Plasma pyridoxal 5′-phosphate in the US population: the National Health and Nutrition Examination Survey, 2003–2004[1–4]

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ABSTRACT

Background: No large-scale, population-based study has considered the descriptive epidemiology of vitamin B-6 status with use of plasma pyridoxal 5′-phosphate (PLP), the indicator of vitamin B-6 adequacy used to set the current Recommended Dietary Allowance, which is ≤2 mg/d for all subgroups.

Objectives: We sought to examine the epidemiology of vitamin B-6 status in the US population.

Methods: In >6000 participants aged ≥1 y in the National Health and Nutrition Examination Survey (2003–2004), we considered relations between plasma PLP and various subject characteristics and examined trends in plasma PLP and homocysteine with vitamin B-6 intake, both overall and in selected subgroups.

Results: In males, plasma PLP decreased with age after adolescence only in nonusers of supplemental vitamin B-6. Regardless of supplement use, plasma PLP concentrations of women of childbearing age were significantly lower than those of comparably aged men, and most oral contraceptive users had plasma PLP <20 nmol/L. The prevalence of low plasma PLP was significantly >3% at vitamin B-6 intakes from 2 to 2.9 mg/d in all subgroups and at intakes from 3 to 4.9 mg/d in smokers, the elderly, non-Hispanic blacks, and current and former oral contraceptive users. Intakes from 3 to 4.9 mg/d compared with <2 mg/d were associated with significant protection from low plasma PLP in most subgroups and from hyperhomocysteinemia in the elderly.

Conclusions: Vitamin B-6 intakes of 3 to 4.9 mg/d appear consistent with the definition of a Recommended Dietary Allowance for most Americans. However, at that intake level, substantial proportions of some population subgroups may not meet accepted criteria for adequate vitamin B-6 status. *Am J Clin Nutr* 2008;87:1446–54.

INTRODUCTION

Vital metabolic functions of vitamin B-6 include its role as a cofactor for enzymes involved in the synthesis and catabolism of neurotransmitters (1), homocysteine (Hcy) transsulfuration (2), and the metabolism of other amino acids (3), fats (3), and glycogen (3, 4). Vitamin B-6 also modulates the action of hormones (4) and affects immune competence (5). Consequently, though no vitamin B-6 deficiency disease is known, a relative lack of the vitamin would be expected to contribute to ill health, including cardiovascular disease. Furthermore, though dietary sources are ubiquitous, status could be adversely affected by alcoholism, age-related gastrointestinal changes, disease, and use of commonly prescribed medications, such as oral contraceptives (OC).

In metabolic studies, various biochemical indicators have been used to shed light on the epidemiology of vitamin B-6 status, and the circulating level of pyridoxal 5′-phosphate (PLP), the biologically active form of the vitamin (6, 7), has emerged as the single best indicator of long-term body stores (8–10). Data published thus far paint a confusing picture of how vitamin B-6 status varies with diet and basic demographic factors such as age and sex. Moreover, relations to race and to lifestyle factors such as smoking and OC use, have not been formally addressed in large-scale population-based studies.

The PLP concentration was measured in plasma samples from nearly 8000 participants in the US National Health and Nutrition Examination Survey (NHANES) that was conducted between 2003 and 2004, providing the best opportunity to date to explore the descriptive epidemiology of vitamin B-6 status.

SUBJECTS AND METHODS

Study population

The NHANES monitors the nation's health and nutritional status through a continuous annual survey (11). Selection of a representative sample of the noninstitutionalized US civilian population is accomplished through use of 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, Centers for Disease Control and Prevention, and informed consent was obtained from all participants.

Trained interviewers used a computer-assisted personal interview system to interview participants in their homes. The

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≈10 122 interviewed participants were also asked to report to a mobile examination center (MEC) to provide further interview data and undergo a physical examination that included phlebotomy and body composition measurements. A detailed description of blood collection and processing can be found in the NHANES Medical Technologists Procedures Manual (12). Female participants aged ≥12 y were interviewed regarding reproductive history at the MEC. Questions concerned age at menarche, menopausal status, and history of OC use, pregnancy, and hormone replacement therapy.

The MEC-examined subjects numbered 9643, of whom 464 were aged <1 y, the age limit for the PLP assay. PLP measurements were obtained for 7822 subjects (85%). From these, we excluded the 249 pregnant women, an additional 787 subjects with incomplete or unreliable dietary data, and 235 women for whom reproductive histories were not obtained.

Determination of daily vitamin B-6 intake

Beginning with the 2003–2004 NHANES, 2 days of dietary intake data were collected (13). 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 US Department of Agriculture 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.

Data on dietary supplement use were collected during the in-home interview (14). Subjects were asked whether they had used any vitamins, minerals, or other dietary supplements within 30 d of the interview. Subjects who had used such products were asked to show the interviewer the supplement containers and provide information on the amount, frequency, and duration of use. The amount of each ingredient in each product used 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 National Institutes of Health's Office of Dietary Supplements. The information in the database came from sources such as the manufacturer or retailer, the Internet, and company catalogs.

For subjects who reported using no dietary supplements, daily vitamin B-6 intake was estimated as the average of the 2 vitamin B-6 intake values derived from the 2 diet recalls and the nutrient databases.

We used the various dietary supplement files to identify all subjects who reported using any supplemental source of vitamin B-6 and to determine each such subject's average daily intake of vitamin B-6 from supplements. This amount was added to the average from food obtained from the 2 diet recalls to yield the daily vitamin B-6 intake for subjects who used supplemental vitamin B-6.

