Vitamin B-6 Intake Is Inversely Related to, and the Requirement Is Affected by, Inflammation Status\textsuperscript{1,2}

Martha Savaria Morris,\textsuperscript{3,*} Lydia Sakakeeny,\textsuperscript{4} Paul F. Jacques,\textsuperscript{3} Mary Frances Picciano,\textsuperscript{5} and Jacob Selhub\textsuperscript{4}

\textsuperscript{3}Nutritional Epidemiology Program and \textsuperscript{4}Vitamin Metabolism Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111; and \textsuperscript{5}Office of Dietary Supplements, NIH, Bethesda, MD 20892

Abstract

Low circulating pyridoxal 5’-phosphate (PLP) concentrations have been linked to inflammatory markers and the occurrence of inflammatory diseases. However, the implications of these findings are unclear. The measurement of PLP and C-reactive protein (CRP) in blood samples collected from participants in the 2003–2004 NHANES afforded us the opportunity to investigate this relationship in the general U.S. population. Dietary and laboratory data were available for 3864 of 5041 interviewed adults, 2886 of whom were eligible (i.e. provided reliable dietary data and were not diabetic, pregnant, lactating, or taking hormones or steroidal antiinflammatory drugs). Vitamin B-6 intake was assessed using 2 24-h diet recalls and supplement use data. After multivariate adjustment for demographics, smoking, BMI, alcohol use, antioxidant vitamin status, intakes of protein and energy, and serum concentrations of creatinine and albumin, high vitamin B-6 intake was associated with protection against serum CRP concentrations >10 mg/L compared with ≤3 mg/L. However, plasma PLP ≥20 nmol/L, compared with <20 nmol/L, was inversely related to serum CRP independently of vitamin B-6 intake (P < 0.001). Among participants with vitamin B-6 intakes from 2 to 3 mg/d, the multivariate-adjusted prevalence of vitamin B-6 inadequacy was <10% in participants with serum CRP ≥3 mg/L but close to 50% in those with serum CRP > 10 mg/L (P < 0.001). In conclusion, higher vitamin B-6 intakes were linked to protection against inflammation and the vitamin B-6 intake associated with maximum protection against vitamin B-6 inadequacy was increased in the presence compared to absence of inflammation. J. Nutr. 140: 103–110, 2010.

Introduction

Low vitamin B-6 status has been linked in studies to cardiovascular disease (CVD)\textsuperscript{6} (1–6). The vitamin acts as a cofactor for >100 enzymes involved in such diverse processes as macromolecular metabolism, immune competence, hormone function, heme biosynthesis, and the synthesis and catabolism of sphingolipids and neurotransmitters (7). Consequently, causal connections between low vitamin B-6 status and age-related diseases such as CVD, Alzheimer’s disease, diabetes, and cancer are plausible.

Thus far, most interest in vitamin B-6 as a disease-preventive factor has focused on its role in homocysteine (Hcy) transulfuration. However, vitamin B-6 status is now known to be a comparatively weak determinant of the Hcy concentration (4,8,9) and studies have shown that the connection between low vitamin B-6 status and CVD is independent of Hcy (3,4,10,11).

Forty-five years ago, McCusick et al. (12) discovered that patients with rheumatoid arthritis had decreased excretion of pyridoxine, a form of vitamin B-6. Along with people affected by other chronic inflammatory conditions (13,14), such patients are at high risk of morbidity and mortality from CVD (15,16). In fact, CVD (17,18) and other age-related chronic diseases (19–21) are increasingly viewed as inflammatory and inflammation is considered to be central to the aging process (20,22). Low plasma concentrations of pyridoxal 5’-phosphate (PLP), the biologically active form of vitamin B-6, in patients hospitalized for myocardial infarction could reflect an acute phase reaction. Specifically, vitamin B-6 is required for production of the cytokines (23) that are among the primary mediators of chronic inflammation (24) as well as for the proliferation/activation of lymphocytes that characterizes the inflammatory response (25,26). In fact, some studies demonstrated a transient lowering of plasma PLP after myocardial infarction (5,27). Furthermore, previous studies from our laboratory and elsewhere favored the hypothesis that plasma PLP concentrations were lowered by inflammation over the idea that low vitamin B-6 status increases the risk of inflammation or inflammatory diseases (28–30).

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\textsuperscript{3} Abbreviations used: CRP, C-reactive protein; CVD, cardiovascular disease; Hcy, homocysteine; MEC, mobile examination center; NSAID, nonsteroidal antiinflammatory drug; OR, odds ratio; PLP, pyridoxal 5’-phosphate.

\textsuperscript{4} To whom correspondence should be addressed. E-mail: martha.morris@tufts.edu.
These findings generate the hypothesis that people experiencing inflammation require higher vitamin B-6 intakes than people not experiencing inflammation to maintain adequate circulating PLP concentrations and thus prevent the effects of vitamin B-6 deficiency. However, the above-described reverse causation scenario could not explain associations that have been reported between low vitamin B-6 intake and inflammatory markers (31,32) and CVD risk (3,4).

