Vitamin B-6 catabolism and long-term mortality risk in patients with coronary artery disease[1,2]

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ABSTRACT

Background: Low vitamin B-6 status has been related to increased risk of coronary artery disease (CAD), which is a condition that is associated with inflammation. The most common status marker, plasma pyridoxal 5′-phosphate (PLP), decreases during inflammation; therefore, causal relations are uncertain.

Objective: We evaluated the vitamin B-6 biomarkers PLP, pyridoxal, and pyridoxic acid (PA) and the pyridoxic acid:(pyridoxal + PLP) ratio (PAr), a proposed marker of vitamin B-6 catabolism during activated cellular immunity, as predictors of mortality.

Design: Associations with risks of long-term all-cause mortality and cardiovascular mortality were evaluated with the use of Cox regression in patients who were undergoing elective coronary angiography for suspected stable angina pectoris (SAP) (n = 4131) and an independent cohort of patients who were hospitalized for acute myocardial infarction (AMI) (n = 3665).

Results: Plasma PLP (AMI patients only) and PA predicted all-cause mortality in models that were adjusted for established risk predictors, but associations were attenuated or nonsignificant after additional adjustment for inflammatory markers. PAr was correlated with biomarkers of inflammation (Pearson's $r \geq 0.37$) and predicted all-cause mortality and cardiovascular mortality after adjustment for established risk predictors. In SAP patients, PAr had greater predictive strength than did current smoking, diabetes, hypertension, apolipoproteins, or C-reactive protein. PAr provided multiadjusted HRs per SD of 1.45 (95% CI: 1.30, 1.63) and 1.31 (95% CI: 1.21, 1.41) in SAP and AMI patients, respectively. In both cohorts, PAr was a particularly strong predictor of all-cause mortality for patients with no previous CAD history (P-interaction $\leq$ 0.04).

Conclusion: PAr may capture unique aspects of inflammatory activation and thus provide new insights into disease mechanisms that may aid in identifying patients at increased risk of future fatal events. *Am J Clin Nutr* 2016;103:1417–25.

Keywords: biomarker, coronary artery disease, inflammation, mortality, vitamin B-6

INTRODUCTION

The importance of vitamin B-6 in metabolism is underlined by its involvement as a cofactor in more than 160 known enzyme activities including the synthesis, interconversion, and degradation of amino acids, the synthesis of neurotransmitters, nucleic acids, porphyrins, and lipids, and the degradation of glycogen (1). Pyridoxal 5′-phosphate (PLP)[7] is the active coenzyme, which is the most abundant form in plasma, and currently the most-frequently used marker of vitamin B-6 status (2–4). Plasma also contains pyridoxal that is able to cross lipid bilayer membranes and the main catabolite pyridoxic acid (PA) (4).

Several studies have reported an association between low intakes of vitamin B-6 and risk of coronary artery disease (CAD) (5–7). These findings were supported by observations of low plasma PLP concentrations in subjects who subsequently developed CAD, stroke, or other vascular conditions (8–11).

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[2] Supplemental Tables 1 and 2 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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[7] Abbreviations used: AMI, acute myocardial infarction; AOX1, aldehyde oxidase 1; apo A-I, apolipoprotein A-I ; CAD, coronary artery disease; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; HPA, hypothalamic-pituitary-adrenal; KTR, kynurenine:tryptophan ratio; NORVIT, Norwegian Vitamin Trial; PA, pyridoxic acid; PAr, pyridoxic acid: (pyridoxal + pyridoxal 5′-phosphate) ratio; PLP, pyridoxal 5′-phosphate; SAP, stable angina pectoris; WECAC, Western Norway Coronary Angiography Cohort.

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Concurrently, a number of studies reported low plasma PLP in inflammatory conditions and inverse relations between PLP and inflammatory markers (12–14). On this basis, it was argued that an association between low plasma PLP and inflammatory-related diseases might not be causal (15). A subsequent prospective study showed that PLP was not related to CAD after adjustment for inflammatory-related variables (16), but the literature has been inconsistent (9, 17).

Several mechanisms have been proposed to explain low plasma PLP during inflammation. Some studies have suggested a redistribution of PLP from plasma to erythrocytes or affected tissues during inflammation (18, 19). Recently, we also showed indications of increased catabolism of PLP to PA under conditions of oxidative stress and activated cellular immunity (20). On the basis of metabolic control analysis and high intraclass correlation coefficients, we proposed the PA:(pyridoxal + PLP) ratio (PAr) as an indicator of vitamin B-6 catabolism during such conditions (20). A large number of studies have reported associations of inflammatory markers with CAD, and inflammatory involvement is now a recognized component of the etiology of CAD (21–24). Notably, PAr was shown to be associated with joint high concentrations of the acute phase marker C-reactive protein (CRP) and the cellular immunity markers neopterin and kynurenine:tryptophan ratio (KTR) (20). These markers represent different inflammatory modalities. Therefore, PAr may be of interest as a novel and potentially powerful inflammatory marker by itself beyond its role as an indicator of altered vitamin B-6 metabolism. Notably, we recently showed that PAr predicted the risk of incident lung and colorectal cancer, which are cancer subtypes with a known inflammatory component (25). The aim of the current study was to evaluate the plasma vitamin B-6 indexes PLP, pyridoxal, PA, and PAr, with and without adjustment for inflammatory markers, as potential predictors of long-term mortality in CAD patients from 2 independent clinical cohorts.

METHODS

Subjects

The data used in this study were derived from 2 large clinical cohorts. The WECAC (Western Norway Coronary Angiography Cohort) consisted of 4164 patients who were undergoing elective coronary angiography because of suspected stable angina pectoris (SAP) between 2000 and 2004. Approximately two-thirds of these patients participated in the Western Norway B-Vitamin intervention Trial, which evaluated the lowering of plasma homocysteine by oral B-vitamin treatment to prevent future clinical events. The NORVIT (Norwegian Vitamin Trial) enrolled 3749 participants during hospitalization for an acute myocardial infarction (AMI) between 1998 and 2002. The study design and treatment protocols were identical for the Western Norway B-Vitamin intervention Trial and the NORVIT and have been described in detail elsewhere (26–28). Only the baseline data were used for the evaluation of the association with mortality at follow-up. Patients with missing data on plasma PLP, pyridoxal, or PA were excluded, which left 4131 patients with suspected SAP and 3666 patients with an AMI for the final analyses.