Laboratory analyses

Plasma PLP was measured through use of a homogeneous, nonradioactive, enzymatic assay (A/C Diagnostics, San Diego, CA) (15). The mean intra- and interassay CVs were 8% and 12%–13%, respectively (16). The detection limit of the assay was 10.05 nmol/L. Results below the detection limit were replaced with the value 7.1 nmol/L (ie, the detection limit divided by the square root of 2). Plasma PLP, which was highly skewed, was logarithmically transformed when used as a continuous outcome variable in multivariate analyses. When the outcome of analyses was low compared with higher plasma PLP, "low" was defined as <20 nmol/L. This definition of low vitamin B-6 status was the index of vitamin B-6 adequacy used to set current Estimated Average Requirements (EARs) and Recommended Dietary Allowances (RDAs) of vitamin B-6 (10). The EAR is defined as the average daily intake level of a nutrient that is estimated to meet the needs of one-half of the members of an age and sex group; the RDA is defined as the intake level estimated to meet the needs of virtually all (ie, 97%–98%) of the members of an age and sex group (17). The current EAR and RDA for vitamin B-6 are all <2 mg/d (10). The daily value of vitamin B-6 used on food and supplement labels to compare the nutrient content of a product to a standardized dietary reference value is 2 mg (10).

Creatinine concentrations were measured in serum samples obtained from survey participants aged ≥12 y (Collaborative Laboratory Services, L.L.C., Ottumwa, IA). The Beckman Synchron LX20 modular chemistry side employed uses the Jaffe rate method.

The fully automated Abbott Hcy fluorescence polarization immunoassay (Abbott Diagnostics, Abbott Park, IL) was used to measure total Hcy in plasma samples from subjects aged ≥3 y. These analyses were carried out at the NHANES central laboratory, the Inorganic Toxicology and Nutrition Branch of the National Center for Environmental Health's Division of Laboratory Sciences (Atlanta, GA).

Statistical analyses

Statistical analyses were performed through use of the SUDAAN release 9.0 (Research Triangle Institute, Research Triangle Park, NC) with appropriate masked variance units (ie, pseudoprimary and pseudostratum) and 2-year sampling weights to account for the survey's complex sampling design (18). $P < 0.05$ was considered statistically significant for all analyses.

We considered associations between several demographic and lifestyle factors and plasma PLP concentration separately for users and nonusers of dietary supplements containing vitamin B-6. These associations were described by differences between least-square means and proportions generated by SUDAAN PROC REGRESS. A multivariate model was used for this purpose, with independent variables of age, sex, race-ethnicity (non-Hispanic white, non-Hispanic black, Mexican-American, other Hispanic, other), cigarette smoking history (current, former, never), body mass index (in kg/m^2), self-reported diabetes status, and intakes of protein, energy, and alcohol. Estimates of associations between these factors and plasma PLP concentration in users of supplemental vitamin B-6 were obtained after additional control for daily intake of vitamin B-6 from supplemental sources and duration of supplement use. The continuous and categorical dependent variables in the analyses were log (plasma PLP) and plasma PLP below (compared with at or above) 20 nmol/L. We also used the multivariate models to evaluate associations between the various subject characteristics and supplement use (yes or no), and to estimate least-square mean plasma PLP and associated 95% CI for 32 vitamin B-6 intake categories, each comprising 3.2% of the 6165 subjects ($n \cong 200$).

We further explored associations between age and plasma PLP by estimating multivariate-adjusted least-square mean plasma PLP for subjects in 12 age-by-sex categories. Along with the previously mentioned covariates, these analyses were controlled

for dietary vitamin B-6. With use of the same multivariate model additionally controlled for serum creatinine where possible (ie, age 13–20 y and higher age categories), we related estrogen status (based on sex, menopausal status, and exogenous estrogen use) to circulating PLP concentration and low (compared with higher) PLP status in 4 age categories of nonusers of supplements that contained vitamin B-6.

Combining supplement users and nonusers, we considered how log (plasma PLP) and log (plasma Hcy) varied over 4 categories of vitamin B-6 intake (ie, <2, 2–2.9, 3–4.9, and ≥5 mg/d) in young children, subjects aged 13–54 y stratified by sex and OC use, the elderly, non-Hispanic black and non-Hispanic white males aged ≥13 y, and male smokers and nonsmokers aged ≥13 y. All analyses involving Hcy were additionally controlled for history of heart attack or stroke and circulating folate and vitamin B-12 concentrations.

Finally, focusing on survey participants aged ≥65 y, we used SUDAAN PROC MULTILOG to estimate multivariate-adjusted odds ratios and associated 95% CI relating the odds of being in each of the 3 highest vitamin B-6-intake categories (compared with consuming <2 mg/d) and the odds of normal compared with low plasma PLP to the odds of elevated plasma Hcy and hyperhomocysteinemia (compared with a normal Hcy concentration) according to the following definitions: normal Hcy, ≤10.2 μmol/L; elevated Hcy, 10.3–14 μmol/L; hyperhomocysteinemia, Hcy >14 μmol/L.

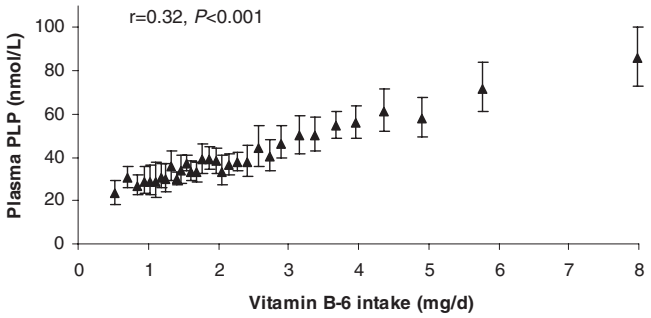

FIGURE 1. Increase in plasma pyridoxal 5′-phosphate (PLP) with increasing vitamin B-6 intake from foods and supplements combined; subjects (n = 6165) were aged ≥1 y. Symbols represent least-square geometric means for 32 categories, each comprising 3.2% ($n \cong 200$) of the subjects. Means are adjusted for age, sex, race-ethnicity, cigarette smoking history, BMI (in kg/m^2), diabetes status, and intakes of protein, energy, and alcohol; error bars represent 95% CI for the means.

