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Plasma B_6 vitamer and plasma and urinary 4-pyridoxic acid concentrations of middle-aged obese black women

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ABSTRACT

Plasma B_6 vitamer and plasma and urinary 4-pyridoxic acid (4-PA) concentrations of fifteen middle-aged obese black women were determined by high-performance liquid chromatography (HPLC). Estimated protein and vitamin B_6 intakes of the subjects, aged 27–52 years, were 64.5 ± 15.6 g and 1.21 ± 0.68 mg (mean ± S.D.), respectively. Mean HPLC-derived plasma B_6 vitamer and 4-PA concentrations for these subjects were 68.9, 3.1, 1.2, 4.1, 3.4, 7.2 and 2.0 nmol/l for pyridoxal 5′-phosphate (PLP), pyridoxine 5′-phosphate, pyridoxamine 5′-phosphate, pyridoxal, pyridoxine, pyridoxamine and 4-PA, respectively. The mean urinary 4-PA/creatinine ratio of the women was 0.88 μmol/mmol. All subjects had plasma PLP levels indicative of adequate vitamin B_6 status. Vitamin B_6 status parameters of the middle-aged obese black women were similar to those previously reported for white nonobese women having adequate vitamin B_6 status.

INTRODUCTION

Several methodologies have been used to determine vitamin B_6 status. Plasma pyridoxal 5′-phosphate (PLP) concentration has been considered to be the most sensitive and reliable indicator of vitamin B_6 status for the last decade or so [1,2]; however, many researchers are now questioning its usefulness and sensitivity. Most researchers in the area have recommended that status be determined using more than one parameter.

Recent interest has focused on the use of high-performance liquid chromatography (HPLC) as a means of determining vitamin B_6 levels in plasma. Several researchers [3–13] have published plasma B_6 vitamer concentrations of a limited number of subjects. Chrisley *et al.* [4] recently reported plasma B_6 vitamer con-

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centrations of men fed controlled diets as determined by HPLC. Plasma B_6 vitamer and 4-pyridoxic acid (4-PA) concentrations of white adolescent females [5] and young women [6] have also been reported. HPLC methodology has also been developed for quantitating urinary 4-PA values [14] and this parameter suggested for use in the assessment of vitamin B_6 status [15]. HPLC-derived plasma B_6 vitamer and urinary 4-PA levels may be of use in the assessment of vitamin B_6 status.

Published plasma B_6 vitamer and 4-PA values are primarily those of white populations living in the USA. Little is known regarding the vitamin B_6 status, requirements and intakes of blacks living in the USA. The purpose of this study was to determine the vitamin B_6 status of American middle-aged obese black women via plasma PLP and urinary 4-PA measurements and vitamin B_6 intake estimations as well as to determine their plasma concentrations of other B_6 vitamers and 4-PA. Obese subjects were used since about half of the black women in the USA have been reported to be obese [16] and these individuals may be more likely to have health problems. A body weight of $\geq 20\%$ above desirable weight constitutes an established health hazard according to a National Institutes of Health Consensus Development Panel [17].

EXPERIMENTAL

Subjects

Obese black female residents of the Petersburg, VA area, aged 27–52 years, volunteered to serve as subjects. They were at least 20% above ideal body weight as calculated using midpoints for frame sizes [18] and in apparent good health. No women taking vitamin and mineral supplements were included in the study. The study was approved by the Human Subjects Committee at Virginia State University; informed consent was obtained from all subjects.

Anthropometric and dietary analyses

Subjects were measured for height and weight while in stocking feet and wearing light clothing. The body mass index (BMI = weight/height^2) [19] was calculated. Two 24-h food recalls fashioned after those of Christakis [20] were obtained from the subjects by trained dietary interviewers; food models were used in estimating portion sizes. The recalls were conducted on two occasions (both weekdays) that were separated by at least a one-week interval. The reported kilocalorie, protein and vitamin B_6 intakes of the subjects were estimated as previously described [4].