Baseline data, clinical data, and laboratory analyses

Medical histories, including cardiovascular disease risk factors and medications, were collected from self-administered questionnaires and validated against medical records. Hypertension and diabetes were defined by pre-existing diagnosis. Diabetes included both type 1 and type 2. Smoking status was based on self-reported smoking habits corrected by plasma cotinine (i.e., patients initially classified as nonsmokers but with plasma cotinine concentrations ≥85 nmol/L were reclassified as smokers) (29). BMI (in kg/m^2) was obtained by dividing weight by height squared. The estimated glomerular filtration rate (eGFR)/1.73 m^2 was calculated on the basis of the chronic kidney disease–epidemiology collaboration formula (30).

Follow-up and clinical endpoints

WECAC participants were followed up from enrollment until death or 31 December 2006, whereas patients included in NORVIT were followed throughout 2007. Data on fatal events was obtained from the Cause of Death Registry at Statistics Norway (www.ssb.no/en). The primary endpoint was all-cause mortality with cardiovascular mortality and noncardiovascular mortality regarded as secondary outcomes. Details on the routines for the collection and classification of endpoints have been described previously (23, 31).

Laboratory analyses

Plasma concentrations of PLP, pyridoxal, PA, creatinine, neopterin, kynurenine, and tryptophan were quantified with the use of liquid chromatography–tandem mass spectrometry at Bevital AS (www.bevital.no) (32). CRP was measured in serum with the use of an ultrasensitive immunoassay (Behring nephelometer II system N Latex CRP mono; Behring Diagnostics). Serum concentrations of apolipoprotein A-I (apo A-I) and apolipoprotein B were measured on Hitachi 917 and 912 systems, respectively (Roche Diagnostics GmbH). Details concerning the handling and storage of blood samples before analysis have been described previously (26, 31, 33). Briefly, baseline venous blood samples were drawn either 1–3 d before or immediately after the coronary angiography (in patients with SAP) or a median of 4 d (5th, 95th percentiles: 2, 7 d) after the incident AMI (in patients with AMI) and stored at −80°C until undergoing laboratory analyses during the period 2006–2008.

Statistical analyses

Continuous variables are reported as medians (5th, 95th percentiles), and categorical variables are reported as counts (percentages). Differences in baseline characteristics between cohorts were assessed with the use of Kruskal-Wallis or Mann-Whitney nonparametrical tests for continuous variables and chi-square or Fisher exact tests for categorical variables. All continuous variables displayed some degree of right-skewed distribution as assessed by distribution plots, quantile-quantile plots, and the Shapiro-Wilk test for normality and, therefore, were log transformed before use in parametric statistical models. Summary scores of inflammatory markers were obtained by summing the standardized, log-transformed variables. We used Cox proportional hazards regression to evaluate B-6 vitamers,

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PAr, and other variables as predictors of all-cause mortality. HRs are reported per 1-SD increment. Model assumptions were tested with the use of survival plots and Schoenfeld residuals. The basic model (model 1) was adjusted for age at study enrollment and sex. A multi-adjusted model (model 2) was obtained by applying stepwise selection to a panel of established risk factors that were common to both cohorts. The initial set of candidate predictors included current smoking, diabetes, hypertension, total cholesterol, BMI, and the eGFR, and adjustments for age and sex were maintained throughout. Modeling was performed separately within each cohort, and model 2 was defined as the set of co-predictors that these models shared. A third model (model 3) consisted of model 2 with the addition of inflammatory markers neopterin, KTR, and CRP (WECAC) or neopterin and KTR (NORVIT). The impact on the model fit of the addition or removal of predictors during the stepwise selection procedure was evaluated with the use of the Bayesian information criterion modified for competing risk as recommended by the developers of the crrstep package in R software (version 3.02 for Macintosh; The R-Foundation for Statistical Computing; www.r-project.org) (34). We applied both forward selection and backward selection when modeling with similar results. Subgroups were obtained from predefined categories or created according to median values of continuous variables. We tested effect modifications by adding product terms to the models. Possible nonlinear risk associations were analyzed with the use of penalized smoothing splines for the term of interest in the Cox model. Model discrimination was explored by calculating the improvement in the C statistic by using the multivariate Cox model with or without the new biomarker. We performed all statistical analyses by using R version 3.02 software for Macintosh with the use of the packages crrstep and survival for the Cox regression analysis and the package pROC for the evaluation of receiver operating characteristics. All reported *P* values are 2 sided.

RESULTS

Patient characteristics

Patients in the 2 cohorts were similar in terms of sex with men representing 71.9% and 73.9% of the SAP and AMI cohorts, respectively ($P = 0.05$). The age distributions were similar for men with a median age of 61 y (5th, 95th percentiles: 44, 77 y) in the SAP cohort and a median age of 61 y (5th, 95th percentiles: 44, 81 y) in the AMI cohort ($P = 0.31$). As expected, women were older than men in both cohorts ($P < 0.001$) and were older in the AMI cohort than in the SAP cohort ($P < 0.001$) (**Table 1**). Compared with patients with SAP, AMI patients had less hypertension and diabetes but had higher total cholesterol and were more frequently current smokers. Moreover, AMI patients had lower concentrations of PLP, pyridoxal, and PA; a higher PAr and KTR; and higher neopterin concentrations. The mortality rate was ~2 times as high in the AMI-patient group as in the SAP patients. Additional characteristics are shown in Table 1. Approximately 52% of SAP patients and 20% of AMI patients had a previous CAD history (i.e., a previous AMI or a coronary revascularization procedure before enrollment). The mortality rate differed considerably by previous CAD history and ranged from 9.9/1000 person-years in SAP patients with no previous CAD history to 64.2/1000 person-years in AMI patients with a previous CAD history (**Supplemental Table 1**). The variables that most closely paralleled this variation in mortality risk were the use of antihypertensive medicine at hospital discharge, PAr, KTR, eGFR, neopterin, and age according to unadjusted Spearman correlations.