RESULTS

Subject characteristics

By design, the demographic makeup of the sample resembled that of the US population. Mean ± SEM intake of vitamin B-6 from foods was 1.86 ± 0.02 mg/d in nonusers of supplements containing vitamin B-6 and 1.94 ± 0.02 mg/d in supplement users (P = 0.001), with teenagers consuming the least and the elderly consuming the most. Thirty-six percent of the population used supplements that contained vitamin B-6, and this habit was associated with higher age, female sex, non-Hispanic white race-ethnicity, nonsmoking status, and nonobese status (data not shown). Among women, use of female hormones was significantly associated with supplement use (odds ratio, 95% CI = 1.5, 1.1–1.9). However, only about one-half of the OC users supplemented their diets with vitamin B-6. Mean ± SEM daily intakes of protein and energy were 81 ± 0.73 g and 2193 ± 16.5 kcal, respectively.

Diet and subject characteristics in relation to plasma PLP

With all covariates and total vitamin B-6 intake controlled for, plasma PLP was not significantly related to energy intake. However, plasma PLP increased by 1 nmol/L for every additional gram of protein ingested per day ($P < 0.001$), and a positive association between serum creatinine and plasma PLP was marginally significant ($P < 0.058$).

After multivariate adjustment, plasma PLP increased by about 12 nmol/L per 1 mg increase in daily vitamin B-6 intake ($P <$ 0.001) (**Figure 1**).

Plasma PLP concentrations <20 nmol/L occurred in 11% of supplement users and 24% of nonusers. However, only 5% of men using supplements had PLP values that low, compared with nearly 32% of women who were not supplementing their diets with vitamin B-6 (**Table 1**).

Among both users and nonusers of supplements containing vitamin B-6, plasma PLP was significantly lower in women compared with men, non-Hispanic blacks compared with non-Hispanic whites, current smokers compared with those who had never smoked, and underweight as opposed to normal-weight subjects.

The preliminary data analyses did not suggest that the elderly had particularly low plasma PLP, regardless of supplement use. Specifically, plasma PLP increased significantly with age in supplement users ($P < 0.001$), even after controlling for daily intake from supplements and duration of supplementation. Furthermore, data for nonsupplement users, as well as data on the prevalence of low PLP in supplement users, provided some evidence that adults aged 21–44 y had particularly low vitamin B-6 status, though teenagers had the lowest vitamin B-6 intakes.

Interaction among supplement use, sex, and age in relation to plasma PLP

Sex-stratified analyses revealed that both the apparent vulnerability to low plasma PLP of young adults and the increase in plasma PLP with increasing age of supplement users were artifacts of combining data for males and females, 2 groups with very different age trends. Among nonusers of supplements, male teens had the highest plasma PLP levels (**Figure 2**) and the lowest prevalence of plasma PLP <20 nmol/L (12%) of all the age × sex categories. In fact, even with serum creatinine controlled for, plasma PLP decreased significantly with increasing age in males from the teen years onward (P = 0.001). Furthermore, 22% of both young boys and men aged ≥65 y had low plasma PLP ($P <$ 0.01 for the comparisons between these age groups and male teens).

Just under 30% of young girls, female adolescents, and elderly women had plasma PLP <20 nmol/L, and geometric mean plasma PLP for these subgroups was, at best, slightly above 30 nmol/L. As low as the plasma PLP concentrations of these female subjects were, they were about 10 nmol/L higher on average than the concentrations we estimated for women aged 21–44 y, in whom the prevalence of low plasma PLP was >40%.

TABLE 1
Vitamin B-6 status according to demographics, body weight, diabetes, and lifestyle in the US population, 2003–2004

	Plasma PLP					
	Nonusers of supplemental vitamin B-6[1]			Users of supplemental vitamin B-6[2]		
Characteristic	Subjects	Geometric mean (95% CI)	Percentage <20 nmol/L (95% CI)	Subjects	Geometric mean (95% CI)	Percentage <20 nmol/L (95% CI)
	n			*n*		
Age						
<13 y	903	34 (31, 36)[3]	25 (20, 30)[3]	275	49 (40, 62)	11 (2, 19)
13–20 y[4]	1317	37 (35, 40)	19 (17, 21)	196	58 (52, 65)	10 (6, 14)
21–34 y	556	32 (29, 35)[3]	27 (22, 32)[3]	197	56 (47, 68)	16 (10, 23)[5]
35–44 y	410	32 (29, 36)[3]	26 (20, 33)[3]	163	57 (50, 66)	15 (9, 21)[5]
45–64 y	632	36 (33, 40)	23 (20, 27)[3]	428	73 (60, 90)[3]	7 (1, 13)
≥65 y	581	36 (31, 41)	24 (18, 30)[5]	501	78 (64, 97)[3]	6 (0, 13)
Sex						
Male[4]	2351	41 (37, 45)	16 (12, 20)	834	81 (73, 89)	5 (3, 7)
Female	2048	29 (26, 31)[3]	32 (28, 36)[3]	926	54 (49, 59)[3]	15 (12, 17)[3]
Race/ethnicity						
Non-Hispanic white[4]	1654	35 (32, 39)	23 (19, 27)	1105	66 (60, 73)	10 (8, 12)
Non-Hispanic black	1357	31 (29, 33)[3]	29 (26, 32)[3]	270	54 (45, 65)[3]	17 (11, 23)[3]
Mexican American	1139	34 (28, 43)	23 (13, 33)	300	63 (52, 76)	13 (5, 22)
Smoking status						
Never[4]	3411	37 (33, 40)	22 (18, 26)	1483	68 (62, 75)	9 (7, 11)
Former	92	33 (27, 39)	26 (16, 36)	16	65 (47, 89)	2 (−3, 6)[3]
Current	896	29 (27, 31)[3]	30 (26, 34)[3]	261	51 (43, 60)[3]	15 (9, 20)[5]
BMI						
<18.5	674	32 (28, 35)[3]	31 (24, 36)[3]	239	81 (67, 100)[3]	4 (−3, 10)[5]
18.5–24.9[4]	1611	36 (32, 39)	23 (19, 27)	583	66 (60, 73)	13 (9, 17)
24.9–29.9	1079	36 (33, 38)	22 (17, 26)	541	67 (56, 80)	9 (4, 13)[5]
≥30	1035	33 (31, 36)	24 (20, 28)	397	56 (49, 65)[3]	10 (3, 17)
Diabetes						
No[4]	4110	34 (32, 37)	24 (20, 28)	1601	64 (58, 71)	11 (9, 13)
Yes	289	35 (30, 40)	20 (13, 27)	159	77 (67, 90)[3]	5 (0, 11)[5]
Alcohol						
Nonuser[4]	3576	34 (31, 37)	24 (20, 28)	1317	65 (59, 71)	10 (8, 12)
User	823	37 (33, 40)[5]	22 (18, 25)	443	66 (60, 73)	10 (6, 15)

