4 nmol/L, below which the inverse relationship between PLP and HK showed the steepest slope (**Fig. 1**; **Table 2**). The relation of PLP with other kynurenines were essentially linear.

*PLP and HK according to levels of inflammatory markers.* Among a total of 153 (5.0%) participants with PLP < 18.4 nmol/L, 143 (93.5%) had baseline serum or plasma concentrations of 1 or more inflammatory markers in the upper tertile (**Fig. 2**). For isolated high CRP, KTR, neopterin, or WBC, the numbers of affected participants were 93, 92, 82, and 75, respectively, all higher than the expected number of 51 (one-third of 153) ($P < 0.001$).

We investigated the dose-response relationship between PLP and HK at baseline according to tertile strata of inflammatory markers CRP, WBC, KTR, and neopterin. Segmented regression analyzes and Davies' test demonstrated a biphasic (2 segments) relationship characterized by a highly significant breakpoint in the plasma PLP concentration range of 16–19 nmol/L and a

**TABLE 2** Plasma HK vs. PLP at baseline according to inflammatory status in cardiovascular patients by segmented regression and Davies' test

| Plasma/serum inflammation marker | Tertile | *n* | Segmented regression | | | Davies' test[2] | |
|---|---|---|---|---|---|---|---|
| | | | Slope 1 (95% CI)[1] | Breakpoint[1] | Slope 2 (95% CI)[1] | Breakpoint[1] | *P* |
| | | | *nmol 3-HK/nmol PLP* | *nmol PLP/L* | *nmol 3-HK/nmol PLP* | | |
| | All | 3035 | −4.5 (−5.4, −3.6) | 18.4 (17.6, 19.3) | −0.06 (−0.07, −0.05) | 18.4 | <0.001 |
| CRP | 3[3] | 1011 | −4.2 (−5.4, −3.0) | 18.9 (17.4, 20.5) | −0.11 (−0.17, −0.06) | 22.5 | <0.001 |
| WBC | 3[3] | 975 | −8.7 (−10.7, −6.8) | 16.4 (15.6, 17.3) | −0.11 (−0.16, −0.06) | 16.3 | <0.001 |
| KTR | 3[3] | 1005 | −4.8 (−6.2, −3.4) | 18.6 (17.2, 20.0) | −0.16 (−0.21, −0.11) | 23.3 | <0.001 |
| Neopterin | 3[3] | 1006 | −5.0 (−6.4, −3.7) | 18.8 (17.4, 20.3) | −0.14 (−0.19, −0.09) | 23.7 | <0.001 |

[1] Adjusted for age, sex, study center, smoking (cotinine), creatinine, and BMI.
[2] Tests for a nonzero difference in slope parameter of a segmented relationship.
[3] Lower limit of each tertile was CRP, 3.1 mg/L; WBC, 7.9 × $10^9$; KTR, 26.6; neopterin, 9.2 nmol/L.

steep slope with a narrow CI at low PLP concentrations in the upper tertile of each inflammatory marker (Table 2). In the middle and lower tertiles, the CI for the lower segments generally were wide and/or Davies' test detected no difference in slope parameters (not shown).

*Effect of pyridoxine supplementation.* Supplementation with pyridoxine (40 mg/d) for 1 mo increased geometric mean plasma PLP 8-fold and reduced geometric mean HK from 29.5 to 20.2 nmol/L ($P < 0.001$) but did not affect the concentrations of inflammatory markers. The concentrations of KA, AA, and HAA were slightly increased, whereas XA was not affected by pyridoxine supplementation (**Table 3**).

The dose-response curve for PLP compared with HK after pyridoxine supplementation was similar to and essentially a continuation of the upper segment found at baseline, showing a modest decrease in HK by increasing PLP (**Fig. 3**).

