

Evidence for increased catabolism of vitamin B-6 during systemic inflammation^{1–3}

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ABSTRACT

Background: Plasma concentrations of PL 5'-phosphate (PLP), which is the active coenzyme form of vitamin B-6, are reduced during inflammation. The underlying mechanisms may include altered tissue distribution or increased catabolism via pyridoxal (PL) to pyridoxic acid (PA). Recently, we showed that catabolic enzyme activity could be assessed by substrate product ratios measured in plasma.

Objective: We evaluated the ratios PA:PL, PA:PLP, and PA:(PL + PLP) as possible markers of vitamin B-6 catabolism.

Design: Cross-sectional and longitudinal data were derived from the Western Norway B-Vitamin Intervention Trial. We analyzed associations of ratios with inflammatory markers and other clinical variables by using multiple linear regression and partial correlation. In addition, intraclass correlation coefficients (ICCs) were used to assess the ability of plasma indexes to differentiate between subjects.

Results: PA:(PL + PLP) had the highest ICC of all vitamin B-6 metabolites and ratios tested. In regression models, the inflammatory markers C-reactive protein, white blood cell count, neopterin, and kynurenine:tryptophan collectively accounted for 28% of the total and > 90% of the explained variation in PA:(PL + PLP). For individual B-6 metabolites, corresponding numbers were 19–25% and 20–44%, respectively, with vitamin supplement intake, smoking, and kidney function (estimated glomerular filtration rate) as additional predictors. In an analysis of receiver operating characteristics, PA:(PL + PLP) discriminated high inflammatory concentrations with an area under the curve (95% CI) of 0.85 (0.81, 0.89).

Conclusions: Broad-specificity enzymes upregulated to reduce oxidative and aldehyde stress could explain increased catabolism of vitamin B-6 during inflammation. The ratio PA:(PL + PLP) may provide novel insights into pathologic processes and potentially predict risk of future disease. *Am J Clin Nutr* 2014;100:250–5.

INTRODUCTION

Vitamin B-6 is involved in >100 enzymatic reactions in the body including the metabolism of amino acids, neurotransmitters, nucleic acids, heme, and lipids. Vitamin B-6 is also involved in energy homeostasis through glycogen degradation and gluconeogenesis. The active coenzyme form of vitamin B-6 is pyridoxal 5'-phosphate (PLP)⁴, and its concentration in plasma is the most frequently used measure of vitamin B-6 status (1, 2). The dephosphorylated form pyridoxal (PL) and the main catabolite 4-pyridoxic acid (PA) are also present in plasma, but normally at lower concentrations.

A number of epidemiologic studies have shown reduced concentrations of circulating PLP in association with chronic or

acute disease (3–6). Inverse associations have been shown between plasma PLP and the acute phase marker C-reactive protein (CRP) (5, 7–9) but also to a wider panel of inflammatory markers (10, 11). Most evidence points to an altered tissue distribution as the main mechanism (5, 6, 8, 9), whereas no conclusive evidence have been provided for increased catabolism (5, 9). Recently, we noted positive associations between PA and 2 markers of T-helper 1 type immune activation neopterin and kynurenine:tryptophan (KTR) both before and during vitamin B-6 treatment. These results suggested an increased catabolism of vitamin B-6 during activated cellular immunity (9).

The enzyme indoleamine 2,3-dioxygenase (IDO) converts tryptophan to kynurenine in hematopoietic and epithelial tissue. On stimulation by inflammatory signals, most importantly interferon- γ , IDO activity increases, leading to an increase in KTR in plasma (12). Recently, we showed the utility of 3'-hydroxykynurenine: xanthurenic acid as an indicator of the PLP-dependent activity of kynurenine aminotransferase (13). These examples illustrate the concept of substrate product ratios as measures of enzyme activity. Conceivably, ratios could be used as indicators of the catabolism of PLP via PL to PA.