[1] Estimates are adjusted for all listed factors plus intakes of protein and energy.
[2] Estimates are adjusted for listed factors, months of supplement use, and intakes of protein, energy, and supplemental vitamin B-6.
[3] $P < 0.05$ for *t* test comparing the indicated category to the referent category.
[4] Referent category.
[5] $P\ 0.05 \leq P < 0.1$ for *t* test comparing the indicated category to the referent category.

Among male supplement users, the geometric mean plasma PLP concentration was consistently high from adolescence onward, and young boys had significantly lower plasma PLP than male subjects in all older age groups. The age pattern for supplement-using women was similar to that observed for nonusers, except that the means were much higher in the supplement users. Among female supplement users, only the elderly had higher plasma PLP concentrations than young children ($P = 0.006$), because, during the young-adult years, the women's PLP concentrations were at their nadir rather than their peak.

Estrogen status and plasma PLP

Although current and former OC users had particularly low plasma PLP concentrations (**Table 2**), the male-female differences revealed in Figure 2 among nonusers of supplemental vitamin B-6 were not entirely explained by exogenous estrogen use. Specifically, estimates of geometric mean plasma PLP for teen girls and young-adult women who had never used exogenous estrogen were significantly lower than those obtained for comparably aged men, and 20–25% of the females in these age groups had plasma PLP below the cutoff point for vitamin B-6 adequacy, compared with <15% of the males.

Geometric mean plasma PLP approximated 25 nmol/L for 3 age ranges of premenopausal former OC users who were not supplementing their diets with vitamin B-6. Although the majority of such women met the criterion for vitamin B-6 adequacy, the proportion with low plasma PLP was, at 40%, much higher than the 12–19% estimated for comparably aged men and boys. Geometric mean plasma PLP was significantly lower than 20 nmol/L among current OC users, about three-quarters of whom would be considered vitamin B-6-deficient based on the currently accepted definition.

Vitamin B-6 intake in relation to plasma PLP and plasma Hcy in selected subgroups

Neither plasma PLP nor plasma Hcy differed between young boys and girls, or between men and women aged ≥65 y. Fur-

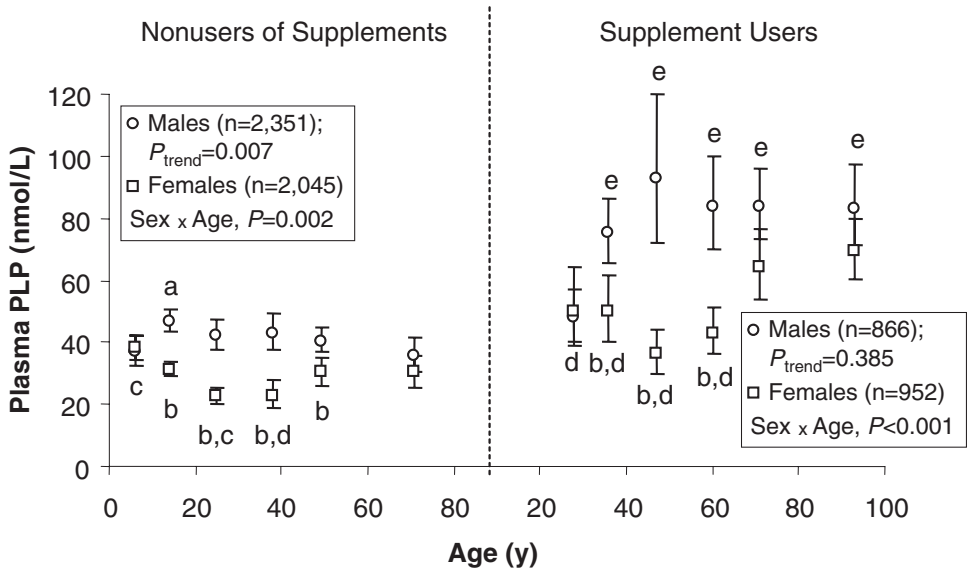

FIGURE 2. Relation between age and plasma pyridoxal 5′-phosphate (PLP) in users and nonusers of dietary supplements containing vitamin B-6 aged ≥1 y. Symbols represent geometric least-square means adjusted for race-ethnicity, cigarette smoking history, BMI (in kg/m^2), diabetes status, and intakes of protein, energy, alcohol, and vitamin B-6; error bars represent 95% CIs for the means. Significant differences according to *t* tests were a) different from all other age groups of men; b) different from men in the same age group; c) different from all other age groups of women; d) different from women in all older age groups; and e) different from young boys; age × supplement use, $P < 0.001$; sex × supplement use, $P = 0.690$, supplement use × sex × age, $P = 0.026$.