Blood and urine sampling and analyses

Venous blood samples were collected as previously described [4] from each fasting subject between 06:30 and 08:30. Random freshly voided urine samples were obtained as previously described [5] during the same time frame. Hematocrit

[21] values were determined. Plasma B_6 vitamer (PLP; pyridoxine 5'-phosphate, PNP; pyridoxamine 5'-phosphate, PMP; pyridoxal, PL; pyridoxine, PN; and pyridoxamine, PM) and 4-PA concentrations were measured as previously reported [4]. The HPLC system (Waters Assoc., Milford, MA, USA) consisted of the following components: Model 730 data module, Model 720 system controller, two Model 45 solvent delivery systems, Model U6K universal injector, column temperature control system and Model 420 E/AC fluorescence detector (300 nm excitation, 375 nm emission). The analytical column was a μBondapak C_{18} octadecylsilane column (300 mm × 3.9 mm I.D., 10 μm porous packing, Waters Assoc.). The mobile phase for gradient elution consisted of 85:15 (v/v) methanol–water and a combination of two ion-pair reagents PIC B-7 and B-8 (0.005 *M* heptanesulfonic acid and octane sulfonic acid, Waters Assoc.).

Deoxypyridoxine (DPN, 5 ng) was added to 2 ml of plasma along with 0.2 ml of 50% trichloroacetic acid; the mixture was incubated at 50°C for 15 min and cooled to 5°C. An equal volume of methylene chloride was added to the mixture followed by centrifugation at 7000 *g* for 20 min at 5°C. The supernatant was removed and an equal volume of freon amine was added, followed by recentrifugation, removal of the supernatant and freon amine treatment again with recentrifugation. The supernatant (at 50°C) was dried using nitrogen followed by reconstitution with 1 ml of the PIC B-7–B-8 solution; the sample was adjusted to pH 2.9 and filtered through a 0.2-μm Acrodisc (Gelman) and then through a C_{18} Sep-Pak (Waters Assoc.). The sample filtrate was then injected into the HPLC system. Representative chromatograms of the B_6 vitamers and 4-PA in combined standard solution and in human plasma given in Figs. 1 and 2 have been previously published [4]. Minimum detectable quantities were 2–5 ng. Recoveries of 88 to 97% were obtained when plasma samples were spiked with each of the B_6 vitamers and 4-PA (both independently and as a mixture) before extractions; the coefficients of variation (C.V.) were $< 5\%$.

Urinary 4-PA analyses were conducted utilizing the above detailed HPLC method [4] and the extraction method of Gregory and Kirk [14]. Recoveries of 105–108% were obtained when urine samples were spiked with 4-PA before extraction; the C.V. was $< 5\%$. Urinary creatinine concentrations were also determined (Stanbio kit, Fisher Scientific, Raleigh, NC, USA).

Fig. 1. Representative chromatogram of B_6 vitamers and 4-PA in combined standard solution containing 5 ng of each. The retention times were as follows: 1.0, 1.6, 4.2, 5.4, 14.0, 16.4, 17.3 and 19.4 min for PLP, PNP, 4-PA, PMP, PL, PN, DPN and PM, respectively.

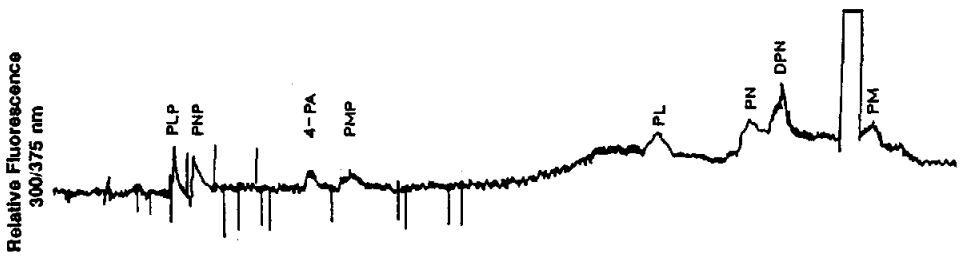

Fig. 2. Representative chromatogram of B_6 vitamers and 4-PA in human plasma. The retention times were as follows: 1.0, 1.6, 4.4, 5.4, 13.8, 16.2, 17.2 and 19.5 min for PLP, PNP, 4-PA, PMP, PL, PN, DPN and PM, respectively.

Statistical analyses

Analysis of variance procedures were used to determine if there were any differences between values obtained by the two 24-h recalls [22]; no differences were found and these data were combined. Pearson *r* coefficients were determined between values obtained by various parameters. Differences and coefficients were considered to be significant at $p < 0.05$. Means and standard deviations (S.D.) were also calculated.