Association of vitamin B-6 indexes with inflammatory markers and other baseline characteristics

The correlations of B-6 vitamers and the PAr index with inflammatory markers and established cardiovascular disease risk factors are shown in **Table 2**. Generally, PLP was negatively associated with all inflammatory markers, whereas PA was positively associated with the 2 cellular immunity markers. Pyridoxal associations were weak and somewhat inconsistent. PAr showed, by comparison, strong positive associations with all inflammatory markers (Table 2). Correlations with established risk predictors were mostly weak with notable exceptions for PA and PAr with eGFR. Finally, PLP, pyridoxal, and PA were correlated with taking vitamin supplements, whereas PAr correlated only weakly or not at all (Table 2).

Vitamin B-6 indexes and all-cause mortality

The association of vitamin B-6 indexes with all-cause mortality was modeled with the use of Cox regression adjusted for age and sex (model 1, and, in addition, for current smoking and diabetes (model 2). In both cohorts, PLP was not, or negatively associated, and PA and PAr were positively associated with all-cause mortality, whereas pyridoxal showed no significant association (**Table 3**). After additional adjustment for inflammatory markers, only PA among the isolated vitamers was significantly associated with mortality risk in SAP patients. In contrast, PAr was a strong predictor of all-cause mortality with HRs per 1-SD increment according to model 2 of 1.45 (95% CI: 1.30, 1.63) and 1.31 (95% CI: 1.21, 1.41) for SAP and AMI patients, respectively. When inflammatory markers were added (model 3), PAr, current smoking, diabetes, and CRP were significant covariates in the model for SAP patients, whereas PAr, current smoking, diabetes, neopterin, and KTR were significant covariates in the model for AMI patients. Risk estimates for cardiovascular mortality were essentially similar to those shown for all-cause mortality in both cohorts (**Supplemental Table 2**).

PAr compared with other risk predictors

We compared risk predictors by analyzing how much they improved the model fit (Δ Aikake's information criterion) after being added to a Cox regression model that included age and sex (**Table 4**). In patients with suspected SAP, PAr was the strongest predictor followed by current smoking, CRP, neopterin, apo A-I, KTR, diabetes, and the eGFR. Forward stepwise selection selected PAr, current smoking, diabetes, apo A-I, and CRP, in that order, as the best model for predicting all-cause mortality (Table 4). In AMI patients, both neopterin and KTR had numerically stronger Δ Aikake's information criterion values than did PAr, and the best model for risk prediction included, in the order of selection, neopterin, current smoking, diabetes, KTR, and PAr (Table 4).

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TABLE 1
Characteristics of study subjects[1]

	Patients with SAP	Patients with AMI	*P*
n	4164	3749	—
Male, *n* (%)	2995 (71.9)	2771 (73.9)	0.05
Age, y	62 (44, 78)[2]	63 (44, 81)	<0.001
Men	61 (44, 77)	61 (43, 80)	0.31
Women	64 (45, 79)	69 (46, 82)	<0.001
BMI, kg/m^2	26.3 (21.1, 33.7)	26.0 (20.8, 33.0)	<0.001
eGFR, mL · min^{-1} · 1.73 m^{-2}	80 (50, 103)	77 (46, 111)	<0.001
apo A-I, g/L	1.30 (0.92, 1.80)	NA	—
apoB, g/L	0.87 (0.57, 1.36)	NA	—
Total cholesterol, mmol/L	4.9 (3.5, 7.1)	5.7 (3.9, 7.9)	<0.001
Hypertension, *n* (%)	1945 (46.7)	1074 (29.0)	<0.001
Current smoker, *n* (%)	1320 (31.7)	1795 (47.9)	<0.001
Diabetes mellitus, *n* (%)	495 (11.9)	368 9.9)	0.005
PLP, nmol/L	41.3 (18.7, 124)	28.4 (12.9, 72.3)	<0.001
Pyridoxal, nmol/L	9.5 (5.1, 31.8)	6.3 (3.4, 13.9)	<0.001
PA, nmol/L	24.4 (13.6, 94.5)	18.7 (9.4, 50.8)	<0.001
PAr, nmol:nmol	0.50 (0.25, 1.06)	0.54 (0.25, 1.24)	<0.001
CRP, mg/L	1.8 (0.4, 12.4)	NA	—
KTR, nmol:mmol	23.8 (15.3, 41.3)	25.0 (15.9, 46.0)	<0.001
Neopterin, nmol/L	8.2 (5.2, 16.2)	8.8 (4.9, 20.9)	<0.001
Previous MI, *n* (%)	1679 (40.3)	628 (17.0)	<0.001
Previous PCI, *n* (%)	798 (19.2)	181 (4.8)	<0.001
Previous CABG, *n* (%)	479 (11.5)	177 (4.7)	<0.001
Extent of CAD at angiography, *n* (%)			—
No significant CAD	1046 (25.1)	NA	
Single vessel disease	966 (23.2)	NA	
Double vessel disease	928 (22.3)	NA	
Triple vessel disease	1223 (29.4)	NA	
Medication at hospital discharge, *n* (%)			
Aspirin	3399 (81.6)	3088 (88.6)	<0.001
Statins	3334 (80.1)	2827 (81.4)	0.15
β Blockers	3014 (72.4)	3175 (91.2)	<0.001
ACE inhibitors	860 (20.7)	1072 (31.0)	<0.001
Loop diuretics	451 (10.8)	614 (17.7)	<0.001
All-cause mortality			
Follow-up time, y	4.7 (2.8, 6.8)	7.0 (1.0, 8.8)	<0.001
Events, *n* (%)	308 (7.4)	777 (20.7)	<0.001
Person-years	19,580	24,647	<0.001
Incidence rate/1000 person-years	15.7	31.5	<0.001

[1]*P* values were determined with the use of the Mann-Whitney test for continuous variables and Fischer's exact test for categorical variables. ACE, angiotensin-converting enzyme; AMI, acute myocardial infarction; apo A-I, apolipoprotein A-I; apoB, apolipoprotein B; CABG, coronary artery bypass graft; CAD, coronary artery disease; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; KTR, kynurenine:tryptophan ratio; MI, myocardial infarction; NA, not applicable; PA, pyridoxic acid; PAr, pyridoxic acid:(pyridoxal + PLP) ratio; PCI, percutaneous coronary intervention; PLP, pyridoxal 5′-phosphate; SAP, stable angina pectoris.
[2]Median; 5th, 95th percentiles in parentheses (all such values).

