Vitamin B-6 Intakes and Plasma B-6 Vitamer Concentrations of Men and Women, 19–50 Years of Age

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Abstract: The vitamin B-6 intakes and plasma B-6 vitamer levels of healthy nonsupplemented men and women, 19–24 and 25–50 years, were compared. The subjects did not take nutrient supplements or medications or use tobacco products. Subjects were grouped as follows: eight, 19–24 y men; nine, 25–50 y men; 11, 19–24 y women; and 13, 25–50 y women. The estimated vitamin B-6 intakes, obtained via 24-h recalls followed by 2-d food records, of the two groups of men were significantly higher ($P < 0.05$) than those of the two groups of women. Thirty-five percent of the women reported consuming less than the Estimated Average Requirement for vitamin B-6. The four gender: age groups had similar B-6 vitamer concentrations of plasma pyridoxal-5'-phosphate, 4-pyridoxic acid, pyridoxine, pyridoxamine, and pyridoxamine-5'-phosphate. Males 25–50 y had significantly higher ($P < 0.05$) plasma pyridoxal concentrations than the two groups of females. All subjects had pyridoxal-5'-phosphate concentrations indicative of vitamin B-6 adequacy. Generally the plasma B-6 vitamer concentrations of these men and women, 19–24 and 25–50 years of age, all having adequate vitamin B-6 status, were similar.

Key words: Vitamin B-6, plasma B-6 vitamers, pyridoxal phosphate, men, women

Introduction

Vitamin B-6 exists in three interconvertible forms: pyridoxine (PN; also known as pyridoxol), pyridoxal (PL), and pyridoxamine (PM), each of which has a corresponding 5'-phosphate (P). 4-pyridoxic acid (4-PA) is the major excretory catabolite. Then nonphosphorylated forms of the vitamin can be converted to their respective phosphorylated forms and vice versa by pyridoxal kinase and pyridoxine (pyridoxamine) phosphate oxidase [1, 2]. Recently, these kinase and oxidase enzymes have been identified as potential targets for pharmacologic agents [3].

Researchers have developed and perfected HPLC techniques for quantitating B-6 vitamers in various tissues. Plasma B-6 vitamer and 4-PA concentrations of healthy individuals, primarily young adults, have been published by several research groups [4–17]; the number of subjects included in each of these studies is low and frequently information was not provided as to gender and age. Many of these researchers did not report the vitamin B-6 intakes

Abbreviations used: PN, pyridoxine; PL, pyridoxal; PM, pyridoxamine; PLP, pyridoxal-5'-phosphate; PMP, pyridoxamine-5'-phosphate; 4-PA, 4-pyridoxic acid; RDA, recommended dietary allowance; EAR, estimated average requirement

of their subjects. Plasma pyridoxal-5'-phosphate (PLP) may also be quantitated using radioenzymatic techniques [18, 19]. Plasma PLP concentration has been considered the most relevant and frequently used direct measure of vitamin B-6 status [19, 20] for the last two decades.

Several researchers [21–28] have reported finding abnormal values for the various biochemical status indices, including plasma PLP concentrations, in their elderly subjects. Lee and Leklem [29] reported that five young ($\bar{x}$ = 24.4 y) and eight middle-aged ($\bar{x}$ = 55.3 y) women had significantly different plasma PLP levels when vitamin B-6 intakes were 2.3 mg/d but not when intakes were 10.3 mg/d. Information exists as to "norms" for plasma PLP concentrations of young adults, with more limited data on plasma concentrations of the other B-6 vitamers. Values for vitamin B-6 indices of the elderly are abnormally low. Little information exists as to "norms" for plasma PLP concentrations (and that of other B-6 vitamers) of healthy men and women between 25 and 50 y. The objective of the present study was to compare the vitamin B-6 intakes and plasma B-6 vitamer levels of healthy non-supplemented men and women, aged 19–24 and 25–50 y.

Materials and Methods

Subjects: Forty-one Caucasian men and women, aged 19–50 y, who were in apparent good health and did not use dietary supplements, medications, or tobacco products volunteered as subjects after the project had been approved by the University's Institutional Review Board For the Protection of Human Subjects. The subjects were grouped as follows: 19–24 y men, 25–50 y men, 19–24 y women, and 25–50 y women; these were the age groupings utilized in the 1989 Recommended Dietary Allowances [30]. Subjects were measured for height and weight (Fairbanks scale, Fisher Scientific Co, St. Louis, MO, USA) while wearing light clothing and no shoes.

Blood collection: Approximately 30 mL venous blood was collected from subjects by a qualified phlebotomist at 0700–0900 after subjects fasted overnight. The samples were kept in ice and protected from light. Blood was centrifuged at 4000× g for 10 min at 5°C and stored at –50°C, 8–10 mo, for later HPLC analyses of plasma B-6 vitamers and 4-PA concentrations.