The NHANES collects data on nutrient intakes from diet and supplements. Furthermore, the inflammatory marker C-reactive protein (CRP) is routinely measured in serum samples. Plasma PLP measurements were also obtained for >7000 NHANES 2003–2004 participants. Using these data, we evaluated associations between both vitamin B-6 intakes and plasma PLP concentrations and serum CRP concentrations. We also investigated the question of whether the vitamin B-6 requirement is affected by inflammation by determining the vitamin B-6 intake associated with minimal prevalence of vitamin B-6 inadequacy in different serum CRP strata.

Materials and Methods

Participants. The NHANES monitors the nation’s health and nutritional status via a continuous annual survey (33). Selection of a representative sample of the noninstitutionalized U.S. civilian population is accomplished using a complex multistage probability design. To increase the precision of estimates derived from the survey, adolescents, the elderly, Mexican-Americans, and Blacks are oversampled. The protocols for conduct of the NHANES were approved by the institutional review board of the National Center for Health Statistics, CDC, and informed consent was obtained from all participants.

Trained interviewers used a computer-assisted personal interview system to interview participants in their homes. During the home visit, participants were asked to produce all dietary supplements, prescription drugs, and nonprescription pain relievers they were taking. In the NHANES 2003–2004, 10,122 participants were interviewed and asked to report to a mobile examination center (MEC) to provide further interview data and undergo a physical examination that included phlebotomy, body composition assessment, and blood pressure measurements. A detailed description of blood collection and processing can be found in the NHANES Laboratory Procedures Manual (34). Female participants were interviewed regarding reproductive history at the MEC. Questions concerned age at menarche, menopausal status, and history of oral contraceptive use, pregnancy, and hormone replacement therapy.

MEC-examined participants aged >19 y numbered 4742. PLP measurements were obtained for 4454 of them (95%). From the 3926 with reliable dietary data for both diet recalls, we excluded 470 who had been diagnosed with diabetes or whose diabetes status was unclear. After additional exclusions for pregnancy (n = 172), breast-feeding (n = 23), hormone replacement therapy (n = 102), and use of oral contraceptives (n = 94) or steroidal antiinflammatory drugs (n = 43), complete data for analyses were available for between 2653 and 2686 participants, depending on the model covariates.

Estimation of usual intake of vitamin B-6 and other nutrients. Beginning with the 2003–2004 NHANES, 2 d of dietary intake data were collected (35). The first day’s data were collected in a 24-h recall interview conducted at the MEC. A second 24-h recall interview was conducted by telephone 3–10 d after the MEC examination. The 2 dietary interviews were administered by trained staff and the USDA’s Food Surveys Research Group was responsible for the dietary data collection methodology, maintenance of the databases used to code and process the data, and data review and processing.

The availability of results from the 2 24-h recalls allowed us to estimate each participant’s usual intake of vitamin B-6, protein, and energy using methods and computer programs developed by the National Cancer Institute (36). The National Cancer Institute method uses the repeat diet assessments to estimate the day-to-day variability in intakes as well as the correlation between intakes reported over consecutive days and changes in diet between weekdays and weekend days.

If the participant had used vitamins, minerals, or other dietary supplements within 30 d of the interview, the interviewer recorded information on the amount taken per use and the frequency and duration of use (37). The amount of each ingredient in each product was determined by matching the name and manufacturer of the supplement to those in a database developed by the National Center for Health Statistics in collaboration with the NIH’s Office of Dietary Supplements. The information in the database came from sources such as the manufacturer or retailer, the Internet, and company catalogs. We used the various dietary supplement files to identify all participants who reported using any supplement source of vitamin B-6 and to determine each such participant’s usual daily intake of vitamin B-6 from supplements. This amount was added to the estimated usual intake of vitamin B-6 from food to yield the total daily vitamin B-6 intake for participants who used supplemental vitamin B-6.

Laboratory analyses. Plasma PLP was measured by A/C Diagnostic- using a homogeneous, nonradioactive, enzymatic assay (38). The following statistics were reported: mean intra-assay CV, 8%; mean interassay CV, 12–13%; detection limit, 10.09 nmol/L; and normal range, 20–120 nmol/L (34). Plasma PLP ≥20 nmol/L was also the definition of vitamin B-6 adequacy used to set the current Estimated Average Requirements and Recommended Dietary Allowances of vitamin B-6 (39). Results below the detection limit of the assay were replaced with the value 7.1 nmol/L (i.e., the detection limit divided by the square root of 2) (40).

Serum CRP was assayed at the University of Washington Medical Center Department of Laboratory Medicine using a Dade Behring Nephelometer II Analyzer system (Dade Behring Diagnostics). The detection limit of the assay was 0.2 mg/mL (CV < 7.5%). Results below the detection limit were coded 0.1 mg/mL. CRP has been called the classic acute-phase reactant and has been advocated for following disease activity in inflammatory conditions (41). The reference range is 0–10 mg/L (41) and we divided participants into 3 groups as follows: ≤3 mg/L, 3.1–10 mg/L, and >10 mg/L.