In this study, we used data from the Western Norway B-Vitamin Intervention Trial (WENBIT), which included measurements of biomarkers in plasma collected at specified time intervals (14). Repeated measurements allow for the evaluation of associations on a longitudinal as well as cross-sectional basis. They also enable the calculation of intraclass correlation

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⁴ Abbreviations used: ALDH, aldehyde dehydrogenase; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; ICC, intraclass correlation coefficient; IDO, indoleamine 2,3-dioxygenase; KTR, kynurenine:tryptophan; PA, pyridoxic acid; PL, pyridoxal; PLP, pyridoxal 5'-phosphate; ROC, receiver operating characteristic; WBC, white blood cell count; WENBIT, Western Norway B-Vitamin Intervention Trial.

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coefficients (ICC) for metabolites and ratios. The ICC is a measure of between-subject variation relative to the total (including within-subject) variation and has been used to assess reliability and within-person reproducibility. The reliability indicates the degree of differentiation between subjects (ie, the ability to tell subjects apart from each other within a population) (15).

The aim of this work was to further explore the possibility of an increased catabolism of PLP during inflammation. Specifically, we evaluated ratios between PA, PL, and PLP concentrations in plasma that could serve as indicators of catabolic enzyme activity. ICCs were calculated and used as an independent assessment of established and novel indexes.

SUBJECTS AND METHODS

Subjects

The WENBIT included 3088 adults (>98% white) who underwent a coronary angiography between 1999 and 2004 at the Haukeland University Hospital (Bergen, Norway) and Stavanger University Hospital (Stavanger, Norway). Details of the WENBIT have been published elsewhere (14). In the current study, participants ($n = 460$) classified as having acute coronary syndrome were excluded, which left 2628 eligible subjects for the final analyses. Of included subjects, 2584 participants had stable angina pectoris, and 44 participants had aortic stenosis. We used data at baseline and after 28 d follow-up for participants randomly allocated to 4 treatment groups in a 2×2 factorial design. The 4 treatment groups consisted of 1) vitamin B-6 (40 mg pyridoxine hydrochloride), folic acid (0.8 mg), and vitamin B-12 (0.4 mg); 2) folic acid and vitamin B-12; 3) vitamin B-6; and 4) a placebo.

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.

Clinical data and laboratory analyses

Nurses or physicians interviewed patients at baseline by using trial-specific questionnaires. Smoking status was assessed by asking participants if they were current or former smokers. Vitamin supplementation was assessed by asking about the regular use of over-the-counter vitamin supplements. Plasma samples obtained at baseline and after 28 d vitamin treatment were stored at -80°C for a mean duration of 5.6 y before analysis. Concentrations of PLP, PL, PA, neopterin, kynurenine, tryptophan, and creatinine were measured by using Liquid chromatography-tandem mass spectroscopy in the laboratory of Bevitall AS (16, 17). KTR was calculated by dividing the plasma concentration of kynurenine (nmol/L) by the concentration of tryptophan ($\mu\text{mol/L}$). The estimated glomerular filtration rate ($\text{eGFR}/1.73 \text{ m}^2$) was calculated on the basis of the Chronic Kidney Disease Epidemiology Collaboration formula (18). CRP was determined in serum by using an ultrasensitive immunoassay with the Behring nephelometer II system (Latex CRP mono; Behring Diagnostics).

Statistical methods

All metabolites and derived ratios had right-skewed distributions in plasma and were, therefore, log-transformed before

inclusion in statistical analyses. Summary scores of inflammatory markers were obtained by summing standardized, log-transformed variables. Associations between variables were assessed by using multiple linear regression and partial Pearson's correlation with adjustment for age, sex, and study center (Haukeland or Stavanger University Hospitals, model 1) and, in addition, for eGFR, smoking [current smoker (yes or no)], and use of vitamin-containing supplements (yes or no) (model 2). Interactions were evaluated by the inclusion of product-terms in multiple linear regression models. ICCs were calculated on the basis of a 2-way mixed-effects model. With observations at days 0 and 28 in a 2-column matrix, the ICC was estimated by the formula

$$\frac{[\text{Mean square (row)} - \text{mean square (error)}] \div [\text{mean square (row)} + \text{mean square (error)}]}{(1)}$$