RESULTS AND DISCUSSION

Fifteen obese black women served as subjects; their age ranges were as follows: four females, 27–29 years; five females, 31–37 years; six females, 42–52 years. Anthropometric measurements of the subjects were as follows: height, 159.8 ± 5.0 cm; weight, 85.9 ± 16.0 kg; BMI, 33.5 ± 5.4; desirable body weight, 59.4 ± 4.2 kg; percentage of desirable body weight, 142 ± 26%. The mean heights of the subjects in this study were similar to median values reported for black women in the NHANES II study [16]. These women also were obese in that they had BMI values well above 30 as well as had body weights ≥20% of desirable weights from the 1983 or 1959 Metropolitan Life Insurance Company Tables [19,23].

The hematocrit values of the subjects were 0.40 ± 0.02 (mean ± S.D.). All of the subjects had acceptable [20] hematocrit values.

Dietary measurements

The estimated mean food energy (1585 ± 335 kcal), protein (64.5 ± 15.6 g), and vitamin B_6 intakes (1.21 ± 0.68 mg) (mean ± S.D.) of the black subjects in this study were similar to mean values for white women and higher than mean values for black women included in the Nationwide Food Consumption Survey [24,25]. All but one of the subjects reported consuming less than two thirds of the 1989 Recommended Dietary Allowance (RDA) [26] for protein while eight of the fifteen subjects reported consuming less than two thirds of the 1989 RDA for vitamin B_6. The vitamin B_6 requirement is known to be related to the protein

intake. The RDA for vitamin B_6 is based on a figure of 0.016 mg vitamin B_6 per g protein; the ratio for the subjects was 0.019 ± 0.011 (mean ± S.D.); six of the fifteen subjects reported consuming less than the 0.016 ratio.

Plasma B_6 vitamer measurements

Plasma B_6 vitamer and 4-PA values of the subjects as determined by HPLC procedures are given in Table I. Table II summarizes the plasma B_6 vitamer and 4-PA concentrations reported by others; the sex, age and diets of the subjects are recorded here as seen in the respective publications. The plasma B_6 vitamer and 4-PA concentrations of the middle-aged obese black subjects in the current study are in general agreement with the published values; large S.D. values were found in this study as well as in those reported by others. Little detail was offered regarding the diets consumed by the subjects. One might expect genetic variability with regard to these B_6 vitamer and 4-PA values as well as variability due to dietary intakes of the vitamin.

PLP is the predominant plasma B_6 vitamer in all subjects examined in this study as well as in subjects by other researchers (Table II). Most of the subjects in the present study had PM and 4-PA in their plasma. The majority of the other researchers (Table II) did not give any details as to how many of their subjects had detectable levels of the various B_6 vitamers and 4-PA.

TABLE I

PLASMA B_6 VITAMER AND 4-PA CONCENTRATIONS OF SUBJECTS

Analyte[a]	*n* detectable/ *n* total[b]	Concentration (nmol/l)	
		Mean ± S.D.[c]	Range
PLP	15/15	68.9 ± 12.4	50.6–80.9
PNP	5/15	3.1 ± 4.7	ND–10.8
PMP	4/15	1.2 ± 2.3	ND–7.7
PL	4/15	4.1 ± 7.4	ND–22.1
PN	6/15	3.4 ± 4.5	ND–10.6
PM	9/15	7.2 ± 7.3	ND–17.8
4-PA	6/15	2.0 ± 2.9	ND–9.3

[a] PLP = pyridoxal 5'-phosphate; PNP = pyridoxine 5'-phosphate; PMP = pyridoxamine 5'-phosphate, PL = pyridoxal; PN = pyridoxine; PM = pyridoxamine; 4-PA = 4-pyridoxic acid.

[b] *n* subjects having detectable levels/total *n* subjects.

[c] Non-detectable levels were calculated as zeros.