Creatinine and albumin concentrations were measured in serum samples by Collaborative Laboratory Services, using the Beckman Synchron LX20 modular chemistry side.

Serum vitamin C (HPLC with an internal standard and electrochemical detection), folate, and vitamin B-12 (Quanaphase II Folate/Vitamin B-12 Radioassay kit, Bio-Rad Laboratories) and plasma Hcy (Abbott Diagnostics, fully automated Hcy fluorescence polarization immunoassay) were assayed at the NHANES central laboratory, the Inorganic Toxicology and Nutrition Branch of the National Center for Environmental Health’s Division of Laboratory Sciences in Atlanta, GA. HPLC with multi-wavelength photodiode-array absorbance detection was used by Craft Technologies to assay vitamins E and A and the carotenoids.

Statistical analyses. Statistical analyses were performed using SUDAAN release 10.0 (Research Triangle Institute) with appropriate masked variance weights (using survey procedures and pweight option) and 2-y sampling weights to account for the survey’s complex sampling design (42). P < 0.05 was considered significant for all analyses.

We first used multivariate-adjusted means (95% CI) and proportions (≥ SE) generated by SUDAAN PROC REGRESS and SUDAAN PROC Crosstabs to describe and compare participants stratified by CRP concentration and vitamin B-6 adequacy according to various participant characteristics, including demographic factors, BMI (weight/height²), blood pressure, dietary and lifestyle factors, plasma Hcy, serum concentrations of creatinine and vitamins, and use of nonsteroidal antiinflammatory drugs (NSAID). An NSAID user was any participant who reported regular use of a prescription drug classified as an NSAID in the prescription drug data file (43) or any of the following nonprescription products: aspirin, Ibuprofen, Excedrin, Vanquish, Feldene, Voltarin, Clinoril, Indocin, Naprosyn, Alleve, and Tolectin. The multivariate model (model 1) used for these analyses included terms for basic demographic factors (i.e., age, sex, and race-ethnicity), correlates of
vitamin B-6 intake and status (i.e. usual intakes of energy and protein) (44), and factors that have been linked to inflammation in studies (i.e. indicators of an unhealthy lifestyle) (45) (e.g. smoking and alcohol use), BMI, and serum creatinine (46). The distributions of serum concentrations of creatinine and vitamins were highly skewed and these values were logarithmically transformed before use as outcome variables in the regression models. Differences among groups in subject characteristics were evaluated using the Wald F test reported from the regression modeling programs.

Next, we considered whether use of supplements containing vitamin B-6, total vitamin B-6 intakes, and plasma PLP concentrations were related to protection against elevated serum CRP concentrations. Although our hypothesis regarding plasma PLP and inflammation was bidirectional, in the interest of consistency with the analyses relating supplement use and vitamin B-6 intakes to serum CRP concentrations, we performed these analyses using multinomial multiple logistic regression as performed by SUDAAN PROC MULTILOG, with the 3-level term for serum CRP as the categorical outcome variable. We estimated the odds ratio (OR) (95% CI) using model 1 described above, and, to simultaneously adjust for additional potentially confounding factors, after adding terms for serum concentrations of folate, albumin, and antioxidant vitamins (model 2). We also performed these analyses after adjusting for plasma PLP. This step was aimed at evaluating the specificity of the association for vitamin B-6 status, because supplements contain vitamins other than vitamin B-6 and because high vitamin B-6 intakes may be associated with high intakes of other vitamins. We also performed the analysis relating plasma PLP category to serum CRP category after adjusting for total vitamin B-6 intake. The rationale behind this step was to determine whether the association between plasma PLP category and serum CRP category was entirely explained by vitamin B-6 intake or whether some of the association might be a reflection of the influence of inflammation on the plasma PLP concentration.

To shed light on the hypothesis that inflammation increases the need for vitamin B-6 via increased utilization of the vitamin in the inflammatory process, we attempted to determine whether the vitamin B-6 intake that was associated with maximal prevention of vitamin B-6 inadequacy varied with inflammation status. To accomplish this, we used SUDAAN PROC REGRESS and model 2 described above to estimate, for participants in highest and lowest serum CRP categories, the multivariate-adjusted prevalence (95% CI) of vitamin B-6 inadequacy associated with total vitamin B-6 intakes of <2, 2–2.9, 3–4.9, and ≥5 mg/d.

P-trend for associations between vitamin B-6 intake and study outcomes was estimated using the medians of the vitamin B-6 intake categories modeled as a continuous exposure variable.