Stratified analyses

Risk estimates for PAr did not differ according to vitamin-treatment (i.e., intervention) group in either cohort ($P \geq 0.23$). Risk estimates also did not vary according to smoking status, diabetes, or concentrations of neopterin or KTR in either patient cohort (all *P*-interaction ≥ 0.18). When evaluated in SAP patients only, there were no effect modifications according to concentrations of CRP and apo A-I or by the extent of coronary artery disease. In SAP patients, PAr was a better prognostic marker for women (*P*-interaction = 0.003) and for those without hypertension (*P*-interaction = 0.01), but such differences were not shown in AMI patients. In AMI patients, study-specific blood samples were taken at a median of 4 d (5th, 95th percentiles: 2, 7 d) after the index AMI. Risk estimates for PAr or any of the other variables did not depend on this time lag (*P*-interaction ≥ 0.23). Notably, PAr was a stronger risk predictor in subjects with no previous CAD history (*P*-interaction = 0.04 and 0.005 for SAP and AMI patients, respectively) (**Table 5**).

Sensitivity analyses

We tested the possibility of reverse causation by excluding the first 365 d of follow-up. Analyses were performed separately according to previous CAD history in both cohorts, and risk estimates were adjusted according to model 2. In patient groups with no previous CAD history, HRs with the use of all data

TABLE 2
Correlation between vitamin B-6 indexes and selected variables[1]

	Patients with SAP				Patients with AMI			
	PLP	Pyridoxal	PA	PAr	PLP	Pyridoxal	PA	PAr
Inflammatory markers								
Neopterin	−11	3	23	45	−15	−3	13	33
KTR	−14	−1	18	42	−6	8	21	33
CRP	−23	−12	1	29	—	—	—	—
Summary score[2]	−22	−5	18	52	−12	3	19	37
Established risk predictors								
Current smoking	−17	−12	−9	8	−17	−7	−1	17
Diabetes	−3	0	4	9	−1	4	6	8
Hypertension	0	1	6	9	−3	4	7	11
Apolipoprotein A-I	21	13	7	−15	—	—	—	—
Apolipoprotein B	0	0	1	2	—	—	—	—
Estimated GFR	−5	−10	−26	−30	−5	−10	−22	−23
Vitamin supplements[3]	22	23	25	5	14	11	11	−2

[1]All values are Pearson correlation coefficients × 100 and were adjusted for age and sex. Correlations with a magnitude >4 were significant at $P < 0.05$. AMI, acute myocardial infarction; CRP, C-reactive protein; GFR, glomerular filtration rate; KTR, kynurenine:tryptophan ratio; PA, pyridoxic acid; PAr, pyridoxic acid:(pyridoxal + PLP) ratio; PLP, pyridoxal 5′-phosphate; SAP, stable angina pectoris.
[2]For CRP + neopterin + KTR (SAP group) or neopterin + KTR (AMI group).
[3]Intake of B-vitamin–containing supplements.

compared with data that excluded the first year of follow-up were similar: 1.69 (95% CI: 1.41, 2.03) compared with 1.67 (95% CI: 1.37, 2.04), respectively, for SAP patients and 1.41 (95% CI: 1.28, 1.54) compared with 1.55 (95% CI: 1.39, 1.72) respectively, for AMI patients. For patient groups with a previous CAD history, risk estimates were 1.34 (95% CI: 1.17, 1.54) compared with 1.18 (95% CI: 1.01, 1.39), respectively, for SAP patients and 1.10 (5th, 95th percentiles: 0.98, 1.24) compared with 1.03 (95% CI: 0.88, 1.20), respectively, for AMI patients.

Nonlinear risk-relations

Nonlinear risk-relations were assessed with the use of Cox regression with the application of a penalized smoothing spline for the main predictor. For SAP and AMI patients with no previous CAD history, the risk association was essentially linear across the distribution of PAr values. Patients with a previous CAD history were at higher baseline risk, and an additional increase in risk according to PAr was shown only at values above the median for SAP patients, whereas PAr did not modulate risk at all for this subgroup of AMI patients (**Figure 1**). Overall risk gradients for PLP, pyridoxal, and PA were less pronounced and tended to level off or reverse at high values (representative findings are shown in **Figure 2**). This pattern was shown in both cohorts and did not depend on a previous CAD history. Notably, distributions of PLP and, in particular, pyridoxal and PA were markedly right skewed as depicted by the density plots at the bottom of each panel in Figure 2.

Model discrimination

As shown in the previous 3 sections, PAr had a considerably lower predictive strength in patient groups with previous CAD. Therefore, we applied a receiver operating characteristic analysis only to the patient groups with no previous CAD history. The base model was model 2, and the addition of PAr to this model resulted in an improvement in the C statistic from 0.721 (95% CI: 0.665, 0.777) to 0.753 (95% CI: 0.698, 0.808) ($P = 0.03$) for SAP patients and from 0.781 (95% CI: 0.758, 0.805) to 0.792 (95% CI: 0.769, 0.815) ($P = 0.003$) for AMI patients.

DISCUSSION

Principal findings

We investigated 4 indexes of vitamin B-6 status and metabolism with respect to long-term mortality in 2 cohorts of patients with suspected SAP, and AMI, respectively. In both patient groups, PLP was a negative predictor and PA was a positive predictor of all-cause mortality after adjustment for established risk predictors, but not after additional adjustment for inflammatory markers. The newly proposed marker of vitamin B-6 catabolism, PAr, was a strong predictor of all-cause mortality. In patients with SAP, PAr was selected before the established risk factors smoking, diabetes, apo A-I, CRP, hypertension, and eGFR by Cox regression using stepwise modeling. Notably, PAr was a particularly strong predictor in patients with no previous CAD in both patient groups.