in accordance with ICC(C,1) (19). We had complete records for 2559 of 2628 study participants (97.4%). Results of analyses on the basis of the handling of missing data by listwise deletion and the use of multiple imputation were similar. Data are presented by using listwise deletion. Because of the partly explorative nature of the study, we applied a 2-sided significance level (α) of 0.01 for all main effects and interaction terms. Analyses were performed with R for Macintosh version 3.01 software and packages pROC for receiver operating characteristic (ROC) analysis, psych for ICC calculations, and mice for multiple imputation (The R-Foundation for Statistical Computing).

RESULTS

Characteristics of the study population

The median (5th–95th percentile) age of the study population was 62.2 y (45.3–77.5 y), and 79.2% of subjects were men. The median (5th–95th percentile) BMI (in kg/m^2) was 26.5 (21.5–33.5), and 12.5% of subjects used B-vitamin-containing supplements. Furthermore, 24.3% of subjects were current smokers, 11.9% of subjects had diabetes, and 6.3% of subjects had a CRP concentration $>10 \text{ mg/L}$. Additional characteristics are shown in **Table 1**.

ICCs

We considered the ratios PA:PL, PA:PLP, and PA:(PL + PLP) as possible markers of vitamin B-6 catabolism. On the basis of measurements at baseline and day 28, we calculated intraclass coefficients for these ratios as well as for each vitamin B-6 metabolite, kynurenine, and tryptophan by using data from the placebo group ($n = 664$; **Table 2**).

For single metabolites, ICCs ranged from 0.46 (PA and PL) to 0.70 (kynurenine). The highest ICC overall was shown for KTR (0.77). For vitamin B-6 indexes, highest ICCs were 0.74 and 0.75 for PA:PLP and PA:(PL + PLP), respectively. When calculated in pyridoxine treatment groups, ICCs for vitamin B-6 indexes were expected to be low because of substantial alterations (increases) in concentrations of vitamin B-6 metabolites at day 28. Notably, the ICC for PA:(PL + PLP) was also comparatively high in these groups (0.44; **Table 2**). Consequently, we focused on PA:(PL + PLP) in subsequent analyses.



TABLE 1
Baseline characteristics of the study population ($n = 2628$)¹

	Values
Sex (M) [n (%)]	2082 (79.2)
Age (y)	62.2 (45.3–77.5) ²
BMI (kg/m^2)	26.5 (21.5–33.5)
Diabetes [n (%)]	314 (11.9)
Hypertension [n (%)]	1242 (47.3)
Current smoker [n (%)]	638 (24.3)
Vitamin-supplement user [n (%)]	329 (12.5)
Cardiovascular history [n (%)]	
Acute myocardial infarction	1151 (43.8)
Percutaneous coronary intervention	568 (21.6)
Coronary artery bypass graft	366 (13.9)
Carotid artery stenosis, TIA, or stroke	174 (6.6)
Other peripheral artery disease	246 (9.4)
Creatinine ($\mu\text{mol}/\text{L}$)	73.4 (53.0–103)
CRP (mg/L)	1.7 (0.3–12.0)
WBC (count/mL) $\times 10^9$	6.9 (4.5–10.7)
KTR ($\text{nmol}/\mu\text{mol}$)	23.8 (15.8–39.4)
Neopterin (nmol/L)	7.8 (5.2–14.5)
PLP (nmol/L)	39.9 (18.6–101)
PL (nmol/L)	9.3 (5.0–23.6)
PA (nmol/L)	24.1 (14.2–68.7)
Tryptophan ($\mu\text{mol}/\text{L}$)	68.0 (47.3–92.5)
Kynurenine ($\mu\text{mol}/\text{L}$)	1.7 (1.1–2.6)

¹CRP, C-reactive protein; KTR, kynurenine:tryptophan; PA, pyridoxic acid; PL, pyridoxal; PLP, pyridoxal 5'-phosphate; TIA, transient ischemic attack; WBC, white blood cell count.

