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NFSC 440

AND Evidence Analysis Worksheet

Citation	Sakakeeny L, Roubenoff R, Obin M, et al. Plasma pyridoxal-5-phosphate is inversely associated with systemic markers of inflammation in a population of U.S. adults 1–3. *J Nutr.* 2012; 142:1280-1285.
Study Design	Cross Sectional
Class	D
Research Purpose	To further the basis for the association between vitamin B-6 and inflammation by examining the relationship between vitamin B-6 status and overall inflammation, functional indicator of inflammation, and individual biomarkers of inflammation.
Inclusion Criteria	• Participants from the Framingham Offspring Study o Seventh examination
Exclusion Criteria	• Participants were excluded if they did not have valid dietary intake • Participants that were missing data on inflammation biomarkers • Participants missing other covariates (age, BMI, sex, smoking, anti-inflammatory drug use, vitamin b-6, energy intake, multivitamin use, circulating concentration of folate, vitamin B12, homocystiene, total cholesterol, and creatinine.) information. • participant reported an energy intake of <2.51 MJ/d (600 kcal/d) or >16.74 MJ/d (4000 kcal/d) for women and >17.58 MJ/d (4200 kcal/d) for men or if the participant left >12 items on the FFQ blank

Description of Study Protocol	Recruitment:
	• Participants from the Framingham Offspring Study
	  o Recruited in 1971, examined every 3-8 years since
	Design:
	• Present study is analysis of seventh examination, 1998-2001
	• 3539 participant but 2229 were available for analysis
	  o 1310 were excluded because of missing data and lab values
	• Framingham Offspring Study protocol was reviewed annually by the Boston University Medical Center Institution Review Board
	  o All participants signed written informed consent

Data Collection Summary	• Patients underwent routine physical examination, medical history, and laboratory assessments • Fasting blood samples were collected from all participants to assess plasma PLP and biochemical inflammation markers o PLP, CRP, homocysteine, B12, cholesterol, creatinine

	Dependent Variables:
	• Plasma PLP
	  o Measured by the tyrosine decarboxylase apoenzyme method assay
	  o Inadequate PLP defined as < 20 nmol/L
	Independent Variables:
	• Inflammatory markers
	  o Plasma CRP
	  ▪ Measured by high-sensitivity assay
	  o Plasma fibrinogen
	  ▪ Measured in duplicate using the clot-time method of Clauss with Diagnostica Stago reagents.
	  o Plasma cluster of differentiation 40 ligand (CD40L), plasma P-selectin, plasma osteoprotegerin, plasma TNFα, plasma TNF receptor 2 (TNFR-2), serum soluble intercellular adhesion molecular-1 (ICAM-1), serum IL-6, serum monocyte chemotactic protein-1 (MCP-1), serum myeloperoxidase, plasma lysosomal phospholipase A2 (LPL-A2) mass and activity, and urinary isoprostanes indexed to urinary creatinine
	  ▪ measured using commercially available enzyme-linked immunoassay kits
	2 types:
	• Scores used to represent inflammation; individual marker values were first standardized as Z-scores and then using Z scores they compute the following:
	  o Overall inflammation score (IS)
	  ▪ sum of the standardized values of all of the inflammatory biomarkers (CRP, fibrinogen, IL-6, TNFα, TNFR-2, osteoprotegerin, P-selectin, CD40L, ICAM-1, MCP-1, myeloperoxidase, LPL-A2 mass, LPL-A2 activity, and isoprostanes indexed to creatinine)
	  o Individual Subscores of IS:
	  ▪ Included CRP and fibrinogen, IS-cytokines included IL-6, TNFα, TNFR-2, and osteoprotegerin. IS-selectins included P-selectin and CD40L. IS-oxidative stress included the biomarkers myeloperoxidase, LPL-A2 mass, LPL-A2 activity, and isoprostanes indexed to creatinine
	Confounder Variables:
	• Age, BMI, sex, smoker, anti-inflammatory drug use, vitamin B-6, protein and energy intake, supplement use
	  • Homocysteine
	  o Measured by HPLC with fluorimetric detection
	• Plasma folate

	○ Measured by a microbial assay (*Lactobacillus casei*) in a 96-well plate • Plasma vitamin B-12 ○ Measured with a (Magic) RIA kit from Ciba-Corning. • Plasma cholesterol concentrations ○ Measured by standard lab procedures • Creatinine ○ Measured in serum and urine from fasting participants by the modified Jaffé method **Statistical Analysis** performed with SAS (version 9.1; SAS Institute). • SAS (Statistical analysis software) is tool to analyze, manage, view, and even prevent complex data in a variety of formats. • Proportions were generated using SAS PROC GLM, to determine *P* values of the regression coefficient for the independent inflammatory marker were derived by entering them into a model as continuous variable to determine a *P*-trend ○ $P < 0.05$ was considered significan**t** • Multivariable regression analysis was preformed to calculate plasma PLP (95% CI) across tertile categories of inflammatory biomarkers • Plasma PLP was normalized by natural log transformation • The relationship among vitamin B-6 intake, inflammatory status, and plasma PLP concentrations was examined by using a graph that showed the geometric means of plasma PLP ○ Adjustments were made for confounding factors and arranged by 3 categories of inflammatory status as indicated by IS and 3 categories of vitamin B-6 intake from diet and supplements which examined the prevalence (95% CI) of inadequate PLP [plasma PLP < 20 nmol/L] across 3 categories of the overall IS • Multivariable regression analysis was used to assess the association between individual biomarkers of inflammation and plasma PLP concentrations. • Each biomarker was entered into the models one at a time where natural log-transformed CRP was entered into the model as a continuous confounder to determine whether the relationship between plasma PLP concentrations and individual biomarkers still were associated after accounting for CRP.
Summary of Results	• As Plasma PLP tertile categories decreased there was an inverse relationship with BMI, cigarette smoking, diabetes, homocysteine, plasma CRP, and overall inflammatory score (IS) • As plasma PLP tertile categories increased there was a positive relationship with multivitamin use, NSAID use, energy intake, protein intake, vitamin B6 intake, plasma folate, plasma B12