Results

Participant characteristics in relation to serum CRP and plasma PLP concentrations. Female gender, current smoking, and low serum concentrations of albumin and a variety of vitamins were positively associated with both inflammation and vitamin B-6 inadequacy (Table 1). Age was not related to either study outcome in men. However, after multivariate adjustment, women with elevated CRP concentrations were somewhat older than women with CRP concentrations in the reference range and women with inadequate vitamin B-6 status were somewhat younger than women with adequate status.

Non-Hispanic Black race-ethnicity and higher Hcy concentrations were associated with inadequate vitamin B-6 status and BMI was positively related to inflammation.

Markers of vitamin B-6 status in relation to serum CRP concentration. After multivariate control for demographic characteristics, lifestyle factors, BMI, serum creatinine, and intakes of energy and protein, use of supplements containing vitamin B-6 was associated with protection against markedly elevated CRP concentrations compared with CRP concentrations in the reference range (Table 2). This association was greatly attenuated by adjustment for either plasma PLP or serum concentrations of albumin and other vitamins found to be associated with protection against inflammation in preliminary analyses. These results suggest that the association between vitamin supplement use and protection against inflammation is partially mediated by vitamin B-6 status and partially by other vitamins contained in the supplements.

The odds of a markedly elevated CRP concentration, compared with a concentration within the reference range, decreased significantly with increasing vitamin B-6 intakes from dietary sources and supplements combined. The magnitude of the inverse association between vitamin B-6 intake and this outcome decreased after terms for serum concentrations of folate, albumin, and antioxidant vitamins were added to the model. However, consuming at least 5 mg/d of vitamin B-6, as opposed to consuming <2 mg/d, remained significantly related to protection against a markedly elevated CRP concentration in all models except the one that controlled for plasma PLP concentration. In other words, the association between higher vitamin B-6 intake and protection against a markedly elevated serum CRP concentration did not appear to be effected by confounding by other aspects of a healthy lifestyle. Furthermore, the OR obtained using model 2 additionally adjusted for plasma PLP was identical to that obtained using model 1 additionally adjusted for plasma PLP (i.e. OR, 0.66; 95% CI, 0.35–1.24).

Finally, even after controlling for all of the potentially confounding factors and vitamin B-6 intake from foods and supplements combined, the OR relating a plasma PLP concentration ≥20 nmol/L compared with <20 nmol/L to both moderately elevated and markedly elevated serum CRP concentrations were significantly <1.0. That is, vitamin B-6 intake did not entirely explain the association between the plasma PLP concentration and inflammation status, which leaves open the possibility that inflammation also lowers the plasma PLP concentration.

Relationship between vitamin B-6 intake and vitamin B-6 inadequacy by inflammation status. The prevalence of vitamin B-6 inadequacy decreased with increasing vitamin B-6 intake regardless of serum CRP concentration (Fig. 1). Specifically, among participants with CRP concentrations in the reference range, the prevalence of vitamin B-6 inadequacy was ~20% in the subgroup with vitamin B-6 intakes <2 mg/d (i.e. the lowest intake category) compared with <10% among those in higher vitamin B-6 intake categories (P < 0.01). However, the prevalence of vitamin B-6 inadequacy was much more common (range, 38–54%; P < 0.01) among participants with serum CRP >10 mg/L and vitamin B-6 intakes <5 mg/d (i.e. all intake categories except the highest). In other words, whereas the prevalence of vitamin B-6 inadequacy was minimized at vitamin B-6 intakes ≥2 mg/d in participants with serum CRP ≤3 mg/L, a comparably low prevalence of inadequacy was observed only at much higher vitamin B-6 intakes among participants whose serum CRP concentrations were markedly elevated.

Discussion

Our study was primarily aimed at confirming in a large population-based survey a link between the plasma PLP concentration and inflammation status. We also aimed to shed light on the direction of the relationship by evaluating the association between vitamin B-6 intake and inflammation and
TABLE 1 Characteristics of adult NHANES 2003–2004 participants by serum CRP and plasma PLP category