thermore, trends in plasma PLP and plasma Hcy across vitamin B-6 intake categories were virtually identical between the 2 sexes in these age groups. Consequently, we combined the sexes for subgroup analyses focused on children and the elderly. Results displayed in Table 2 showed that estrogen status was strongly correlated with plasma PLP in adolescence and earlier adulthood. However, because the sample size did not permit simultaneous stratification by estrogen status and other factors, we

TABLE 2
Estrogen status and plasma pyridoxal 5′-phosphate (PLP) among nonusers of supplemental vitamin B-6 in the US population, 2003–2004[1]

	Age			
Estrogen status	<13 y	13–20 y	21–44 y	≥45 y
Males (*n*)	473	700	542	626
Plasma PLP (nmol/L)	35 (31–40)[2]	44 (41–47)	43 (39–47)	39 (36–43)
<20 nmol/L (%)	24 (18–30)	12 (8–16)	14 (10–18)	19 (15–23)
Females				
Never used hormones (*n*)	440	494	97	157
Plasma PLP (nmol/L)	36 (32–42)	35 (32–39)[3]	30 (26–35)[3]	37 (30–47)
<20 nmol/L (%)	23 (13–32)	20 (13–26)[3]	25 (13–37)	23 (13–32)
Past OC use, menstruating (*n*)	0	59	227	65
Plasma PLP (nmol/L)[4]		25 (16–38)[3]	24 (19–29)[3,5]	27 (20–35)[3,5]
<20 nmol/L (%)		37 (15–59)[3]	40 (33–47)[3,5]	38 (26–49)[3,5]
Past OC use menopausal (*n*)	0	1	25	113
Plasma PLP (nmol/L)			30 (21–41)[3]	32 (26–38)[3]
<20 nmol/L (%)			31 (7–55)	25 (14–35)
Past HRT Use (*n*)[6]	0	0	6	163
Plasma PLP (nmol/L)				33 (28–38)[3]
<20 nmol/L (%)				27 (16–36)
Current hormone use (*n*)[6,7]	0	50	47	48
Plasma PLP (nmol/L)		13 (10–15)[3,5]	11 (8–14)[3,5]	31 (19–47)
<20 nmol/L (%)		73 (63–83)[3,5]	78 (59–96)[3,5]	27 (16–46)

[1] OC, oral contraceptive; HRT, hormone replacement therapy. Tabulated values were estimated from multivariate models adjusted for age, race/ethnicity, BMI, smoking status, diabetes status, serum creatinine (last 3 columns only), and intakes of protein, energy, alcohol, and vitamin B-6.
[2] $\bar{x}$; range in parentheses (all such values).
[3] $P < 0.05$ for the *t* test comparing females in the subgroup to males in the same age range.
[4] $P = 0.009$ for the comparison between former OC users and females who never used hormones when ages 13–44 y were combined.
[5] $P < 0.05$ for the *t* test comparing females in the subgroup to females in the same age range who had never used hormones.
[6] Some of those on HRT had used OCs when premenopausal; analyses were controlled for this past use.
[7] Aged <45 y, OC use; aged ≥45 y, HRT.

TABLE 3
Plasma pyridoxal 5′-phosphate (PLP) and plasma homocysteine (Hcy) by vitamin B-6 intake and population subgroup[1]