	• IS (inflammatory score) was significantly higher when plasma PLP levels were lower. At the lowest plasma PLP category that participants were placed in the overage inflammation score was an 80. At the highest PLP levels the average IS was 61 ($P<0.001$). • IS-acute phase reactants were a 76 when plasma PLP was in the lowest level category for participants. At the highest PLP category, the IS-acute phase reactants were an average of 61 ($P<0.001$). • IS-cytokines levels were 76 with the lowest category of plasma PLP levels in the participants and 61 at the highest PLP levels ($P<0.001$). • IS-oxidative stress was 72 at the lowest category of plasma PLP and a 63 at the highest PLP levels ($P<0.001$). • ICAM-1 was at a 72 with the lowest plasma PLP category and 64 with the highest level of plasma PLP ($P<0.001$). • Individuals with high intake of B6 have higher plasma PLP levels • Individuals with the greatest degree of inflammation have significantly lower plasma PLP regardless of intake. Even when more B6 was ingested levels still remained low.
Author Conclusion	Overall inflammation is inversely associated with plasma PLP concentrations and that there is a strong association with vitamin B-6 inadequacy increased with inflammation and inflammatory diseases. These results expand on previous findings that showed inverse relationships between plasma PLP and inflammatory diseases and/or individual biomarkers of inflammation.
Reviewer Comments	**Strengths:** • Large sample size • Well implemented • The study has a unique demonstration of the inverse correlation of plasma PLP with overall IS and scores representing the many different biochemical inflammation markers **Limitations:** • Socioeconomic status, ethnicity, and demographics of participants were not provided • The design is not experimental, which would be a better way to examine because you can induce inflammations and assess the direct impact on B6 or modify B6 and examine impact on inflammation • There was no assessment to determine if participants had disease states • It was generalized that with high inflammation, plasma PLP levels decrease but it is not considered that low plasma PLP levels cause inflammation **Funding source:** • Pharmavit, USDA, and Framingham Heart Study Core contract

What is the relationship between vitamin B6 and inflammatory markers?

Evidence Summary:

In a cross-sectional study, Folsom et al (2003) looked at survivors of the Atherosclerosis Risk in Communities (ARIC) cohort study's sample, which included healthy individuals between the ages of 45 and 64 (n=519), and found that plasma PLP was not associated with CRP or fibrinogen concentration, and that there is no strong correlation between B vitamin status and circulating inflammatory markers, cellular adhesion molecules or thrombogenic factors. Limitations of these findings include: participants are listed as healthy even though authors admit they did not ask whether any subjects had an inflammatory disease, only single measurements were made of the blood markers, and some statistical comparisons could have been significant, at $p<0.05$, by chance.

In a cross-sectional study, Simonetta et al (2001) looked at data from the 20th examination of subjects participating in the Framingham Heart Study cohort, whose ages ranged between 30 and 62 upon their entry into the study between the years 1948 and 1950(n=892). They found that individuals with higher levels of CRP (≥6mg/L) had lower levels of PLP (36.5 nmol/L) than individuals with lower levels of CRP (<6mg/L, PLP=55.8nm/L, $p<0.001$). These results may reflect vitamin B6 utilization in the presence of an underlying inflammatory process, and represent a possible mechanism to explain the decreased vitamin B6 levels in CVD.

A Case-control study, by Friso S, et al. (2004), showed that low plasma PLP concentrations (<36.3nmol/L) are related to high levels of major markers of inflammation (hs-CRP, fibrinogen, t-Hcy) ($p<0.001$). 742 participants were recruited from Northern Italy, there was a medium BMI of 26 among participants, and 36% were male and 64% female. The

hypothesis was that there is a common link between low plasma concentrations of B6 and development of atherosclerosis. Genetics, lifestyle, and nutrition were not assessed; as well as generalizing to other ethnicities may not be merited.

A Cross Sectional study, by Sakakeeny L, et al. (2012),showed that overall high inflammation is associated with low plasma PLP levels (<36.3nmol/L) and there is a strong relationship that with high levels of inflammation there is low levels of plasma PLP, regardless of high B6 intake in the diet ($p<0.001$). Participants were 55% women, 45% men, had a median age of 61 years old, and were mostly white Europeans (n=2229). An implication for the practice was to further understand the basis of the association between vitamin B6 and Inflammation. The observational nature of the study prevented interference; therefore examining the phenomenon by an experimental model, where one can induce inflammation and examine the impact on vitamin B6 would be a better approach. Participants were included in the study without consideration of having an illness which makes it possible that presence of chronic diseases were not controlled for, and participants were mostly white Europeans so generalizing to other ethnicities is limited.

Conclusion:

Circulating vitamin B6, or plasma PLP, has a strong inverse relationship with inflammation markers, most notably, C-reactive protein.

Grade III