²Median; 5th–95th percentiles in parentheses (all such values).

KTR and neopterin were highly correlated ($r = 0.68$). Therefore, we made a summary score of neopterin and KTR to be used as an additional marker of cellular immune activation. With the use of data from all subjects ($n = 2628$), the ICC of the summary score was slightly higher than the ICCs of KTR and neopterin (0.75 compared with 0.74 and 0.70, respectively).

Association of vitamin B-6 metabolites and PA:(PL + PLP) with inflammatory markers

We modeled vitamin B-6 metabolites and PA:(PL + PLP) by using multiple linear regression. The variables CRP, white blood cell count (WBC), neopterin, and KTR collectively accounted for a modest part of the variation in PA, PL, and PLP (7.4%, 3.8%, and

9.4%, respectively). In contrast, the 4 inflammatory markers explained 27.9% of the variation in PA:(PL + PLP) (Table 3). Moreover, although the variables eGFR and smoking explained an additional 3–4% of the variation in PA, PL, and PLP; they contributed an additional 0.1%, only in the model explaining PA:(PL + PLP). Intake of vitamin supplements was the most-important single predictor of PA, PL, and PLP but only a weak predictor of PA:(PL + PLP) (Table 3). When supplement users were excluded, the 4 inflammatory markers explained 29.7% of the variation in PA:(PL + PLP), and the total explained was 32.3%.

The correlation between vitamin B-6 indexes and the 4 inflammatory markers are shown in Table 4. PA was positively associated with neopterin and KTR, PL was negatively associated, mainly with CRP and the WBC, and PLP was negatively associated with all 4 inflammatory markers. The association of PA:(PL + PLP) with inflammatory markers was, in all cases, stronger than for individual B-6 metabolites. Strongest correlations were shown between PA:(PL + PLP) and neopterin, KTR, and their summary score ($r = 0.36, 0.37, \text{ and } 0.41$, respectively, according to model I).

PA:(PL + PLP) as a marker of systemic inflammation

The ability of PA:(PL + PLP) to discriminate high circulating concentrations of inflammatory markers was further analyzed by using an ROC analysis. All data at baseline were used for this analysis. AUCs for detecting high (>95th percentile) CRP, WBC, KTR, neopterin, and KTR plus neopterin summary scores were 0.73, 0.58, 0.80, 0.80, and 0.82, respectively. We also made a summary score of neopterin plus KTR plus CRP. PA:(PL + PLP) detected high concentrations (>95th percentile) of this score with an AUC (95% CI) of 0.85 (0.81, 0.89) (Figure 1). In groups who received 40 mg pyridoxine hydrochloride for 28 d, the corresponding AUC (posttreatment) was 0.74 (0.69, 0.78).

Longitudinal associations

We related the change in PA:(PL + PLP) from days 0 to 28 in the placebo group ($n = 664$) to corresponding changes in inflammatory markers with the change (Δ) defined as

$$\text{Log}(\text{variable at day 28} - \text{log}(\text{variable at day 0})) \quad (2)$$

Correlations of Δ PA:(PL + PLP) with Δ CRP, Δ KTR, Δ neopterin, and Δ (neopterin + KTR summary score) were 0.19,

TABLE 2
ICCs¹

	Placebo group ($n = 664$)			Pyridoxine-treatment groups ($n = 1313$)		
	PA	PL	Kynurenine	PA	PL	Kynurenine
PL	0.46	0.46	0.70	0.06	0.01	0.67
PLP	0.46	0.61 ²	0.53	0.01	0.26 ²	0.04
PLP + PL	0.67	0.74 ²	0.72	0.30	0.30	0.39
Tryptophan	0.63	0.75 ²	0.69	0.08	0.44 ²	0.16
	0.48	0.51	0.48	0.41	0.11	0.04
			0.77 ¹			0.72 ²

¹All values are ICCs calculated from measurements at baseline and 28 d into the vitamin-treatment period. Calculations were performed separately for placebo and pyridoxine treatment groups. ICCs for individual metabolites are shown in the first row and first column. The remaining numbers are ICCs for ratios defined by column:row. ICC, intraclass correlation coefficient; PA, pyridoxic acid; PL, pyridoxal; PLP, pyridoxal 5'-phosphate.