B-6 vitamers and long-term prognosis

A number of previous reports have shown lower circulating PLP concentrations or lower vitamin B-6 intakes in subjects who subsequently developed CAD or stroke (8, 9, 11). Our study confirmed that plasma PLP was inversely related to all-cause mortality incidence across patient categories. However, we found no significant association after including inflammatory markers in the model, which supports the view that low plasma PLP may be secondary to inflammatory activation and that inflammatory processes, rather than low PLP, may be causally related to all-cause mortality (16).

TABLE 3
Vitamin B-6 indexes and all-cause mortality[1]

	Model 1	Model 2	Model 3
Patients with SAP			
PLP	0.83 (0.71, 0.96)	0.89 (0.77, 1.02)	0.98 (0.86, 1.13)
Pyridoxal	1.02 (0.89, 1.17)	1.06 (0.95, 1.18)	1.07 (0.95, 1.20)
PA	1.18 (1.08, 1.30)	1.20 (1.10, 1.30)	1.13 (1.02, 1.25)
PAr	1.52 (1.37, 1.69)	1.45 (1.30, 1.63)	1.29 (1.11, 1.51)
Patients with AMI			
PLP	0.83 (0.76, 0.90)	0.87 (0.80, 0.95)	0.93 (0.86, 1.01)
Pyridoxal	1.00 (0.93, 1.07)	1.03 (0.96, 1.09)	1.03 (0.96, 1.10)
PA	1.09 (1.03, 1.15)	1.09 (1.03, 1.16)	1.03 (0.97, 1.10)
PAr	1.37 (1.27, 1.47)	1.31 (1.21, 1.41)	1.12 (1.03, 1.22)

[1]All values are HRs per 1-SD increment of the predictor; 95% CIs in parentheses. Model 1 was adjusted for age and sex. Model 2 was adjusted as for model 1 and for current smoking and diabetes. Model 3 was adjusted as for model 2 and for neopterin, KTR, and CRP (patients with SAP) and for neopterin and KTR (patients with AMI). Risk estimates were obtained by Cox regression. AMI, acute myocardial infarction; CRP, C-reactive protein; KTR, kynurenine:tryptophan ratio; PA, pyridoxic acid; PAr, pyridoxic acid: (pyridoxal + PLP) ratio; PLP, pyridoxal 5′-phosphate; SAP, stable angina pectoris.

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TABLE 4
Comparison of predictive strength for selected variables[1]

	Patients with SAP			Patients with AMI		
	ΔAIC[2]	Order of strength[3]	Order of selection[4]	ΔAIC[2]	Order of strength[3]	Order of selection[4]
PAr	−59.7	1	1	−66.9	3	5
Current smoking	−41.4	2	2	−29.5	4	2
Diabetes	−11.5	6	3	−21.3	6	3
Hypertension	NS	NS	NS	−4.2	8	NS
Apolipoprotein A-I	−24.0	5	4	NA	NA	NA
C-reactive protein	−38.3	3	5	NA	NA	NA
Neopterin	−31.9	4	NS	−91.1	1	1
KTR	−15.5	7	NS	−74.4	2	4
Estimated GFR	−11.4	8	NS	−28.5	5	NS
Total cholesterol	1.8	9	NS	−9.9	7	NS
BMI	1.9	10	NS	0.8	9	NS

[1]AIC, Aikake's information criterion; AMI, acute myocardial infarction; GFR, glomerular filtration rate; KTR, kynurenine:tryptophan ratio; NA, not applicable; PAr, pyridoxic acid:(pyridoxal + PLP) ratio; PLP, pyridoxal 5′-phosphate; SAP, stable angina pectoris.
[2]Reduction in the AIC after the inclusion of the variable in a Cox regression model that was adjusted for age and sex. The AIC for the starting model was 4760.1 (SAP group) and 11,063.6 (AMI group). ΔAIC values can be compared vertically but not horizontally.
[3]Order of predictive strength according to the ΔAIC.
[4]Order of selection in a Cox regression model with the use of forward stepwise selection.

PAr and long-term prognosis

As reported previously (20), PAr was strongly correlated with the established inflammatory markers CRP, neopterin, and KTR. These markers were highly significant predictors of all-cause mortality in simple models. However, in the SAP-patient group, PAr was a stronger predictor and was selected in preference to both neopterin and KTR during the stepwise modeling. In addition, established risk predictors including current smoking, diabetes, hypertension, CRP, lipoprotein fractions, BMI, and kidney function (eGFR) were all weaker predictors than was PAr. In the AMI-patient group, the cellular immunity markers neopterin and KTR rivaled PAr as predictors of all-cause mortality, and all 3 variables were retained in the final multiadjusted model. Thus, this result highlights an involvement of inflammatory processes at progressed stages of CAD. Moreover, PAr, along with neopterin and KTR, apparently, captured overlapping but distinct aspects of inflammatory activation that was related to future mortality risk in this patient group.

TABLE 5
PAr and all-cause mortality risk by strata of other risk predictors[1]

	Patients with SAP			Patients with AMI		
	n	HR (95% CI)[2]	*P*	*n*	HR (95% CI)[2]	*P*
Sex			0.003			0.84
F	1162	2.01 (1.65, 2.46)		950	1.26 (1.10, 1.45)	
M	2968	1.35 (1.19, 1.54)		2687	1.34 (1.22, 1.46)	
Hypertension			0.01			0.93
No	2199	1.78 (1.46, 2.17)		2542	1.32 (1.19, 1.46)	
Yes	1931	1.32 (1.15, 1.52)		1033	1.27 (1.13, 1.42)	
Previous CAD			0.04			0.005
No	1999	1.69 (1.41, 2.03)		2834	1.41 (1.28, 1.54)	
Yes	2131	1.34 (1.17, 1.54)		727	1.10 (0.98, 1.24)	

[1]Risk estimates were obtained by Cox regression. AMI, acute myocardial infarction; CAD, coronary artery disease; PAr, pyridoxic acid:(pyridoxal + PLP) ratio; PLP, pyridoxal 5′-phosphate; SAP, stable angina pectoris.
[2]Per 1-SD increment of PAr adjusted for age, sex, current smoking, and diabetes.