Dietary analyses: A 24-h recall for each subject was conducted by a trained interviewer using food models and cross-checking [31]. In addition, the subjects were asked to complete and return a self-recorded food intake record for the following 2 d. The reported intakes for food energy, protein, and vitamin B-6 over the three consecutive days were estimated utilizing a food composition database developed by our laboratory which uses values given in the US Department of Agriculture food composition database [32] and label information. The estimated food energy, protein, and vitamin B-6 intakes of the subjects obtained from 24-h recalls were compared with those obtained from 2-d food records and no significant differences were observed, so data from the 3 d were averaged.

HPLC analyses: All HPLC methods were performed using a Waters Associates (600E, Milford, MA, USA) solvent delivery system, column heater, Ultramex 3 μm, 250× 3.2 mm C_{18} column (Phenomenex, Torrance, CA, USA), and model 470 scanning fluorescence detector. Plasma B-6 vitamers and 4-PA concentrations were measured using the extraction method of Chrisley *et al* [10] and the HPLC method of Sampson and O'Connor [12]. Proofs of identities and pre-extraction fortifications were conducted as described by Chrisley *et al* [10]. The standards were satifactorily separated in 45 min. Percent recoveries of the vitamers and 4-PA from pre-extraction fortifications (means of three replications) were as follows: PLP, 97.0%; 4-PA, 96.7%; PMP, 95.3%; PL, 96.0%; PN, 97.0%; and PM, 92.0%. Nondetectable concentrations were recorded as zeros. Interassay variability was within 8%.

Statistical analyses: All data were analyzed by the general linear models (GLM) module of SAS (SAS Institute, Cary, NC, USA), Duncan's multiple-range tests, and Pearson correlation coefficients (using data of all subjects combined). Means and SD were also calculated. Differences were considered significant at $P < 0.05$.

Results

The subjects included eight men aged 19–25 y; nine men, 25–50 y; 11 women, 19–24 y; and 13 women, 25–50 y. The height and weight values of men were significantly greater ($P < 0.05$) than those of women (Table I).

Estimated food energy, protein, and vitamin B-6 intakes: There were no significant differences in the estimated protein intake and ratio of vitamin B-6 to protein (mg/g) among the four groups, though differences ($P < 0.05$) existed in estimated food energy intake (Table I). The estimated vitamin B-6 intakes of the two groups of men were significantly higher ($P < 0.05$) than those of the two groups of women. Significant correlations were observed between intakes of vitamin B-6 and protein (r =

Table I: Height and weight values and daily estimated food energy, protein, and vitamin B-6 intakes of subjects

	Men		Women	
	19–24 y (n = 8)	25–50 y (n = 9)	19–24 y (n = 11)	25–50 y (n = 13)
Height (cm)	180.1[a] ± 8.3	174.8[a] ± 6.2	166.0[b] ± 5.9	168.1[b] ± 7.1
Weight (kg)	82.5[a] ± 10.4	78.7[a] ± 12.7	61.5[b] ± 5.0	63.7[b] ± 11.1
Food energy (MJ)	13.1[a] ± 3.6	10.7[ab] ± 3.3	8.8[b] ± 2.1	9.0[b] ± 2.9
Protein (g)	112.1 ± 47.0	100.8 ± 31.0	76.5 ± 23.1	73.3 ± 20.7
Vitamin B-6 (mg)	2.87[a] ± 1.49	2.99[a] ± 1.77	1.52[b] ± 0.63	1.77[b] ± 0.72
Vitamin B-6: protein (mg)	0.027 ± 0.013	0.030 ± 0.015	0.021 ± 0.009	0.025 ± 0.009

Values are means ± SD. Values in a row with different superscript letters are significantly different, $P < 0.05$.

0.55, $P < 0.0002$), vitamin B-6 and food energy ($r = 0.64$, $P < 0.0001$), and protein and food energy ($r = 0.70$, $P < 0.0001$) of all subjects combined. All subjects indicated that their 3-d dietary records were typical of their normal food consumption.

Plasma B-6 vitamers and 4-pyridoxic acid: Plasma B-6 vitamers and 4-PA concentrations were not significantly different among groups with one exception (Table II). Men 25–50 y had significantly higher ($P < 0.05$) plasma PL levels than the two groups of women. The predominant plasma B-6 vitamer in all subject groups was PLP. Plasma PN and PL concentrations were correlated ($r = 0.37$, $P < 0.05$); but all other correlations between plasma B-6 vitamer concentrations were nonsignificant. Plasma B-6 vitamer levels (as well as 4-PA) were not correlated to vitamin B-6 intakes. Standard deviations for all of these variables were large as much individual-to-individual variation existed in these variable values.

