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Vitamin B_6 status assessment in relation to dietary intake in high school students aged 16–18 years

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The vitamin B_6 status of high school students and its relationship with dietary intake were investigated in this cross-sectional study by face-to-face interview. A total of 157 healthy students aged 16–18 years (eighty-three boys and seventy-four girls) were randomly recruited from two out of nineteen senior high schools in Tainan, Taiwan. Vitamin B_6 intakes were calculated from three 24-h dietary records. Direct and indirect vitamin B_6 status indicators were measured in plasma, erythrocytes and urine. The anthropometric data, being similar to those of the first Nutrition and Health Survey in Taiwan (1993–1996), showed the normal growth and development of these students. All students except one girl (28·7 nmol/l) had plasma pyridoxal-5′-phosphate (PLP) levels >35 nmol/l, indicating an adequate vitamin B_6 status. The mean dietary vitamin B_6 intakes of boys and girls were 1·04 (SD 0·29) and 0·96 (SD 0·27) mg/d, respectively. Vitamin B_6 status indicators, including plasma PLP, erythrocyte alanine aminotransferase activity coefficient (EALT-AC), aspartate aminotransferase activity coefficient (EAST-AC) and urinary 4-pyridoxic acid (4-PA), were correlated with vitamin B_6 intake. Students with adequate values of plasma PLP (>35 nmol/l), EALT-AC ($<1·25$), EAST-AC ($<1·8$) and urinary 4-PA ($>3·0$ μmol/d) had median intakes of 1·08 and 1·01 mg/d, respectively, for boys and girls. This study suggests that vitamin B_6 requirements for boys and girls aged 16–18 years were approximately 1·1 and 1·0 mg/d, respectively.

Vitamin B_6 intake: Nutritional status: High school students

The Dietary Reference Intakes Committee in Taiwan (Wei *et al.* 2003) and in the USA (Institute of Medicine, 1998) extrapolated the vitamin B_6 estimated average requirement and RDA for children and adolescents aged 1–18 years from adult values due to limited information. We found that levels of plasma pyridoxal-5′-phosphate (PLP) and total aldehyde B_6 in 7–12 and 13–18-year-old groups were the lowest in all age groups studied in the first Nutrition and Health Survey in Taiwan 1993–1996 (NAHSIT) (Chang *et al.* 1999). Unfortunately, vitamin B_6 intakes were not analysed due to the lack of dietary information in NAHSIT. Therefore, these age groups were further studied by our laboratory concerning vitamin B_6 requirements because the status of this nutrient is possibly marginal among various population groups. We have reported the vitamin B_6 requirement for children aged 7–12 years (Chang *et al.* 2002) and adolescents aged 13–15 years (Chang *et al.* 2003). The dietary intake, nutritional status and functional consequences of a certain range of intake are suggested in determining nutrient requirements (King, 1996). In the present study, vitamin B_6 status and its relationship to dietary intake of high school students was studied to investigate the requirement. We evaluated the effect of vitamin B_6 intake on adequate vitamin B_6 status indicators in plasma, erythrocytes and urine of high school students determined to be healthy by anthropometric measurements. The median intake of vitamin B_6 was determined from the adequate levels of all status indicators and the requirement was suggested.

Materials and methods

Subjects

Healthy senior high school students (eighty-three boys and seventy-four girls) aged 16–18 years from two out of nineteen high schools in Tainan, the southern part of Taiwan, were randomly recruited in the present study. The experimental procedures were reviewed and approved by the Review Board of the Department of Life Sciences, National Cheng Kung University, Tainan, Taiwan, and explained to the students and parents before submission of the consent form. Informed consent was obtained from both the students and their parents. Students who were not in good health, had any diseases interfering with vitamin B_6 metabolism or were taking medicines and/or supplements altering vitamin B_6 status were not included in this study. A total of 157 students participated and 127 (sixty-five boys and sixty-two girls) completed the study with both anthropometric and biochemical data. Those who withdrew from the study only had anthropometric data. All measurements, including dietary intake, anthropometry

Abbreviations: 4-PA, 4-pyridoxic acid; EALT-AC, erythrocyte alanine activity coefficient; EAST-AC, aspartate aminotransferase activity coefficient; NAHSIT, the first Nutrition and Health Survey in Taiwan; PLP, pyridoxal-5′-phosphate.

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and vitamin B_6 status, were made at the beginning and end of the semester and averaged to determine the data for each student at the age studied.

Dietary intake assessment

Dietary records (3 d; one record was for a weekend day and the other two for weekdays) were obtained with a follow-up by personal interview to minimize uncertainties (Reynolds, 1990). A trained dietary interviewer assisted the students in using the chart of food portion sizes provided by the Department of Health, Executive Yuan, Taiwan. A computer program, Nutritionist IV (N-squared, Salem, OR, USA), was used and foods unique to Taiwan were added into the program to calculate dietary energy and nutrient intakes.