Nonlinear risk associations and stratified and sensitivity analyses

In both cohorts, patients with previous CAD had considerably higher mortality rates and higher median PAr values than did subjects with no known CAD at study enrollment. These observations may raise the question of whether PAr merely reflected more advanced disease at baseline. However, a noticeable and consistent finding of the current study was that PAr provided the best risk prediction in subjects with no previous CAD history. In these patients, mortality risk increased through the entire distribution of PAr. In contrast, in subgroups with previously diagnosed CAD, elevations in risk were confined to PAr values that were above the median, or could not be shown. Notably, a similar difference in risk estimates across subgroups based on previous CAD history was observed even after exclusion of events occurring during the first year of follow-up. Finally, the HRs were of similar magnitude to those shown for the total follow-up period. Hence, the association of PAr with an adverse prognosis was probably not explained by reverse causation.

Risk relations for the individual B-6 vitamers PLP, pyridoxal, and PA tended to level off at high concentrations. Generally, high concentrations are observed shortly after taking pyridoxin-containing supplements (19). Such high concentrations are short-lived (a few hours), do not reflect vitamin status, and probably explain the right-skewed distributions of B-6 vitamer concentrations. Consequently, risk associations at higher percentiles of the vitamin-metabolite distributions could be expected to tend toward mean risk as was observed. In contrast, the PAr index showed

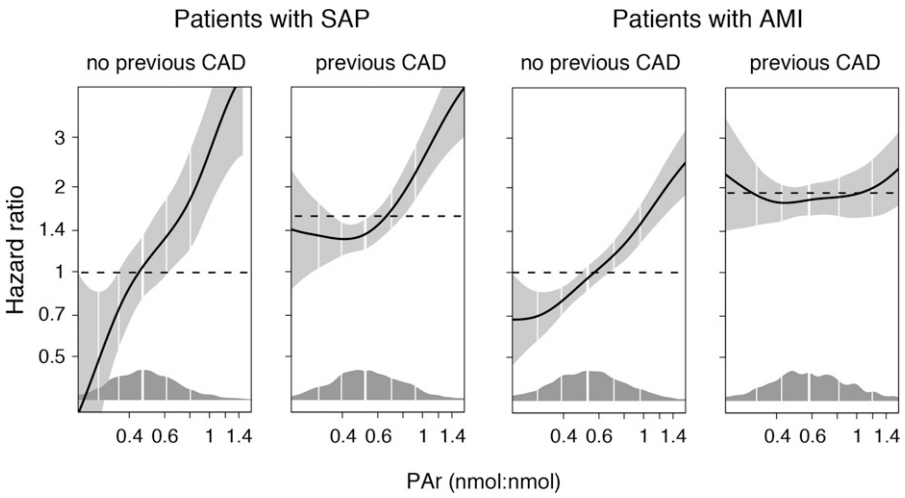

FIGURE 1 Associations of PAr with all-cause mortality by Cox regression and penalized smoothing splines. Risk associations were adjusted for age, sex, current smoking, and diabetes. Shaded areas around central estimates denote 95% confidence bands. Horizontal broken lines show relative differences in the adjusted mean risk by previous CAD history with the group with no previous CAD history as the reference (adjusted mean HR: 1). Density plots show the distribution, and vertical white lines indicate 10th, 25th, 50th, 75th, and 90th percentiles of PAr. For SAP patients, the numbers of subjects in the group with no CAD history compared with those in the group with previous CAD history were 1999 and 2137, respectively, and for AMI patients, the numbers were 2834 and 727, respectively. AMI, acute myocardial infarction; CAD, coronary artery disease; PAr, pyridoxic acid:(pyridoxal + pyridoxal 5′-phosphate) ratio; SAP, stable angina pectoris.

essentially no correlation with vitamin supplement intake and exhibited a nearly symmetrical distribution in plasma. Hence, potential confounders that proportionally affect PA and pyridoxal plus PLP, such as supplement intake, are apparently corrected for by the ratio, thereby contributing to the robustness and specificity of the PAr index.

PAr, oxidative stress, and cellular immunity

In the current study as well as in a previous study (20), PAr showed the strongest correlations with the 2 cellular immunity markers neopterin and KTR. A number of mechanisms may explain these observations. Reactive aldehydes are formed during the course of normal metabolism; however, oxidative stress accelerates this process, and the detrimental accumulation of reactive aldehyde species has been shown in atherosclerotic plaques (35). One protective response is the increased expression of aldehyde-scavenging oxidases such as aldehyde oxidase 1 (AOX1). This enzyme was recently shown to be regulated by the Nrf2/ARE transcription pathway, which is a key pathway in the oxidative stress response (36, 37). AOX1 has also been recognized as the enzyme that oxidizes pyridoxal to PA (38). The expression of AOX1 has been mainly shown in the liver, but activities have also been reported in the epithelia of lung, intestine, and kidneys (39). Conceivably, members of the inducible aldehyde dehydrogenase protein family (40, 41) could also contribute to the oxidation of pyridoxal to PA either as an unrecognized activity or as a result of modifications of enzyme specificities or kinetics by the local microenvironment. Concentrations of the 2 established cellular immunity markers neopterin and KTR depend on interferon-γ but also, extensively, on redox signaling (42, 43). Furthermore, neopterin, plays a role