Table II: Plasma B-6 vitamer and 4-PA concentrations of subjects

	Men		Women	
	19–24 y (n = 8)	25–50 y (n = 9)	19–24 y (n = 11)	25–50 y (n = 13)
	(nmol/L)			
Pyridoxal-5'-phosphate	95.0 ± 28.8	90.0 ± 50.2	70.1 ± 27.5	74.1 ± 25.4
Pyridoxamine-5'-phosphate	6.4 ± 8.7	11.0 ± 12.4	4.9 ± 7.3	9.7 ± 19.1
Pyridoxal	7.8[ab] ± 3.3	9.3[a] ± 4.6	5.8[b] ± 2.1	6.2[b] ± 1.7
Pyridoxine	4.3 ± 3.2	5.4 ± 1.7	3.0 ± 3.6	2.9 ± 3.9
Pyridoxamine	13.0 ± 4.9	12.8 ± 8.0	9.7 ± 10.2	12.6 ± 8.7
4-Pyridoxic acid	8.5 ± 7.2	10.9 ± 5.9	8.3 ± 6.0	5.9 ± 4.1

Values are means ± SD. Groups with different superscripts for the variable are significantly different, $P < 0.05$.

Discussion

The mean dietary vitamin B-6 intakes of the subjects in the current study were higher than the mean intakes of 2.17 mg/d and 1.52 mg/d observed for men and women, 20–49 y, respectively in the CSFII survey of 1994–96 [33]. All of our subjects consumed meats and cereals daily. Also, none of our subjects were vegetarians.

The 1998 Recommended Dietary Allowance (RDA) [34] for vitamin B-6 is 1.3 mg daily for adults 19–50 y. The Estimated Average Requirement (EAR) may be used to estimate the prevalence of inadequate nutrient intake [34]. The 1998 EAR for vitamin B-6 is 1.1 mg for adults 19–50 y. Utilizing the method of Guenther *et al* [35] in calculating usual intakes, 35.3% of women (37.5% of women 19–24 y and 33.3% of women 25–50 y) and none of the men in the current study reported consuming less than the EAR.

The mean dietary food energy and protein intakes of the subjects in the current study were also higher than means observed for men (11.0 MJ, 98.6 g) and women (7.4 MJ, 63.9 g) 20–49 y in the CSFII survey [33]. The requirement for vitamin B-6 may be related to the protein intake, with 0.016 mg vitamin B-6/g protein consumed being indicative of adequacy [29, 36–38]. However, other studies showed no effect of protein intake on vitamin B-6 status parameters [34, 39]. Two 19–24 y and two 25–50 y women as well as one 25–50 y man in the current study had ratios of vitamin B-6 to protein (mg/g) < 0.016.

The mean plasma B-6 vitamer concentrations of the four groups of subjects in the present study, with one exception, were within the ranges of means for healthy adults derived using several different HPLC techniques which have been previously reported [4–10, 12–17] and were as follows (nmol/L): for PLP, 29.5–88.0; for PMP, nondetectable to 14.9; for omit-our error, PL, 1.0–41.4; for PN, nondetectable to 41.4; and for PM, nondetectable to 17.6. The plasma 4-PA values of subjects in the current study were within the ranges of means (2–90 nmol/L) reported by others [4, 7–11, 13, 15, 16]. As noted earlier, most of these researchers did not provide information as to whether their subjects took supplements containing vitamin B-6.

The plasma PLP values for the two groups of men in

the current study were higher than the reported ranges of means obtained using HPLC techniques. Thirty percent of the male subjects reported consuming over 5 mg of dietary vitamin B-6 daily. Vitamin B-6 supplements have been reported to increase radioenzymatically determined plasma PLP values of men and women [40], with concentrations of 74.0 ± 45.6 and 125.1 ± 38.0 nmol reported for adults consuming 3.5 ± 0.5 and 6.3 ± 1.3 mg of the vitamin daily from diet plus supplement. Elderly men and women taking vitamin B-6 supplements have also been reported to have higher plasma PLP concentrations than those not taking supplements [27]. Hence, finding higher than usual plasma PLP concentrations in men consuming an average of almost 3 mg of dietary vitamin B-6 daily is not unexpected.

Plasma PL concentrations of 25–50 y men were significantly higher than those of women, but not the 19–24 y men. The men, especially those 25–50 y, having the higher plasma PL concentrations also had the higher vitamin B-6 intakes.

Plasma PLP remains the indicator of choice for the assessment of vitamin B-6 status, although its use has been questioned because plasma PLP can be affected by certain physiological conditions and certain drugs. None of the subjects in the current study reported having health problems or used drugs.

All of the subjects in the present study, even the subject that reported consuming 0.7 mg of vitamin B-6 daily, had plasma PLP concentrations indicative of vitamin B-6 adequacy, regardless of whether < 30 nmol/L [19, 20] or < 20 mnol/L [34] was used as the "cutoff" for inadequacy. Plasma PLP concentrations of elderly men and women [21–23, 25–28] have been reported to be abnormal or at least different than values for young adults. Plasma PLP and other B-6 vitamer concentrations of both men and women in the current study who were 25–50 y of age were similar to those who were 19–24 y, thus indicating that adults in these age groups, having adequate vitamin B-6 status, also have similar values for vitamin B-6 status indices. This suggests that "normal" values for vitamin B-6 status established for young adults can also be utilized for those who are 25–50 years of age and that "norms" established for men can be utilized for women.

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