	Vitamin B-6 intake (mg/d)			
Category	<2	2–2.9	3–4.9	≥5
Boys and girls aged <13 y [*n* (%)][2]	717 (56)	301 (26)	161 (17)	
Plasma PLP, nmol/L; $P_{trend} < 0.001$	36 (32, 41)	40 (33, 47)	54 (44, 66)[3,4]	
Plasma PLP < 20 nmol/L; %; $P_{trend} = 0.002$	25 (19, 30)	19 (11, 28)	3 (0, 7)[3,4]	
Plasma Hcy, μmol/L; $P_{trend} = 0.711$	4.7 (4.5, 4.9)	4.8 (4.6, 4.9)	4.8 (4.6, 4.9)	
Males aged 13–54 y [*n* (%)]	750 (35)	507 (28)	332 (21)	207 (16)
Plasma PLP, nmol/L; $P_{trend} < 0.001$	40 (36, 44)	49 (43, 55)[3]	62 (56, 69)[3,4]	108 (94, 124)[3,4]
Plasma PLP < 20 nmol/L; %; $P_{trend} = 0.005$	16 (10, 21)	10 (6, 14)	7 (3, 10)[3]	5 (1, 8)[3,4]
Plasma Hcy, μmol/L; $P_{trend} < 0.001$	8.4 (8.3, 8.6)	8.1 (7.6, 8.6)	8.1 (7.8, 8.4)	7.5 (7.2, 7.8)[3,4]
Menstruating females aged 13–54 y				
Never used OCs [*n* (%)]	482 (65)	114 (15)	62 (11)	38 (9)
Plasma PLP, nmol/L, $P_{trend} = 0.008$	31 (28, 35)	34 (29, 40)	62 (49, 78)[3,4]	51 (35, 75)[3,4]
Plasma PLP < 20 nmol/L; %; $P_{trend} = 0.008$	24 (18, 30)	20 (11, 30)	7 (1, 13)[3,4]	4 (−6, 13)[3]
Plasma Hcy, μmol/L; $P_{trend} = 0.556$	7.3 (7.0, 7.6)	7.2 (6.9, 7.5)	6.8 (6.1, 7.7)	6.8 (6.4, 7.2)
Using OCs [*n* (%)]	83 (47)	25 (17)	22 (17)	22 (20)
Plasma PLP, nmol/L; $P_{trend} < 0.001$	10 (8, 13)	10 (7, 13)	17 (12, 28)[3,4]	45 (28, 70)[3,4]
Plasma PLP < 20 nmol/L; %; $P_{trend} = 0.025$	76 (59, 93)	93 (80, 106)	49 (27, 71)[3,4]	39 (22, 56)[3,4]
Plasma Hcy, μmol/L; $P_{trend} = 0.173$	7.2 (6.9, 7.5)	6.8 (5.9, 7.8)[3]	6.7 (6.3, 7.1)	6.7 (6.4, 7.0)
Used OCs in the past [*n* (%)]	282 (53)	90 (18)	76 (17)	43 (12)
Plasma PLP, nmol/L; $P_{trend} < 0.001$	23 (21, 25)	30 (24, 37)[3]	40 (33, 47)[3,4]	53 (38, 84)[3,4]
Plasma PLP < 20 nmol/L; %; $P_{trend} < 0.001$	40 (36, 44)	29 (19, 39)	24 (17, 30)[3]	9 (−2, 20)[3,4]
Plasma Hcy, μmol/L; $P_{trend} = 0.165$	7.8 (7.5, 8.2)	7.6 (7.3, 7.9)	7.2 (6.7, 7.8)	7.0 (6.3, 7.7)
Men and women aged ≥ 65 y [*n* (%)]	449 (37)	134 (13)	270 (27)	212 (22)
Plasma PLP, nmol/L; $P_{trend} < 0.0001$	32 (28, 37)	46 (39, 53)[3]	68 (62, 75)[3]	109 (91, 130)[3]
Plasma PLP < 20 nmol/L; %; $P_{trend} < 0.001$	26 (20, 33)	14 (6, 22)[3]	6 (4, 9)[3]	6 (2, 9)[3]
Plasma Hcy, μmol/L; $P_{trend} < 0.001$	11.6 (11.1, 12.1)	11.1 (10.7, 11.6)	10.2 (9.8, 10.6)[3,4]	10.4 (10, 10.8)[3,4]
Non-Hispanic white males aged ≥ 13 y [*n* (%)]	402 (39)	287 (20)	282 (22)	253 (20)
Plasma PLP, nmol/L; $P_{trend} < 0.001$	40 (36, 44)	47 (40, 55)[3]	65 (60, 70)[3,4]	112 (98, 129)[3,4]
Plasma PLP < 20 nmol/L; %; $P_{trend} < 0.001$	16 (10, 22)	12 (6, 18)	5 (3, 7)[3,4]	3 (1, 5)[3,4]
Plasma Hcy, μmol/L; $P_{trend} < 0.001$	9.7 (9.1, 10.3)	9.1 (8.6, 9.7)	8.8 (8.7, 9)[3]	8.4 (8.3, 8.6)[3,4]
Non-Hispanic black males aged ≥ 13 y [*n* (%)]	327 (59)	140 (19)	96 (14)	48 (8)
Plasma PLP, nmol/L; $P_{trend} < 0.001$	31 (27, 36)	43 (36, 51)[3]	50 (42, 60)[3]	105 (78, 140)[3,4]
Plasma PLP < 20 nmol/L, $P_{trend} < 0.001$	27(21, 33)	13 (7, 19)[3]	10 (4, 16)[3]	2 (0, 4)[3,4]
Plasma Hcy, μmol/L; $P_{trend} = 0.321$	8.8 (8.3, 9.4)	8.8 (8.3, 9.3)	8.5 (8.3, 8.7)	8.5 (8.3, 8.7)
Male nonsmokers aged ≥ 13 y [*n* (%)]	742 (40)	428 (20)	366 (21)	303 (19)
Plasma PLP, nmol/L; $P_{trend} < 0.001$	40 (35, 46)	51 (45, 57)[3]	64 (58, 71)[3,4]	124 (110, 139)[3,4]
Plasma PLP < 20 nmol/L, $P_{trend} < 0.001$	16 (10, 22)	9 (5, 13)	5 (3, 7)[3]	1 (−1, 3)[3,4]
Plasma Hcy, μmol/L; $P_{trend} < 0.001$	9.4 (9.2, 9.6)	9.0 (8.7, 9.4)	8.7 (8.3, 9)[3]	8.2 (7.9, 8.6)[3,4]
Male smokers aged ≥ 13 y [*n* (%)]	282 (49)	175 (21)	131 (17)	71 (13)
Plasma PLP, nmol/L; $P_{trend} < 0.001$	33 (29, 37)	40 (33, 47)	54 (45, 64)[3,4]	77 (63, 93)[3,4]
Plasma PLP < 20 nmol/L; %; $P_{trend} < 0.001$	21 (15, 27)	15 (5, 25)	9 (5, 13)[3]	6 (0, 12)[3]
Plasma Hcy, μmol/L; $P_{trend} = 0.016$	9.7 (9.1, 10.3)	9.2 (8.5, 10)	9.1 (8.8, 9.5)	8.7 (8.0, 9.4)[3]

[1] OC, oral contraceptive. Values are least-square geometric means (and 95% CIs) adjusted for age, body mass index (in kg/m^2), diabetes status, serum creatinine, and intakes of protein, energy, and alcohol; means and proportions are also adjusted for estrogen status, race/ethnicity, and smoking status, except where results are shown stratified by these factors; plasma Hcy values are additionally adjusted for serum concentrations of folate and vitamin B-12 and for history of heart attack or stroke.

[2] The 2 highest intake categories were combined for young children (and placed under the "3–4.9" heading), because of the small number consuming ≥5 mg vitamin B-6/d.

[3] $P < 0.05$ for *t* test comparing the indicated category to the category that consumed <2 mg/d.

[4] $P < 0.05$ for *t* test comparing the indicated category to the category that consumed 2–2.9 mg/d.

included males only in analyses stratified by race-ethnicity and smoking status (**Table 3**).

The proportion of subjects who consumed <2 mg/d of vitamin B-6 ranged from 35% in men aged 13–54 y to 65% in menstruating women who had never used exogenous estrogen.