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>≤3</th>
<th>3.1–10</th>
<th>&gt;10</th>
<th>P-value</th>
<th>≥20</th>
<th>&lt;20</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants</td>
<td>n (% ± SE)</td>
<td>n (% ± SE)</td>
<td>P-value</td>
<td>n (% ± SE)</td>
<td>n (% ± SE)</td>
<td>P-value</td>
<td></td>
</tr>
<tr>
<td>Participants</td>
<td>1787 (71 ± 1.37)</td>
<td>678 (22 ± 0.91)</td>
<td>221 (7 ± 0.92)</td>
<td>0.002</td>
<td>2206 (82 ± 1.38)</td>
<td>480 (17 ± 1.38)</td>
<td>0.046</td>
</tr>
<tr>
<td>Female gender</td>
<td>699 (42 ± 2)</td>
<td>327 (47 ± 3.19)</td>
<td>133 (52 ± 5.65)</td>
<td>0.181</td>
<td>873 (43 ± 1.77)</td>
<td>286 (48 ± 4.61)</td>
<td>0.924</td>
</tr>
<tr>
<td>Race-ethnicity</td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Non-Hispanic black</td>
<td>1048 (77 ± 3.41)</td>
<td>362 (73 ± 3.84)</td>
<td>111 (69 ± 6.98)</td>
<td>0.19</td>
<td>1286 (77 ± 3.21)</td>
<td>236 (69 ± 6.04)</td>
<td>0.034</td>
</tr>
<tr>
<td>Non-Hispanic white</td>
<td>315 (10 ± 1.91)</td>
<td>131 (10 ± 1.81)</td>
<td>68 (15 ± 4.52)</td>
<td>0.273</td>
<td>377 (10 ± 1.71)</td>
<td>137 (16 ± 3.51)</td>
<td>0.002</td>
</tr>
<tr>
<td>Mexican-American</td>
<td>315 (7 ± 1.84)</td>
<td>146 (9 ± 2.39)</td>
<td>34 (6 ± 4.69)</td>
<td>0.181</td>
<td>408 (7 ± 1.76)</td>
<td>87 (6 ± 2.7)</td>
<td>0.924</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Never</td>
<td>1391 (75 ± 1.63)</td>
<td>470 (64 ± 2.87)</td>
<td>161 (62 ± 5.95)</td>
<td>0.002</td>
<td>1678 (73 ± 1.66)</td>
<td>306 (62 ± 3.18)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Former</td>
<td>19 (1 ± 0.32)</td>
<td>6 (1 ± 0.61)</td>
<td>2 (2 ± 1.34)</td>
<td>0.241</td>
<td>23 (1 ± 0.25)</td>
<td>4 (1 ± 0.65)</td>
<td>0.08</td>
</tr>
<tr>
<td>Current</td>
<td>417 (4 ± 1.68)</td>
<td>202 (36 ± 2.89)</td>
<td>58 (36 ± 6.45)</td>
<td>0.004</td>
<td>507 (26 ± 1.74)</td>
<td>170 (37 ± 3.27)</td>
<td>0.001</td>
</tr>
<tr>
<td>Alcohol user</td>
<td>1232 (73 ± 2.38)</td>
<td>421 (73 ± 2.91)</td>
<td>130 (72 ± 5.93)</td>
<td>0.896</td>
<td>1485 (74 ± 2.54)</td>
<td>298 (72 ± 4.37)</td>
<td>0.691</td>
</tr>
<tr>
<td>NSAID user</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Aspirin</td>
<td>213 (11 ± 0.99)</td>
<td>94 (9 ± 2.44)</td>
<td>20 (6 ± 2.52)</td>
<td>0.367</td>
<td>278 (10 ± 1.14)</td>
<td>49 (9 ± 1.13)</td>
<td>0.169</td>
</tr>
<tr>
<td>Nonaspirin</td>
<td>213 (14 ± 1.13)</td>
<td>94 (13 ± 1.5)</td>
<td>35 (17 ± 4.6)</td>
<td>0.703</td>
<td>270 (13 ± 0.83)</td>
<td>78 (16 ± 2.57)</td>
<td>0.34</td>
</tr>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>48 (46–50)</td>
<td>51 (49–53)</td>
<td>47 (43–51)</td>
<td>0.12</td>
<td>48 (47–50)</td>
<td>48 (47–50)</td>
<td>0.787</td>
</tr>
<tr>
<td>Women</td>
<td>45 (43–47)</td>
<td>49 (47–51)</td>
<td>48 (44–51)</td>
<td>0.056</td>
<td>43 (42–45)</td>
<td>41 (39–42)</td>
<td>0.079</td>
</tr>
<tr>
<td>BMI</td>
<td>26 (26.27)</td>
<td>30 (30–32)</td>
<td>32 (31–34)</td>
<td>&lt;0.001</td>
<td>28 (28.28)</td>
<td>28 (27–29)</td>
<td>0.481</td>
</tr>
<tr>
<td>Energy intake, kJ/d</td>
<td>9400 (9037–9491)</td>
<td>9479 (9224–9734)</td>
<td>0.169</td>
<td>9433 (9433–9433)</td>
<td>9454 (9454–9454)</td>
<td>0.718</td>
<td></td>
</tr>
<tr>
<td>Protein intake, g/d</td>
<td>86.3 (85.2–87.3)</td>
<td>85.2 (84–86.3)</td>
<td>85 (83.1–86.8)</td>
<td>0.233</td>
<td>86.2 (85.3–87)</td>
<td>84.7 (83.4–86)</td>
<td>0.002</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>123 (122–124)</td>
<td>124 (122–126)</td>
<td>125 (122–128)</td>
<td>0.236</td>
<td>124 (122–126)</td>
<td>123 (122–124)</td>
<td>0.214</td>
</tr>
<tr>
<td>Serum albumin, g/L</td>
<td>43.8 (43.6–44)</td>
<td>42.8 (42.3–43.2)</td>
<td>41.9 (41.2–42.6)</td>
<td>&lt;0.001</td>
<td>43.5 (43.3–43.7)</td>
<td>43 (42.7–43.3)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