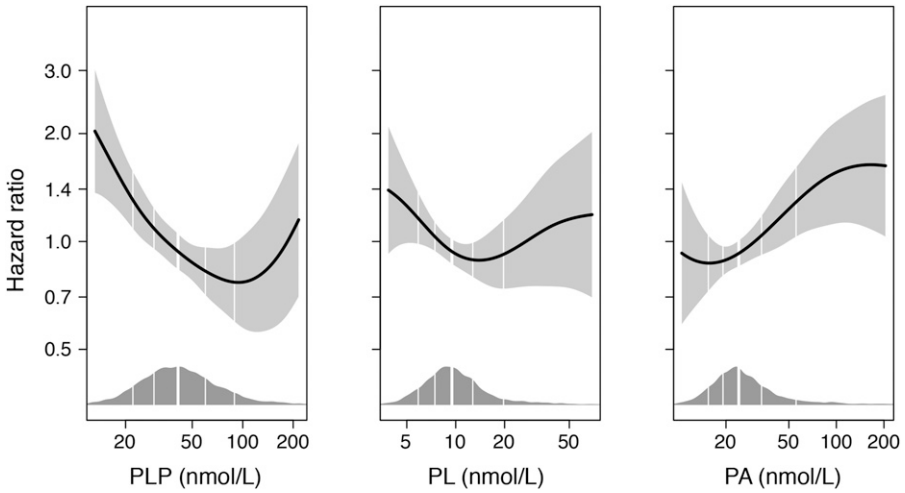

FIGURE 2 Associations of B-6 vitamers with all-cause mortality by Cox regression and penalized smoothing splines. Data for stable angina pectoris patients (Western Norway Coronary Angiography Cohort) are shown. Risk associations were adjusted for age, sex, current smoking, and diabetes. Shaded areas around central estimates denote 95% confidence bands. Density plots show the distribution, and vertical white lines indicate 10th, 25th, 50th, 75th, and 90th percentiles of the predictors. The first to 98th percentile ranges of the predictors are shown. PA, pyridoxic acid; PL, pyridoxal; PLP, pyridoxal 5′-phosphate.

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in potentiating the oxidative capacity of reactive oxygen and chloride species (44). Thus, oxidative and aldehyde stress seems to be a common denominator that explains the correlation of PAr with neopterin and KTR, whereas the direct mechanism that explains a high PAr may involve the associated upregulation of broad-spectrum aldehyde scavenging enzymes such as AOX1 and members of the inducible aldehyde dehydrogenase protein family.

PAr and stress response

PAr was also correlated with CRP, which is a marker of the IL-1b/TNF-α/IL-6 pathway. IL-1b and IL-6 are both activators of the hypothalamic-pituitary-adrenal (HPA) axis (45, 46). The HPA axis is the main regulator of the physiologic stress response with widespread effects including the upregulation of gluconeogenesis and the degradation of protein in muscle, gut, and connective tissue. The liberated amino acids may then be used for energy production, synthesis of immunomodulating proteins, immune cell proliferation, and tissue repair. All of these processes require and may, therefore, increase the cellular demand for PLP. Moreover, an increase in intracellular PLP has been implicated in the modulation of the cell's response to glucocorticoids (47). Notably, IL-6 also upregulates alkaline phosphatase that is necessary for the uptake of PLP from the circulation (48, 49). PAr was associated with CRP mainly through a reduction in PLP and not through an increase in PA. Therefore, some of the increase in PAr might have been attributed to elevated intracellular demands for PLP associated with the stress response.

Strengths and limitations

The study included 2 large cohorts, and all data were obtained prospectively. We had detailed information on patient characteristics including the angiographic extent of coronary artery disease in patients with suspected SAP. Follow-up was ascertained through the use of a population-based registry. Limitations include the observational nature of the study. However, some of these limitations are mitigated by the inclusion of the independent validation cohort (NORVIT). Unfortunately, because of a lack of data, we were unable to replicate some analyses (e.g., analyses that included CRP and apolipoproteins in the NORVIT). Another limitation of the NORVIT cohort is blood sampling a few days after the incident AMI. This period represents a short time lag after a major traumatic event, which could have affected blood values. Specifically, plasma concentrations of several vitamins including vitamin B-6 were shown to decrease substantially but transiently after an AMI (50). This is a probable explanation for the low concentration of B-6 vitamers in the AMI patients. Intermittent changes in food and supplement intakes and the limited ability to smoke during the hospital stay could also have affected vitamin concentrations and inflammatory markers as previously reported (51). These factors might have generated noise in the data that could have affected (mainly attenuated) the predictive value of all or some of the plasma indexes. Despite this fact, we observed a high degree of consistency of risk associations across patient groups (i.e., cohorts) and subgroups. Traditional risk factors including diabetes, hypertension, and dyslipidemia were actively treated in both patient cohorts; thus, risks associated with these variables should be regarded as risks residual to ongoing treatment. Therefore, it remains to be shown how PAr compares with conventional risk factors in populations with low to moderate risk where the majority of individuals do not receive any treatment, medication, or other intervention.

In conclusion, we evaluated 4 vitamin B-6 biomarkers for their ability to predict future mortality in 2 independent cohorts of CAD patients. None of the B-6 vitamers were significant predictors after adjustment for inflammatory markers. In contrast, the newly described inflammation-associated marker PAr showed strong and independent associations with all-cause mortality and cardiovascular mortality. The PAr biomarker is particularly interesting in terms of possible underlying mechanisms. Elevations in PAr could reflect the induction of aldehyde scavenging enzymes during oxidative and aldehyde stress and activated cellular immunity. An additional mechanism implicates the HPA-linked stress response. The current findings should motivate additional studies of PAr as a long-term prognostic marker especially in patient groups at perceived low to intermediate risk and in healthy populations.

The authors' responsibilities were as follows—AU: drafted the the manuscript and had primary responsibility for the final content of the manuscript; AU and ERP: analyzed the data; AU, ON, and PMU: created the study concept and design; ERP, GFTS, and ØM: acquired the data; ERP, GFTS, AM, ØM, ON, and PMU: critically revised the manuscript for important intellectual content; and all authors: read and approved the final manuscript. None of the authors reported a conflict of interest related to the study. Alpharma Inc. played no role in the design, implementation, analysis, or interpretation of the study.

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Online Supplemental Material

Supplemental table 1. Characteristics of the study subjects according to prior CHD history.