Despite the finding of a strong, graded increase in plasma PLP with increasing vitamin B-6 intake in all population subgroups, plasma PLP was often low at vitamin B-6 intakes between 2 and 2.9 mg/d (above the current RDA), with no significant difference in geometric mean plasma PLP between the 2 lowest-intake categories. Furthermore, the entire 95% CI for the prevalence of plasma PLP <20 nmol/L was >3% among subjects consuming between 2 and 2.9 mg/d in all population subgroups. On the other hand, geometric mean plasma PLP was always significantly higher for subjects consuming 3–4.9 mg/d of vitamin B-6 than it was for the subjects consuming <2 mg/d, and in most subgroups, the prevalence of low plasma PLP was not significantly different from 3% at that intake level. Current and former OC users, the

elderly, non-Hispanic black males, and male smokers were exceptions to this rule. However, the prevalence of low plasma PLP was not significantly different from 3% at vitamin B-6 intakes ≥5 mg/d in all of these subgroups, except current OC users, in whom the prevalence of low plasma PLP was about 40%, even at the highest intake level.

In the population aged ≥12 y, plasma Hcy decreased by 0.5 μmol/L per 62 nmol/L increase in plasma PLP ($P < 0.001$). An equivalent decrease in plasma Hcy was associated with a 1 mg/d increase in vitamin B-6 intake ($P = 0.003$). However, the association between vitamin B-6 status and plasma Hcy was not apparent in all population subgroups. Specifically, a significant decrease in plasma Hcy with increasing vitamin B-6 intake was not observed in young children, women of childbearing age, or non-Hispanic blacks.

Despite a large difference in plasma PLP concentration between current OC users and women of childbearing age who had never used exogenous estrogen, the 2 groups had similar plasma Hcy concentrations. On the other hand, after multivariate adjustment for the full set of covariates, including vitamin B-6 intake, geometric mean plasma Hcy (95% CI) for premenopausal former OC users aged 13–54 y was 7.1 (7.0–7.2) μmol/L, which was significantly higher than the value obtained for comparably aged menstruating women who had never used OCs 6.6 (6.4–6.9).

In elderly men and women combined, although consuming ≥2 mg/d of vitamin B-6 was associated with significantly higher plasma PLP than consuming less, plasma Hcy did not differ between subjects in these 2 low-intake categories. However, geometric mean plasma Hcy was ≥1 μmol/L lower in elderly subjects who consumed 3–4.9 mg/d or ≥5 mg/d of vitamin B-6 than it was in elderly subjects who consumed less.

Vitamin B-6 status and hyperhomocysteinemia in elderly subjects

Among subjects aged ≥65 y, consuming 2–2.9 mg/d compared with less vitamin B-6 was not associated with significant protection from elevated Hcy or hyperhomocysteinemia (**Table 4**). However, consuming ≥3 mg/d compared with <2 mg/d was associated with significant protection from both outcomes, as was plasma PLP ≥20 nmol/L compared with a lower value.

TABLE 4
Vitamin B-6 intake category and plasma pyridoxal 5′-phosphate (PLP) category in relation to elevated homocysteine and hyperhomocysteinemia in 1057 NHANES (2003–2004) participants age ≥ 65 y[1]

	Plasma homocysteine (μmol/L)		
Vitamin B-6 status indicator	≤10.2	10.3–14[2]	>14[3]
Vitamin B-6 intake (mg/d)			
<2	Referent	Referent	Referent
2–2.9	1.0	0.6 (0.3, 1.2)	0.6 (0.3, 1.3)
3–4.9	1.0	0.5 (0.3, 0.8)	0.4 (0.2, 0.8)
≥5	1.0	0.4 (0.2, 0.6)	0.3 (0.1, 0.9)
Plasma PLP (nmol/L)			
<20	Referent	Referent	Referent
≥20	1.0	0.5 (0.3, 0.96)	0.3 (0.2, 0.6)

[1] NHANES, National Health and Nutrition Examination Survey. Values are odds ratios (95% CI) generated by polytomous logistic regression after control for age; sex; BMI (kg/m^2); diabetes status; intakes of protein, energy, and alcohol; history of heart attack or stroke; and serum concentrations of folate, vitamin B-12, and creatinine.
[2] Elevated.
[3] Hyperhomocysteinemia.

DISCUSSION

Important findings of this investigation include the persistence of plasma PLP levels recognized as increasing the risk of clinically significant low vitamin B-6 status at intakes near the current RDA in all subgroups and the comparatively low geometric mean plasma PLP concentrations and high prevalence of low plasma PLP associated with some characteristics.

Reports of previous studies have been quite mixed regarding the ways in which age (7, 19–28) and sex (21, 22, 24, 25, 28, 29) relate to vitamin B-6 status, which partly reflects variation across studies in B6-status indicators. However, our study also illustrates the importance of population characteristics (eg, age range and sex distribution) and the power to stratify analyses by key covariates. The most comprehensive previously published study (25) of the descriptive epidemiology of vitamin B-6 status involved 617 participants in the Baltimore Longitudinal Study of Aging, a study of men only. Results were remarkably similar to ours in that plasma PLP decreased by ≅4 nmol/L per 10-year increase in age in nonsupplemented men, whereas no significant effect of age was found in supplement users. The low plasma PLP concentrations of elderly men found in some studies could have resulted from poor diet or biochemical change caused by illness, but the NHANES and the Baltimore Longitudinal Study of Aging focused on free-living people, and we controlled for diet, diabetes, creatinine, smoking, and alcohol intake. Also, excluding people with signs of renal dysfunction and those with heart attack or stroke history did not alter results (data not shown).