## Discussion

*Principal findings.* The primary objective of the present study was to investigate if low plasma PLP in patients with inflammation is associated with impaired cellular function of vitamin B-6. We demonstrated that plasma HK meets some fundamental criteria of a candidate metabolic marker of vitamin B-6 status, i.e. an inverse association with plasma PLP and a marked reduction following supplementation with pyridoxine. Further, inflammation was inversely related to PLP and positively related to HK, but pyridoxine supplementation did not affect the plasma concentration of any of the inflammatory markers. The combination of low plasma PLP and a steep inverse relationship between PLP and HK was essentially confined to participants with inflammatory markers in the upper tertiles. Taken together, these results suggest that low plasma PLP during inflammation reflects tissue PLP depletion to an extent that affects its function as cofactor of (some) cellular enzyme reactions.

*Markers of inflammation.* The strong correlation between KTR and neopterin is in agreement with published results (19,27) and is explained by IFNγ being the main inducer of both neopterin and kynurenine formation during activation of the cellular immune system (19). KTR and neopterin showed weaker correlations with CRP and WBC, which are both part of the acute response of the innate immune system induced primarily by the cytokine IL-6 (33,34). Thus, correlations between inflammatory markers included in this study may reflect the pluridimensional aspects of the inflammatory response.

*Plasma HK as a systemic vitamin B-6 marker.* Among the kynurenines, only HK was inversely related to plasma PLP, with the steepest part of the PLP-HK curve at low PLP. This may reflect that HK is the only kynurenine where further metabolism, but not formation, requires PLP (18). HK is metabolized by 2 PLP-dependent enzymes, kynurenine transaminase and kynureninase, and the activity of the latter enzyme has been shown to be particularly responsive to dietary vitamin B-6 restriction (35,36).

There are 3 lines of evidence in favor of plasma HK as a metabolic marker of vitamin B-6 status. First, the PLP-dependent pathways of HK metabolism provide a biochemical mechanism. Second, we observed an inverse relationship between PLP and HK. Segmented regression and GAM demonstrated that the PLP-HK curve was steep at plasma PLP concentrations < 16–19 nmol/L when including participants with each inflammatory marker in the upper tertile. Finally, our longitudinal data showed a marked reduction in plasma HK following pyridoxine supplementation, demonstrating that plasma HK responds to improved vitamin B-6 status.

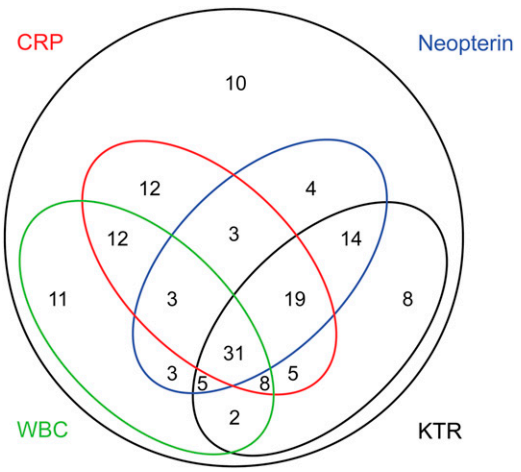


**FIGURE 2** Venn diagram showing the distribution of cardiovascular patients with low plasma PLP (<18.4 nmol/L) according to inflammatory status given as any combination of inflammatory markers in the respective upper tertile. The number of individuals in the upper tertile of each inflammatory marker is indicated inside each ellipse. The total number of individuals with low PLP was 153 and among these 143 had 1 or more inflammation marker in the upper tertile. For isolated high CRP, KTR, neopterin, or WBC, the numbers were 93, 92, 82, and 75, respectively.

**TABLE 3** Circulating concentrations of PLP, inflammatory markers, and tryptophan metabolites in cardiovascular patients who received placebo or pyridoxine (40 mg/d) for 1 mo[1]