1 Values are n (% ± SE), mean (95% CI), or geometric mean (95% CI). n = 2686. 
2 P-value from the Wald F-test.
3 All values were estimated from multivariate models including sampling weights for applicability to the U.S. population and terms for gender, age, race-ethnicity, BMI, alcohol use, smoking status, serum creatinine, and intakes of energy and protein.
4 Participants were nonpregnant, nonlactating adults who had not been diagnosed with diabetes and were not taking oral contraceptives, estrogen-replacement therapy, or steroid antiinflammatory drugs.
5 Relative SE > 30% (i.e. the estimate of percent may be unreliable).
6 BMI is calculated as weight in kg/height in m².
7 SBP, systolic blood pressure.

investigating how vitamin B-6 intake related to vitamin B-6 inadequacy as inflammation status varied. The cross-sectional design of our study did not permit causal inference. However, we found results that would be expected if higher vitamin B-6 intakes protected against inflammation. We also found results that would be expected if inflammation resulted in an increased need for vitamin B-6.

Previous studies showed that low plasma PLP concentrations were characteristic of people with inflammatory conditions (29,47,48). Furthermore, inverse relationships have been found between vitamin B-6 status and markers of inflammation in animal models (49), patient groups (46–48,50), and general population samples (28,31,51). A cross-sectional association between low plasma PLP concentrations and inflammation could mean that vitamin B-6 protects against inflammation or that inflammation adversely affects vitamin B-6 status. However, 1 population-based study found no association at all (11). Moreover, some of the positive studies, including the population-based Framingham Study (28), did not link vitamin B-6 intakes to inflammation status (28,31). Such results argued against an antiinflammatory role for higher vitamin B-6 status. In contrast to these studies, our study did link higher vitamin B-6 intakes to decreased odds of inflammation and results suggested that the association was not confounded by other aspects of a healthy lifestyle. That the association did not remain after the Framingham study may relate to a difference in exposure, the specificity of the association for vitamin B-6 status. The plasma PLP concentration was adjusted for further illustrates that inflammation adversely affects vitamin B-6 status. Howev-er, 1 population-based study found no association at all (11). Moreover, some of the positive studies, including the population-based Framingham Study (28), did not link vitamin B-6 intakes to inflammation status (28,31). Such results argued against an antiinflammatory role for higher vitamin B-6 status. In contrast to those studies, our study did link higher vitamin B-6 intakes to decreased odds of inflammation and results suggested that the association was not confounded by other aspects of a healthy lifestyle. That the association did not remain after the Framingham study may relate to a difference in exposure, because the data used in the Framingham investigation were

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collected in the late 1980s, when the use of supplements was much less common than it is today (52). The mechanism by which low vitamin B-6 status might foster inflammation, or certain inflammatory diseases such as CVD, is unclear. Hcy has been proposed as a likely mediator (53). However, cross-sectional studies, including our own, have found the inverse association between vitamin B-6 status and CRP to be independent of Hcy (28,31,51). Studies have also failed to support the idea that Hcy mediates the association between low vitamin B-6 status and vascular disease (10,54). Consistent with those results, low vitamin B-6 status remains a risk factor for CVD (6%). Studies have shown that critically ill people experiencing inflammation have low plasma PLP concentrations but normal erythrocyte PLP concentrations. Studies in our laboratory have corroborated this finding (47,50) and demonstrated no correlation between the plasma PLP concentration and indicators of vitamin B-6 status in erythrocytes (50). We did, however, find that the plasma PLP concentrations observed during inflammation did not reflect low vitamin B-6 status. Specifically, McMillan et al. (56–59) have repeatedly shown that critically ill people experiencing inflammation have low plasma PLP concentrations but normal erythrocyte PLP concentrations. Studies in our laboratory have corroborated this finding (47,50) and demonstrated no correlation between the plasma PLP concentration and indicators of vitamin B-6 status in erythrocytes (50). We did, however, find that the plasma PLP concentrations observed during inflammation did not reflect low vitamin B-6 status. Specifically, McMillan et al. (56–59) have repeatedly shown that critically ill people experiencing inflammation have low plasma PLP concentrations but normal erythrocyte PLP concentrations. Studies in our laboratory have corroborated this finding (47,50) and demonstrated no correlation between the plasma PLP concentration and indicators of vitamin B-6 status in erythrocytes (50).