The causes and consequences of low vitamin B-6 status in the elderly are unclear. Decreased absorption, increased catabolism, and defective phosphorylation of B-6 vitamers are all possible causal mechanisms (7, 26). Regarding consequences, we found that plasma PLP concentrations ≥20 nmol/L and vitamin B-6 intakes ≥3 mg/d were significantly associated with protection from Hcy concentrations linked in previous studies to health risks. Specifically, Spence (30) concluded from a review that a doubling of coronary risk was associated with Hcy levels >10.2 μmol/L, and Seshadri (31) linked Hcy levels >14 μmol/L to a doubling of Alzheimer's disease risk. Studies also suggest that vitamin B-6 deficiency affects humoral and cell-mediated immune responses and possibly tumor growth and disease processes (5).

Opinions vary about the need for dietary supplements to prevent vitamin B-6 deficiency in the elderly (23, 32). Löwik et al (23) recommended merely consuming more food sources of vitamin B-6. However, in the US population, only 6% of elderly people consuming ≥3 mg/d of vitamin B-6 did not use supplements. About 90% of the users with intakes ≥3 mg/d took conventional multivitamin/multiminerals; the other 10% took a B-complex mixture or vitamin B-6.

Although the low plasma PLP levels of pregnant and lactating women are well recognized (33, 34), the general vulnerability of women of reproductive age to lower plasma PLP concentrations has not been widely reported. In fact, a 1990 report on the state of the art of evaluating vitamin B-6 status did not list sex among factors related to vitamin B-6 status (20). Recent epidemiologic studies have mainly focused on older people because of the

connection between low B-vitamin status and hyperhomocysteinemia (28). In view of our results, it is not surprising that such studies failed to identify female sex as a risk factor for low vitamin B-6 status (23, 24, 35, 36). A 1999 British study of children and adolescents included teenagers old enough to reveal a postpubertal male-female difference in plasma PLP and a decrease from early childhood to adolescence in girls (25), but no published study was large enough to precisely estimate plasma PLP concentrations in males and females across the life span. The age pattern of the male-female difference in plasma PLP (ie, its onset at menarche and its gradual disappearance as more and more women entered menopause) as well as the link to OC use strongly suggest that estrogen plays a role in determining plasma PLP concentrations in women. It is important to stress, however, that, despite significant differences in plasma PLP between nonsupplemented adult men and nonsupplemented menstruating women who had never used OCs, the prevalence of low plasma PLP was minimized in both groups at vitamin B-6 intakes ≥ 3 mg/d.

Our findings corroborate several reports of abnormalities of tryptophan metabolism in OC users that were corrected by megadoses of vitamin B-6 (37–40). Although some authors (37, 41–44) urged the routine supplementation of OC users with vitamin B-6, the abnormality was later attributed directly to the high doses of estrogen delivered by first-generation OCs rather than drug-induced vitamin B-6 deficiency (45), particularly because the difference in plasma PLP levels between OC users and nonusers was so small as to be potentially explained by diet (46). More recently, lower-dose OCs were linked to abnormal tryptophan metabolism (47), and a relatively large study (48) showed a large and significant difference in plasma PLP concentration between OC users and nonusers. Furthermore, symptoms of vitamin B-6 deficiency have been noted at plasma PLP concentrations consistent with those of OC-using NHANES participants (49). However, the low plasma PLP levels of OC users have been hypothesized to represent merely a redistribution of PLP between extracelluar and intracellular compartments (50) or a shift of the total vitamin B-6 from PLP to the pyridoxal vitamer (51). The normal Hcy levels of OC users despite low plasma PLP tend to suggest protection from cardiovascular disease risk and seem to support the view that the low PLP levels of OC users are benign. On the other hand, both OC use (52) and low vitamin B-6 status (52) have been linked to inflammation. A true vitamin B-6 deficit might not result in low Hcy levels during OC use if estrogen lowered plasma Hcy and plasma PLP simultaneously by different mechanisms. Low Hcy concentrations have, in fact, been noted in both women on hormone replacement therapy (53, 54) and OC users during the high-hormone phase (55). Considering the many functions of vitamin B-6 and the possibility that cardiovascular disease is related to vitamin B-6 status independently of Hcy (29, 56), any Hcy advantage might be outweighed, in both the user and her unborn children (57), by the effects of long-term vitamin B-6 deficiency, which the low plasma PLP concentrations and elevated Hcy concentrations of former OC users may reflect.

The suggestion of low vitamin B-6 status in blacks corroborates findings of 2 earlier studies (58, 59). Also consistent with our results, Serfontein et al (59) showed a relation between vitamin B-6 intake and Hcy in South Africans that was restricted to whites, which led the authors to speculate that Hcy is metabolized efficiently at low vitamin B-6 status in blacks.

The low plasma PLP concentrations of cigarette smokers have also been noted before. However, the relevance of this finding to the smokers' vitamin B-6 status has been questioned, even though, when corrected for hematocrit, erythrocyte PLP was also found to be significantly lower in smokers compared with nonsmokers (60). Smokers in our study lagged behind nonsmokers in both plasma PLP increases and Hcy lowering as vitamin B-6 intake increased.

Strengths of this study included the large, multi-ethnic sample, the experience and training of the NHANES researchers, the administration of 2 diet recalls, and the extensive data on supplement use. The rich NHANES data set allowed us to perform stratified analyses and consider many covariates, both to clarify findings and control bias. Weaknesses included variations across subgroups in the usefulness of plasma Hcy as an indicator of vitamin B-6 function, indirect assessment of estrogen status, and the lack of data on other B-6 vitamers. Future studies should attempt to confirm and clarify our findings by overcoming these limitations.

In conclusion, our findings were inconsistent with the idea that the current RDAs for vitamin B-6 guarantee adequate vitamin B-6 status for nearly everyone. Furthermore, intakes between 3 and 4.9 mg/d would likely leave some smokers, blacks, seniors, and current and former OC users with inadequate status.

The authors' responsibilities were as follows—JS and MFP: conceived of this study; MSM: designed and performed the statistical analyses and drafted the manuscript; PFJ: provided expert input on epidemiologic and statistical aspects; and all authors contributed to the final version of the manuscript. None of the authors had a conflict of interest.

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