TABLE II

CONCENTRATIONS OF B_6 VITAMERS AND 4-PA IN HUMAN PLASMA

Research group	*n*	Concentration (mean ± S.D.) (nmol/l)			
		PLP	PNP	PMP	PL
Chauhan and Dakshinamurti [3][a]	27	72 ± 11	–	15 ± 3	251 ± 51
Chrisley *et al.* [4]	22	88 ± 18	6 ± 7	12 ± 27	38 ± 23
Chrisley *et al.* [5]	28	78 ± 19	2 ± 4	1 ± 2	1 ± 3
Driskell and Chrisley [6]	21	78 ± 18	1 ± 3	0.4 ± 1	2 ± 3
Coburn and Mahuren [7]	38	57 ± 26	ND	8 ± 8	23 ± 10
Hollins and Henderson [8]	1	84	9	9	29
	10	61 ± 34	ND	ND	5 ± 9
Liu *et al.* [9]	9	30 ± 17	ND	0.4 ± 0.4	13 ± 5
Lumeng *et al.* [10][b]	6	60 ± 10	–	2 ± 1	15 ± 5
Shephard *et al.* [11]	27	51 ± 25	–	0.4 ± 1	9 ± 7
Shephard *et al.* [12]	10	45 ± 17	–	ND	6 ± 3
Vanderslice *et al.* [13]	2	74 ± 35	ND	31 ± 10	ND
Present study	15	69 ± 13	3±5	1 ± 2	4 ± 7

[a] Serum.

[b] Estimated from figure.

Urinary 4-PA measurements

The urinary 4-PA/creatinine ratio of the subjects from freshly voided random collections was 0.88 ± 0.31 μmol/mmol (mean ± S.D.) with a range of 0.42–1.60. The urinary 4-PA/creatinine values of the women in the present study are similar to values reported for adults by Schuster *et al.* [15] and for women reported by Driskell and Chrisley [6].

Vitamin B_6 status assessment

Several researchers [27–30] have proposed guidelines for plasma PLP levels measured by radioassay which are indicative of inadequate, marginal, or low vitamin B_6 status. Plasma PLP concentrations determined using HPLC techniques have previously been reported [6,31] to be similar to those obtained by radioassay. All of the subjects in the current study had plasma PLP concentrations higher than any of the levels suggested as being indicative of inadequate status.

Urinary 4-PA/creatinine values of all the subjects in the current study exceeded the <0.20 value suggested by Sauberlich *et al.* [32] as being indicative of marginal vitamin B_6 status. The correlation ($r = 0.48$) between plasma PLP levels and the urinary 4-PA/creatinine ratios (μmol/mmol) of the subjects approached significance ($p < 0.07$).

Type subject			
PN	PM	4-PA	
ND	164 ± 38	–	Not given
41 ± 35	18 ± 9	39 ± 54	Men, 20–37 years, fed 0.78–0.98 mg B_6 per day
1 ± 4	4 ± 5	8 ± 13	Females, 12–15 years, 1.48 ± 0.66 mg B_6 per day estimated
1 ± 2	6 ± 4	4 ± 3	Women, 21–27 years, 1.60 ± 0.52 mg B_6 per day estimated
19 ± 33	2 ± 2	49 ± 19	Not given
ND	ND	74	Normal volunteer
ND	ND	25 ± 9	Normal individuals
2 ± 2	1 ± 10	57 ± 20	Healthy adults, 25–45 years
30 ± 10	8 ± 3	40 ± 8	Five men, one woman, 23–38 years
1 ± 2	1 ± 1	29 ± 21	Healthy volunteers, thirteen males, fourteen females, 31 ± 10 years
ND	1 ± 2	38 ± 32	Normal lab volunteers, three males, seven females, 36 ± 10 years
180 ± 51	6 ± 8	–	Column not applicable
3 ± 5	7 ± 7	2 ± 3	Women, 27–52 years, 1.21 ± 68 mg B_6 per day estimated

The middle-aged obese black subjects in the current study had plasma B_6 vitamer and 4-PA values, urinary 4-PA concentrations and estimated vitamin B_6 intakes which were statistically similar to those obtained for white females, 12–15 years [5], and white women, 21–27 years [6], but significantly lower ($p < 0.05$) than plasma values for adult men, 20–37 years [4], except for PNP and PMP (values given in Table II). All of the subjects in these four studies (refs. 4–6 and present study) conducted by our research group had plasma PLP levels and coenzyme stimulation of erythrocyte alanine aminotransferase activities and/or urinary 4-PA/creatinine values indicative of adequate vitamin B_6 status.

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