²ICC was significantly higher for the ratio than its component metabolites ($P < 0.05$).



TABLE 3The variation in vitamin B-6 indexes explained by groups of variables by using multiple linear regression¹

	PA	PL	PLP	PA:(PL + PLP)
CRP + WBC + KTR + neopterin	7.4	3.8	9.4	27.9
eGFR + smoking ²	4.0	2.7	3.5	0.1
Age + sex + center ²	3.2	3.0	1.5	2.3
Use of vitamin supplements ²	10.7	9.8	6.8	0.2
Total explained	25.3	19.2	21.2	30.6
Percentage of explained variation attributable to inflammatory markers ³	29.4	19.7	44.6	91.3

¹ All values are percentages (according to an adjusted R^2). CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; KTR, kynurenine:tryptophan; PA, pyridoxic acid; PL, pyridoxal; PLP, pyridoxal 5'-phosphate; WBC, white blood cell count.

² Increments in R^2 (percentage points) after the sequential addition of groups of variables indicated.

³ Obtained by the following equation: (row 1 ÷ row 5) × 100.

0.24, 0.20, and 0.25, respectively (all $P < 0.0001$). There was no significant correlation between Δ PA:(PL + PLP) and Δ WBC ($r = -0.02$, $P = 0.23$). In regression analyses, Δ CRP, Δ KTR, and Δ neopterin collectively explained 7.9% of the variation in Δ PA:(PL + PLP) compared with 2.4 ($P = 0.0008$), 0.1 (NS), and 0.4% (NS) of the variation in Δ PLP, Δ PL, and Δ PA, respectively.

DISCUSSION

Principal findings

We investigated the possibly increased catabolism of vitamin B-6 during inflammation. Our main focus was to evaluate product substrate ratios (measured in plasma) as markers of enhanced catabolic enzyme activity. The ratio PA:(PL + PLP) had, like the previously established KTR, a high ICC (≥ 0.75), which is measure of the ability to differentiate between subjects. In multiple linear regression models, PA:(PL + PLP) was almost exclusively explained by the 4 inflammatory markers CRP, WBC, neopterin, and KTR. In the ROC analysis, PA:(PL + PLP) discriminated a summary score of neopterin plus KTR plus CRP with an AUC (95% CI) of 0.85 (0.81–0.89).

Product substrate ratios as indicators of enzyme activity

Metabolic control theory predicts that a change in flux through a metabolic pathway is dependent on the coordinate regulation of all enzymes in that pathway (20). In such cases, concentrations of intermediates are minimally affected. However, metabolite concentrations may be substantially affected if only a part of a pathway (eg, a single enzyme) is activated or repressed (20). Generally, increased activity leads to decreases in upstream and increases in downstream metabolites and vice versa. Hence, the ratio between a downstream metabolite and upstream metabolite could be used as indicator of the state (activation or suppression) of that enzyme. The KTR is one such example (12). In addition, we recently showed that 3-hydroxykynurenine:xanthurenic acid may be an indicator of the intracellular PLP availability, and, hence, the activity of kynurenine aminotransferase (13).

PL:PLP compared with PA:(PL + PLP)

PLP has limited ability to cross cell membranes (21). Therefore, PLP has to be dephosphorylated to PL by various

membrane-bound phosphatases before entering the cell. Once inside the cell, PL is metabolically trapped by PL kinase which reforms PLP (22). Intracellular PLP-specific phosphatases also exist in most tissues (23). Thus, the interconversion between PL and PLP is reversible and widely distributed. Because the ratio PL:PLP would reflect several enzymes with opposing activities, a low ICC for this ratio could be expected. The oxidation of PL to PA is, in contrast, irreversible. From the previous discussion, it seems reasonable to view plasma PL + PLP as a single pool of vitamin B-6. Therefore, we considered PA:(PL + PLP) as a possible a priori candidate indicator of the oxidation of PL to PA. Notably, this ratio showed the highest ICC and was the only vitamin B-6 index that had an ICC well above 0.40 after the challenge of the pyridoxine intervention.