| | Baseline | | After 28 d | | | | |
|---|---|---|---|---|---|---|---|
| Variable | Placebo | Vitamin B-6 | Placebo | Vitamin B-6 | $P^2$ | $P^3$ | $P^4$ |
| *n* | 763 | 759 | 633 | 639 | | | |
| PLP, *nmol/L* | 39.7 (38.3, 41.1) | 41.5 (39.9, 43.2) | 40.3 (38.8, 41.8) | 333.9 (321.3, 347.0) | <0.001 | 0.56 | 1.00 |
| CRP, *mg/L* | 2.2 (2.0, 2.3) | 2.1 (1.9, 2.3) | 2.1 (1.9, 2.3) | 2.0 (1.8, 2.2) | 0.69 | 1.00 | 1.00 |
| WBC, $x10^9$/*L* | 7.1 (6.9, 7.2) | 6.9 (6.8, 7.0) | 6.7 (6.6, 6.9) | 6.6 (6.5, 6.8) | 0.99 | 0.64 | 0.001 |
| KTR, *nmol/μmol* | 23.9 (23.4, 24.4) | 24.1 (23.6, 24.6) | 25.8 (25.2, 26.4) | 26.0 (25.4, 26.5) | 0.63 | 1.00 | <0.001 |
| Neopterin, *nmol/L* | 8.3 (8.1, 8.5) | 8.1 (8.0, 8.3) | 8.7 (8.4, 8.9) | 8.6 (8.4, 8.8) | 0.10 | 0.80 | 0.002 |
| Trp, *μmol/L* | 67.0 (66.0, 68.0) | 67.7 (66.7, 68.7) | 67.8 (66.8, 68.8) | 67.4 (66.5, 68.3) | 0.15 | 1.00 | 1.00 |
| Kyn, *μmol/L* | 1.60 (1.57, 1.63) | 1.63 (1.60, 1.66) | 1.75 (1.72, 1.78) | 1.75 (1.72, 1.78) | 0.32 | 1.00 | <0.001 |
| KA, *nmol/L* | 47.2 (45.9, 48.5) | 48.0 (46.6, 49.3) | 50.0 (48.6, 51.5) | 56.0 (54.4, 57.6) | <0.001 | 1.00 | <0.001 |
| AA, *nmol/L* | 13.9 (13.5, 14.3) | 13.9 (13.5, 14.3) | 13.5 (13.2, 13.9) | 14.4 (14.1, 14.8) | <0.001 | 1.00 | 0.18 |
| HK, *nmol/L* | 29.0 (28.2, 29.9) | 29.5 (28.7, 30.3) | 29.9 (28.8, 31) | 20.2 (19.5, 20.9) | <0.001 | 1.00 | 0.23 |
| XA, *nmol/L* | 13.5 (13.0, 13.9) | 14.0 (13.5, 14.5) | 14.5 (13.9, 15) | 14.3 (13.8, 14.8) | 0.06 | 0.81 | 0.001 |
| HAA, *nmol/L* | 33.1 (32.1, 34.2) | 33.7 (32.7, 34.8) | 36.3 (35.2, 37.5) | 42.7 (41.5, 43.9) | <0.001 | 1.00 | <0.001 |

[1] Data are geometric means (95% CI).
[2] Test for group (placebo vs. vitamin B-6) by time (d 0 vs. d 28) effect on plasma concentrations by using mixed models.
[3] Difference in concentration ratios in the placebo group vs. the vitamin B-6 group at baseline, by linear mixed models and post hoc tests (Bonferroni corrected).
[4] Difference in concentration ratios in the placebo group at baseline vs. day 28, by linear mixed models and post hoc tests (Bonferroni corrected).

The steep slope of the PLP-HK curve at low PLP demonstrates that HK is a responsive measure of impaired vitamin B-6 status. Method accuracy and precision of PLP measurement at low PLP may be insufficient to capture differences in vitamin B-6 status in deficient individuals. Measurement of the HK response may therefore improve the assessment of vitamin B-6 status and thereby lead to classification improvement in epidemiological studies, where the tryptophan load test is not feasible.

HK was positively correlated with all the inflammatory markers but most strongly to KTR. Therefore, our data do not exclude the possibility that increased HK is restricted to patients with the combination of impaired vitamin B-6 status and immune response linked to IDO activation. However, the observations of reduced urine (21–23) and plasma HK concentrations due to pyridoxine supplementation, and low kynureninase and transaminase activity during dietary vitamin B-6 restriction (35) point to HK as a candidate systemic marker of vitamin B-6 status. Thus, the combination of elevated HK and low PLP suggests that inflammation leads to impaired vitamin B-6 status.