	Patients with SAP				P-value[1]	Patients with AMI				P-value[1]
	no prior CHD		prior CHD			no prior CHD		prior CHD		
n	2012		2151			2949		750		
Age y	61	(44, 77)	64	(45, 79)	< 0.001	62	(43, 80)	70	(39, 109)	< 0.001
Sex n (%)	726	(73.9)	442	(79.5)	< 0.001	2170	(73.6)	566	(75.5)	0.31
Body mass index (kg/m^2)	26.3	(20.9, 33.9)	26.4	(21.4, 33.5)	0.17	25.9	(21.0, 33.0)	26.1	(20.4, 32.2)	0.76
Estimated GFR (ml/min/1.73m^2)	82	(54, 106)	79	(47, 101)	< 0.001	78	(49, 112)	70	(39, 109)	< 0.001
ApoA1 (g/L)	1.34	(0.96, 1.86)	1.26	(0.90, 1.71)	< 0.001					
ApoB (g/L)	0.91	(0.58, 1.39)	0.83	(0.56, 1.29)	< 0.001					
Total cholesterol (mmol/L)	5.2	(3.6, 7.4)	4.7	(3.4, 6.8)	< 0.001	5.8	(4.1, 8.0)	5.2	(3.4, 7.5)	< 0.001
Hypertension n (%)	913	(45.4)	1032	(48.0)	0.09	748	(25.5)	308	(42.2)	< 0.001
Current smoker n (%)	595	(29.6)	725	(33.7)	0.005	1505	(51.0)	262	(34.9)	< 0.001
Diabetes mellitus n (%)	205	(10.2)	290	(13.5)	0.001	231	(7.9)	132	(17.7)	< 0.001
PLP (nmol/L)	43.3	(19.7, 119)	39.7	(17.7, 128)	< 0.001	28.5	(13.2, 71.1)	28.4	(11.7, 80,1)	0.31
PL (nmol/L)	9.7	(5.2, 30.0)	9.4	(5.1, 34.3)	0.09	6.1	(3.4, 13.3)	6.7	(3.6, 16.5)	< 0.001
PA (nmol/L)	23.9	(13.3, 82.9)	25.0	(13.8, 107)	< 0.001	18.4	(9.4, 45.7)	20.4	(10.0, 73.8)	< 0.001

PAr (nmol/nmol)	0.47	(0.24, 1.00)	0.53	(0.26, 1.15	< 0.001	0.53	(0.25, 1.16)	0.58	(0.27, 1.57)	< 0.001
CRP (mg/L)	1.7	(0.4, 12.2)	1.9	(0.4, 12.6)	0.003					
KTR (nmol/μmol))	23.1	(14.7, 38.6)	24.6	(15.8, 44.3)	< 0.001	24.5	(15.9, 43.2)	27.6	(16.4, 56.2)	< 0.001
Neopterin (nmol/L)	8.1	(5.1, 14.9)	8.3	(5.2, 18.2)	0.02	8.6	(4.8, 19.3)	9.6	(5.1, 25.6)	< 0.001
Prior MI n (%)			1679	(78.1)				627	(83.6)	
Prior PCI n (%)			798	(36.9)				181	(24.1)	
Prior CABG n (%)			478	(22.2)				173	(23.1)	
No significant CAD n (%)	813	(40.4)	233	(10.8)	< 0.001					
Single vessel disease n (%)	404	(20.1)	562	(26.1)	< 0.001					
Double vessel disease n (%)	372	(18.5)	556	(25.8)	< 0.001					
Triple vessel disease n (%)	423	(21.0)	800	(37.2)	< 0.001					
Medication at hospital discharge										
Aspirin	1473	(73.2)	1926	(89.5)	< 0.001	2515	(90.5)	537	(81.5)	< 0.001
Statins	1361	(67.6)	1973	(91.7)	< 0.001	2253	(81.4)	540	(81.8)	0.87
Beta blockers	1257	(62.5)	1757	(81.7)	< 0.001	2550	(91.8)	586	(88.7)	0.01
ACE inhibitors	252	(12.5)	608	(28.3)	< 0.001	799	(28.9)	258	(39.3)	< 0.001

Loop diuretics	116	(5.8)	335	(15.6)	< 0.001	392	(14.2)	207	(31.6)	< 0.001
All-cause mortality										
Follow-up time (y)	4.6	(2.9, 6.7)	4.9	(2.4, 6.9)	< 0.001	7.1	(0.9, 8.8)	7.0	(1.5, 8.8)	< 0.001
Events	92	(4.6)	212	(10.5)	< 0.001	485	(16.4)	273	(36.4)	< 0.001
Person-years	9295		10141		< 0.001	20097		4251		< 0.001
Incidence rate/1000 person years	9.9		20.9		< 0.001	24.1		64.2		< 0.001

Numbers are medians (5th, 95th percentile), or n (%)

[1]Difference according to prior CHD history. Mann Whitney test for continous variables and Fisher's exact test for count data

Online Supplemental Material

Supplemental table 2. Vitamin B-6 indices and cardiovascular mortality

	Model 1		Model 2		Model 3	
	HR	(95% CI)	HR	(95% CI)	HR	(95% CI)
Patients with SAP						
PLP	0.84	(0.68, 1.03)	0.90	(0.74, 1.10)	1.02	(0.85, 1.22)
PL	1.04	(0.87, 1.24)	1.08	(0.94, 1.25)	1.09	(0.94, 1.27)
PA	1.25	(1.12, 1.38)	1.25	(1.14, 1.38)	1.18	(1.05, 1.33)
PAr (PA/(PL+PLP))	1.62	(1.42, 1.84)	1.52	(1.32, 1.76)	1.36	(1.10, 1.70)
Patients with AMI						
PLP	0.86	(0.78, 0.96)	0.89	(0.80, 0.99)	0.95	(0.86, 1.06)
PL	1.02	(0.94, 1.11)	1.03	(0.95, 1.12)	1.03	(0.94, 1.13)
PA	1.10	(1.02, 1.19)	1.09	(1.01, 1.18)	1.03	(0.94, 1.12)
PAr (PA/(PL+PLP))	1.35	(1.23, 1.48)	1.28	(1.17, 1.41)	1.08	(0.98, 1.20)

Risk estimates were obtained by Cox regression. Numbers are HRs per 1 SD increment of the predictor.

Model 1: Age and sex

Model 2: Model 1 + current smoking and diabetes

Model 3: Model 2 + neopterin, KTR, and CRP (SAP), + neopterin and KTR (AMI)

Abbreviations: AMI, acute myocardial infarction; PA, pyridoxic acid; PL, pyridoxal; PLP, pyridoxal 5´- phosphate; SAP, stable angina pectoris