Table 2: Association between vitamin B-6 status and serum CRP concentration in U.S. adults (NHANES, 2003–2004)

<table>
<thead>
<tr>
<th>Participants, n (%) ± SE</th>
<th>Categories of serum CRP, mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤3</td>
</tr>
<tr>
<td>Participants, n (%)</td>
<td>2686 (100)</td>
</tr>
<tr>
<td>Vitamin B-6 supplement users, n (%)</td>
<td>969 (37 ± 2.04)</td>
</tr>
<tr>
<td>Model 1, OR (95% CI)</td>
<td>1 [Reference]</td>
</tr>
<tr>
<td>Model 1 plus plasma PLP, OR (95% CI)</td>
<td>1 [Reference]</td>
</tr>
<tr>
<td>Model 2, OR (95% CI)</td>
<td>1 [Reference]</td>
</tr>
<tr>
<td>Model 2 plus plasma PLP, OR (95% CI)</td>
<td>1 [Reference]</td>
</tr>
</tbody>
</table>

Total vitamin B-6 intake, mg/d

Model 1, OR (95% CI)

<table>
<thead>
<tr>
<th>Participants, n (%)</th>
<th>1077 (38 ± 1.8)</th>
<th>692 (26 ± 1.32)</th>
<th>533 (20 ± 1.63)</th>
<th>380 (16 ± 1.24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2</td>
<td>1 [Reference]</td>
<td>1.26 (1.06–1.56)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2–3.9</td>
<td>1 [Reference]</td>
<td>0.93 (0.66–1.46)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4–4.9</td>
<td>1 [Reference]</td>
<td>0.73 (0.47–1.18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥5</td>
<td>1 [Reference]</td>
<td>0.73 (0.47–1.18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P_{trend}$</td>
<td>0.299</td>
<td>0.044</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Model 2, OR (95% CI)

<table>
<thead>
<tr>
<th>Participants, n (%)</th>
<th>1064 (38 ± 1.8)</th>
<th>684 (26 ± 1.33)</th>
<th>523 (20 ± 1.68)</th>
<th>378 (16 ± 1.26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2</td>
<td>1 [Reference]</td>
<td>1.26 (1.06–1.56)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2–3.9</td>
<td>1 [Reference]</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>4–4.9</td>
<td>1 [Reference]</td>
<td>0.73 (0.47–1.18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥5</td>
<td>1 [Reference]</td>
<td>0.73 (0.47–1.18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P_{trend}$</td>
<td>0.641</td>
<td>0.049</td>
<td></td>
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</table>

Model 2 plus plasma PLP, OR (95% CI)

<table>
<thead>
<tr>
<th>Participants, n (%)</th>
<th>1064 (38 ± 1.8)</th>
<th>684 (26 ± 1.33)</th>
<th>523 (20 ± 1.68)</th>
<th>378 (16 ± 1.26)</th>
</tr>
</thead>
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<tr>
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<td></td>
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</tr>
<tr>
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<td>1 [Reference]</td>
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<tr>
<td>4–4.9</td>
<td>1 [Reference]</td>
<td>0.73 (0.47–1.18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥5</td>
<td>1 [Reference]</td>
<td>0.73 (0.47–1.18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$P_{trend}$</td>
<td>0.913</td>
<td>0.332</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

With plasma PLP ≥20 nmol/L, n (%)

<table>
<thead>
<tr>
<th>Participants, n (%)</th>
<th>1565 (87 ± 1.54)</th>
<th>517 (79 ± 2.96)</th>
<th>124 (62 ± 5.96)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1, OR (95% CI)</td>
<td>1 [Reference]</td>
<td>0.48 (0.28–0.84)</td>
<td>0.14 (0.08–0.26)</td>
</tr>
<tr>
<td>Model 2, OR (95% CI)</td>
<td>1 [Reference]</td>
<td>0.51 (0.28–0.95)</td>
<td>0.16 (0.09–0.30)</td>
</tr>
<tr>
<td>Model 2 plus vitamin B-6 intake, OR (95% CI)</td>
<td>1 [Reference]</td>
<td>0.52 (0.28–0.96)</td>
<td>0.17 (0.10–0.31)</td>
</tr>
</tbody>
</table>

1 Percentages are sample-weighted for applicability to the U.S. population given the complex sampling scheme.
2 Participants were nonpregnant, nonlactating adults who had not been diagnosed with diabetes and were not taking oral contraceptives, estrogen-replacement therapy, or steroidal antiinflammatory drugs.
3 All OR (95% CI) are adjusted for intake of vitamin B-6 from food.
4 Adjusted for gender, age, race-ethnicity, BMI, smoking status, alcohol use, serum creatinine, and intakes of energy and protein.
5 Adjusted for covariates in model 1 plus serum concentrations of albumin, folate, β-carotene, lycopene, lutein-zeaxanthin, and vitamins A and C.