*Plasma PLP.* The observed median concentration of PLP (38.7 nmol/L) was in the range previously reported for healthy individuals (37–39). Notably, the breakpoint of the PLP-HK curves occurred at PLP slightly <20 nmol/L, which is close to the lower 5th percentile of our study population and also close to the lower bound of PLP concentrations previously suggested by others to define vitamin B-6 deficiency (12,14,40,41). In our study population, the PLP concentration breakpoint was stable only for the participant groups with an inflammation marker in the upper tertile.

*Vitamin B-6 status during inflammation.* Our data confirm published reports on an association between elevated CRP and low PLP in healthy individuals (5,42) and patients with inflammatory conditions (2,6,9), including CVD (43,44). We also observed inverse associations between PLP and 2 markers of Th1-mediated immune activation, KTR and neopterin. Notably, 143 of 153 individuals with baseline plasma PLP < 18.4 nmol/L had at least 1 inflammatory marker in the upper tertile.

Vitamin B-6 depletion during inflammation and immune activation may be related to the involvement of PLP in cytokine production (45) and lymphocyte proliferation (45,46). Furthermore, the PLP-dependent steps of downstream kynurenine metabolism may also increase vitamin B-6 consumption during inflammation, but additional mechanistic studies are required to test this hypothesis. Uptake of vitamin B-6 into cells is facilitated by hydrolysis of PLP to PL by alkaline phosphatase (ALP). The reported positive correlation of ALP with CRP and WBC (47) may thus suggest that low plasma PLP concentrations in inflammation are mediated in part by elevated ALP. Unfortunately, ALP was not measured in the present study.

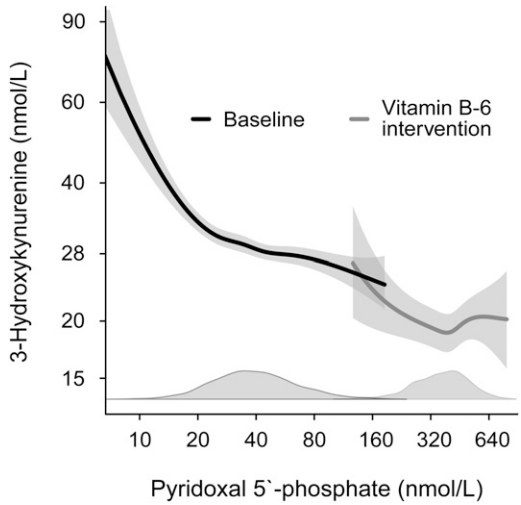


**FIGURE 3** The association of plasma PLP and 3-HK in 759 cardiovascular patients before (baseline) and after pyridoxine supplementation for 1 mo. The shaded area around each GAM curve shows the 95% CI of the model fitted. The distribution of plasma PLP is shown at the bottom of the panel.

We observed reduced plasma HK and no change in the concentration of any inflammatory marker following pyridoxine supplementation for 1 mo. This is in line with previous findings that pyridoxine supplementation does not affect proinflammatory cytokine production in healthy individuals (48) and patients with rheumatoid arthritis (49) or inflammatory markers of atherosclerosis in patients with stable coronary artery disease (50). Notably, pyridoxine supplementation gave null results on clinical outcomes in patients with coronary artery disease in large randomized controlled trials (29,51–53).

*Strength and limitations.* The main strengths of the current study are the large sample size and a well-characterized study population with variable inflammatory status as assessed by 4 different markers reflecting IL-6 as well as INF$\gamma$ mediated immune activation. We measured vitamin B-6 status by plasma PLP concentration as well as a potential functional metabolic marker, plasma HK, and obtained longitudinal data on the effect of pyridoxine treatment on vitamin B-6 status and inflammatory markers. One limitation was that although we had reliable data on B vitamin supplement use, we had no data on dietary intake of vitamin B-6 at baseline. Another limitation was that the study population consisted of patients with coronary artery disease, which includes conditions affecting short-term concentrations of inflammatory markers. Some of our observations on the link between vitamin B-6 status and inflammatory markers may therefore not be directly applicable to healthy populations.