PA:(PL + PLP) and PL-oxidizing enzymes

PA:(PL + PLP) was most strongly associated with neopterin and KTR. These plasma indexes are markers of activated cellular immunity and increased metabolic and oxidative stress (12). As a case in point, KTR is determined by both as T-helper 1-type

TABLE 4Correlations of vitamin B-6 indexes with inflammatory markers¹

	PA	PL	PLP	PA:(PL + PLP)
Model 1				
CRP	NS	-16	-25	30
WBC	NS	-14	-20	21
Neopterin	19	NS	-12	36
KTR	16	NS	-16	37
KTR + neopterin ²	19	NS	-15	41
Model 2				
CRP	NS	-14	-24	28
WBC	NS	-8	-15	19
neopterin	11	-5	-17	32
KTR	7	-9	-22	34
KTR + neopterin ²	10	-8	-22	37

¹ All values are partial correlation coefficients (Pearson's) × 100. Model 1 included adjustment for age, sex, and study center. Model 2 was adjusted as for model 1 and for the estimated glomerular filtration rate, smoking, and vitamin supplementation. CRP, C-reactive protein; KTR, kynurenine:tryptophan; NS, nonsignificant association; PA, pyridoxic acid; PL, pyridoxal; PLP, pyridoxal 5'-phosphate; WBC, white blood cell count.

² Sum of standardized variables.



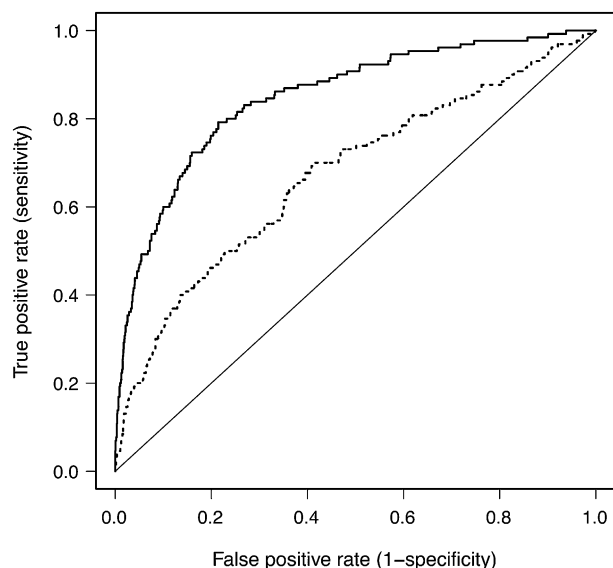


FIGURE 1. Nonparametric receiver operating characteristic plot for the diagnostic accuracy of pyridoxic acid:(PL + PL 5'-phosphate) (solid line) and PL 5'-phosphate (dashed line) to detect a high (>95th percentile) summary score of C-reactive protein, neopterin, and kynurenine:tryptophan. Corresponding AUCs (95% CIs) were 0.85 (0.81, 0.89) and 0.67 (0.62, 0.73), respectively. PL, pyridoxal.

cytokines stimulate the expression of IDO, and the oxygen radical superoxide is the preferred co-substrate for the enzyme (24, 25).

The oxidation of PL to PA is believed to mainly take place in the liver by aldehyde oxidase 1 (26, 27). However, the expression of aldehyde oxidase 1 has also been detected in other tissues, including epithelial tissue (26). Recently, the *AOX1* gene was shown to be regulated by oxidative stress-related signal pathways in rats (28). The enzyme has broad specificity, and in many cases, subtypes of aldehyde dehydrogenase (ALDH) share the same substrates. Specifically, the oxidation of PL by ALDH was detected in a range of mouse tissues (29). ALDHs are cytoprotective enzymes that are upregulated during oxidative or aldehyde stress (30). Taken together, this points to mechanisms whereby several enzymes may contribute to the catabolism of PL, both in the liver and extrahepatically, and activities may increase during immune activation.