Needed for the cytokine production (23) and lymphocyte proliferation (25,26) that characterize the inflammatory response. The other hand, some evidence suggests that the low plasma PLP concentrations observed in the presence of inflammation do not reflect low vitamin B-6 status. Specifically, McMillan et al. (56–59) have repeatedly shown that critically ill people experiencing inflammation have low plasma PLP concentrations but normal erythrocyte PLP concentrations. Studies in our laboratory have corroborated this finding (47,50) and demonstrated no correlation between the plasma PLP concentration and indicators of vitamin B-6 status in erythrocytes (50). We did, however, find that the plasma PLP concentration reflected the availability of vitamin B-6 during metabolic challenges (e.g. methionine and tryptophan loads) better than erythrocyte PLP did (50). The low plasma PLP concentrations observed during inflammation could result from increased vitamin B-6 utilization in the liver at the same time that vitamin B-6 is used normally in erythrocytes.
The strengths of our study included the large sample, the collection of dietary data in 24-h recalls, and the extensive information on use of drugs and supplements. The comprehensiveness of the survey also allowed us to consider many possible alternative explanations for our findings. Of course, residual confounding by imperfect measurement of covariates or failure to control for important but unknown confounders is always possible. Our study’s main limitations were its cross-sectional design, the availability of a single, nonspecific (74) inflammation marker, and the lack of high-quality data on chronic inflammatory diseases. Furthermore, despite the improvements in dietary assessment instituted with the 2003–2004 survey and the development of statistical methods to estimate day-to-day variation in intake around the time of the survey, we may not have captured the relevant data if diet prior to the survey was important. Finally, our method of combining the usual intake of vitamin B-6 from foods with data from the supplement survey instrument assumes that day-to-day variation in vitamin B-6 intake from supplement sources is zero, which may not be the case.

In conclusion, our large, general-population study confirmed the association between inflammation and low plasma PLP concentration but challenged the notion that vitamin B-6 intake is unrelated to inflammation. If the associations we found reflect cause-and-effect relationships, higher vitamin B-6 intakes might be of value in preventing inflammation and maintaining vitamin B-6 adequacy when inflammation is present.

**Acknowledgments**
J.S., L.S., M.S.M., and P.F.J. designed research; M.S.M. and L.S. analyzed data and wrote the paper; and M.S.M. had primary responsibility for final content. All authors read and approved the final manuscript.

**Literature Cited**

10. Robinson K, Mayer EL, Miller DP, Green R, van Lente F, Gupta A, Kottke-Marchant K, Savon SR, Selhub J, et al. Hyperhomocysteinemia and vitamin B-6 intake associated with maximal prevention of vitamin B-6 inadequacy among participants with normal serum CRP was ≥2 mg/d compared with the current Recommended Dietary Allowance for adults of 1.3–1.7 mg/d (39). These findings may be consistent with depletion/repletion studies suggesting that the vitamin B-6 requirement is ~2 mg/d (60,61). In the subgroup with markedly elevated serum CRP concentrations, the vitamin B-6 intake associated with maximal prevention of vitamin B-6 inadequacy was even higher. If the low plasma PLP concentrations associated with inflammation are clinically relevant, our findings are thus also consistent with the hypothesis that the vitamin B-6 requirement is increased in the presence of inflammation.

Preventing inflammation is important because several chronic diseases of considerable public health importance are characterized by chronic inflammation (62). Preventing inflammation might also help prevent America’s number 1 killer, CVD (63), the risk of which is increased in association with systemic inflammation and immune-inflammatory disease. The public health importance of inflammation may actually extend well beyond its contribution to these conditions, however, because chronic inflammation is suspected of playing a role in other age-related diseases, including heart failure, type II diabetes, cancer, frailty, cognitive decline/dementia, and osteoporosis (64).

An inflammation-induced reduction in the plasma PLP concentration would be important because of the critical role that PLP plays in immune function (25,65–68), Hcy catabolism (69), hemoglobin synthesis (70), neurotransmitter synthesis (71), and macromolecular metabolism (72,73). It accomplishes these roles by acting as a cofactor for hundreds of enzymes (39).

**FIGURE 1** Decrease in the prevalence of vitamin B-6 inadequacy with increasing vitamin B-6 intake. Participants (n = 1981) were aged ≥20 y. Pregnant and lactating women were excluded, along with participants diagnosed with diabetes and those on oral contraceptives, hormone replacement therapy, and steroidal antiinflammatory drugs; error bars represent 95% CI; adjusted for gender, age, race-ethnicity, BMI, alcohol use, smoking status, intakes of energy and protein, and serum concentrations of creatinine, albumin, folate, β-carotene, lycopene, lutein-zeaxanthin, and vitamins A and C.

CRP > 10 mg/L. The OR relating such a low plasma PLP concentration to a serum Hcy concentration >12 μmol/L was 7.29 (P = 0.006) in the group with markedly elevated serum CRP concentrations after controlling for demographic factors, smoking status, and serum concentrations of folate, vitamin B-12, and creatinine. That finding illustrates the clinical relevance of the low plasma PLP concentrations that occur in connection with inflammation.

An inflammation-induced reduction in the plasma PLP concentration would be important because of the critical role that PLP plays in immune function (25,65–68), Hcy catabolism (69), hemoglobin synthesis (70), neurotransmitter synthesis (71), and macromolecular metabolism (72,73). It accomplishes these roles by acting as a cofactor for hundreds of enzymes (39).