In conclusion, we identified plasma HK as a potential marker of functional vitamin B-6 status by demonstrating its inverse relationship with plasma PLP and reduction of HK following supplementation with pyridoxine. We then demonstrated in this study population of patients with coronary artery disease that inflammation is the main predictor of impaired vitamin B-6 status, and 94% of participants with PLP below the 5th percentile had increased concentrations of 1 or several markers of inflammation. This suggests increased metabolic consumption of vitamin B-6 during inflammation. Additional studies are needed to investigate if this relation is also present in other populations, including healthy individuals. Further investigation of HK as a marker of vitamin B-6 status should assess preanalytical variability and individuality as well as the effects of age, nutritional status, and various diseases.


### Acknowledgments

O.N. and P.M.U. designed the research; Ø.M., E.R.P., M.E., Ø.B., and H.S-H. conducted the research; E.R.P., M.E., and H.S-H. provided the database; Ø.M., A.U., R.M.N., and P.M.U. analyzed the data; Ø.M., A.U., and P.M.U, wrote the paper; and Ø.M. had primary responsibility for final content. All authors read and approved the final manuscript.


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Online Supporting Material

Supplemental Table 1. Spearman correlations between age and plasma indices in cardiovascular patients at baseline[1]

| | Age | Cot | Creat | PLP | CRP | WBC | KTR | Neopt | Trp | Kyn | KA | AA | HK | XA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cot | -0.28* | 1.00 | | | | | | | | | | | | |
| Creat | 0.27* | -0.08* | 1.00 | | | | | | | | | | | |
| PLP | -0.08* | -0.18* | 0.07* | 1.00 | | | | | | | | | | |
| CRP | -0.03 | 0.14* | 0.01 | -0.28* | 1.00 | | | | | | | | | |
| WBC | -0.17* | 0.27* | -0.03 | -0.22* | 0.36* | 1.00 | | | | | | | | |
| KTR | 0.31* | -0.05* | 0.38* | -0.18* | 0.15* | 0.05* | 1.00 | | | | | | | |
| Neopt | 0.28* | -0.07* | 0.36* | -0.15* | 0.23* | 0.08* | 0.46* | 1.00 | | | | | | |
| Trp | -0.13* | -0.04* | 0.02 | 0.26* | -0.09* | -0.07* | -0.41* | -0.21* | 1.00 | | | | | |
| Kyn | 0.23* | -0.09* | 0.43* | 0.02 | 0.09* | 0.001 | 0.67* | 0.31* | 0.32* | 1.00 | | | | |
| KA | 0.13* | -0.08* | 0.42* | 0.13* | 0.001 | 0.003 | 0.29* | 0.08* | 0.20* | 0.46* | 1.00 | | | |
| AA | 0.22* | -0.15* | 0.28* | 0.15* | 0.01 | -0.05* | 0.25* | 0.21* | 0.13* | 0.37* | 0.32* | 1.00 | | |
| HK | 0.15* | 0.002 | 0.33* | -0.20* | 0.20* | 0.11* | 0.43* | 0.25* | 0.11* | 0.55* | 0.39* | 0.20* | 1.00 | |
| XA | -0.09* | -0.04* | 0.27* | 0.19* | -0.06* | -0.03 | 0.02 | -0.06* | 0.36* | 0.29* | 0.63* | 0.21* | 0.41* | 1.00 |
| HAA | -0.06* | -0.05* | 0.11* | 0.10* | 0.09* | 0.02 | 0.09* | -0.01 | 0.42* | 0.42* | 0.44* | 0.16* | 0.50* | 0.56* |

[1] Adjusted (where appropriate) for age, sex, study center and creatinine. All patients were included, n=2985.
Cot, cotinine; Creat, creatinine; Neopt, neopterin; Trp, tryptophan; Kyn, kynurenine.
An asterisk indicate significant correlations: * P< 0.05