Longitudinal associations

Changes in PA:(PL + PLP) over 28 d correlated with corresponding changes in inflammatory status measured as CRP, neopterin, and KTR but not with changes in WBC. Weaker associations with WBC were also shown in the cross-sectional analyses. The WBC changes rapidly in response to sporadic infections, stress, and wounds and is also sensitive to medications including antibiotics and aspirin (31), which might dilute the association between the WBC and PA:(PL + PLP). Notwithstanding the lack of association with the Δ WBC, these analyses showed that PA:(PL + PLP) was considerably more sensitive to short-term changes in inflammatory status than were individual vitamin B-6 metabolites.

Increased catabolism of PLP compared with altered tissue distribution

Although an increase in PA relative to PL plus PLP could simply be interpreted as evidence of increased vitamin B-6

catabolism, a positive association with PA was shown only for neopterin and KTR. PLP, in contrast, was negatively associated with all inflammatory markers but, like PL, more strongly with the acute-phase reactants CRP and WBC. Thus during the acute-phase response, the increase in PA:(PL + PLP) would appear to be explained by decreases in plasma PL and PLP, which would presumably reflect increased tissue uptake of these metabolites (8, 9). Notably, CRP and WBC were more strongly related to PA:(PL + PLP) than to PL or PLP, which indicated that catabolism also plays a significant but minor role during this inflammatory mode. Similarly, both mechanisms could contribute to the increase in PA:(PL + PLP) during activated cellular immunity. If this reasoning is correct, PA:(PL + PLP), rather than being a specific marker of vitamin B-6 catabolism, appears as a more general marker of alterations in vitamin B-6 metabolism that occurs during inflammation.

Strengths and limitations

The study population consisted of middle-aged to elderly subjects, most of whom were men with coronary artery disease. The majority of subjects used medications including platelet inhibitors, antihypertensives, and statins. Statins are known to affect inflammation status; however, associations of vitamin B-6 metabolites or ratios with inflammatory markers were not changed or modified by these treatments.

Strengths of the study included a large and homogenous study population and the measurement of several inflammatory markers. Most biomarkers were analyzed in a single laboratory by using multiplexing methods. The stability of plasma metabolites according to sample handling and storage conditions was validated previously (32, 33). Vitamin B-6 treatment was part of the study protocol of the parent study, and repeated measurements allowed us to calculate ICCs and analyze associations on a longitudinal basis. With the exclusion of WENBIT participants diagnosed with acute coronary syndrome, inflammatory markers were at close to normal concentrations. Therefore, results may extend to healthy populations of similar age.

In conclusion, we have shown evidence to suggest the increased catabolism of vitamin B-6 during inflammation. After the evaluation of different substrate product ratios as markers of catabolic enzyme activity, PA:(PL + PLP) emerged as the most promising candidate. This ratio had a high ICC and was determined almost exclusively by the inflammatory markers CRP, WBC, neopterin, and KTR in multiple linear regression models. By comparison, individual vitamin B-6 metabolites were modestly associated with inflammation and showed stronger associations with vitamin intake, smoking, and kidney function (eGFR). PA:(PL + PLP) may reflect the activity of one or several enzymes upregulated in response to oxidative or aldehyde stress but may also increase as a result of the redistribution of plasma PLP to tissue compartments. With the measurement of PL and PA in addition to PLP, researchers are provided with indicators of vitamin B-6 status and metabolism as well as a sensitive marker of systemic inflammation. PA:(PL + PLP) presents as a promising candidate to be evaluated for disease incidence or other clinical endpoints and could provide new insights into pathogenic processes.

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The authors' responsibilities were as follows—AU, ON, and PMU: study concept and design; ØM: acquisition of data; AU: analysis of data, drafting of the manuscript, and primary responsibility for the final content of the manuscript; and all authors: critical revision of the manuscript for important intellectual content and reading and approval of the final manuscript. None of the authors had a conflict of interest.

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