Anthropometric measurements

Anthropometric measurements including height, weight, midarm circumference, tricep skin-fold thickness and percentage body fat, were made by a trained interviewer. Body weight and height were measured with subjects wearing school uniforms but without shoes. Body weight and percentage body fat were measured simultaneously using a body fat monitor/scale (TBF-531; Tanita, Tokyo, Japan) Weight and height measurements were used to calculate BMI (kg/m^2). Midarm muscle circumference was calculated from the midarm circumference. Tricep skin-fold thickness was measured by skin-fold calipers (Lafayette Instrument, Lafayette, IN, USA). Anthropometric data were compared with that of the NAHSIT 1993–1996 (Kao *et al.* 1999) for the appropriate age group.

Sample collection

Venous blood was collected from fasting students in vacutainer tubes containing EDTA between 0800 and 0900 hours on the second day of the 3-d dietary record, kept in crushed ice and protected from light. Blood samples were centrifuged at 3000 ***g*** and 4°C for 10 min. Plasma was removed and aliquots were frozen at −40°C for plasma PLP analyses. Erythrocytes were washed three times with saline and an aliquot of packed cells was removed for assay of erythrocyte alanine activity coefficient (EALT-AC) and aspartate aminotransferase activity coefficient (EAST-AC).

A 24-h urine collection was obtained on the same day of blood sample collection using toluene as preservative. Aliquots of urine were stored at −40°C for urinary 4-pyridoxic acid (4-PA) analyses.

Laboratory analysis

Plasma PLP concentrations were determined by HPLC with fluorometric detection (Kimura *et al.* 1996). The recovery of added PLP from plasma was 102·3 (SD 3·3) %. Within- and between-day reproducibilities were 1·46 and 2·46 %, respectively. EALT-AC and EAST-AC were measured with and without added PLP (Woodring & Storvick, 1970) on the same day that blood was drawn. The EALT-AC and EAST-AC were calculated as the ratio of simulated (PLP added) to unstimulated (no PLP added) activities. Urinary 4-PA was analysed by HPLC with fluorometric detection (Gregory & Kirk, 1979).The recovery of added 4-PA from urine was 90·5 (SD 0·3) % and the 4-PA data were corrected for recovery. Within- and between-day reproducibilities were 4·13 and 4·13 %, respectively.

Statistical analyses

Data were analysed using the SAS statistical analysis computer program (version 6.12; SAS Institute, Cary, NC, USA) and expressed as means and standard deviations unless otherwise stated. The general linear model was performed to determine the differences between group means at the beginning and end of the semester and between boys and girls for daily dietary intakes and vitamin B_6 status measures. One-way ANOVA was used to test the differences among the means of vitamin B_6 intakes of students who had adequate vitamin B_6 status indicators. Pearson correlation coefficients were computed to determine relationships among vitamin B_6 status measures and vitamin B_6 intakes. The level of significance was considered to be $P<0·05$, unless otherwise stated. The percentages of students with adequate plasma PLP, EALT-AC, EAST-AC and urinary 4-PA (Leklem, 1990) were calculated. Median intakes for those who had adequate status of B_6 indicators were determined and used for the suggestion of vitamin B_6 requirement.

Results

Anthropometric data

Most anthropometric data (Table 1) were similar to those of NAHSIT, 1993–1996 (Kao *et al.* 1999), indicating normal growth of the students.

Dietary intakes

Energy and protein intakes of boys were higher than those of girls, but vitamin B_6 intake was similar (Table 2).

Biochemical status of vitamin B_6

Plasma PLP concentration, EALT-AC, EAST-AC and urinary 4-PA excretion measured at the beginning and end of the semester were not significantly different and were averaged to obtain the individual biochemical indicators of vitamin B_6 (Table 3).

Plasma PLP concentrations for all students except one girl (28·7 nmol/l) were >35 nmol/l (Table 4). Adequate plasma PLP concentration >35 nmol/l was used in the present study because a 20 nmol/l of PLP concentration suggested by the dietary reference intakes for adults (Institute of Medicine, 1998) may not reflect normal status for this age group. The percentages of students with adequate vitamin B_6 status evaluated by PLP >35 nmol/l EALT-AC <1·25, EAST-AC <1·8 and urinary 4-PA excretion >3·0 − μmol/d (Leklem, 1990) were quite different among these four indicators in both boys and girls. However, the vitamin B_6 intakes of the students who had adequate values of the different indicators were not different from one another and ranged from 1·04

Table 1. Anthropometric measurements of high school boys and girls aged 16–18 years*†
(Mean values and standard deviations)

	Age		Height (cm)		Body weight (kg)		BMI (kg/m ^{2})		Body fat (%)		TSF (mm)		MAC (cm)		MAMC (mm)	
Sex	Years	*n*	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Boys	16	27	169·93	6·77	63·65	9·67	22·03	2·93	19·27	5·53	13·20	4·41	26·24	2·66	220·92	17·79
	17	28	173·90	7·23	70·27	15·17	23·14	3·99	20·99	6·13	14·97	4·85	27·41	3·12	227·14	20·93
	18	28	171·13	5·21	69·36	10·73	23·76	3·23	20·96	5·57	15·05	5·25	27·81	3·13	230·79	20·88
	Total	83	171·67	6·40	67·81	11·89	22·99	3·39	20·42	5·75	14·42	4·84	27·16	2·97	226·35	19·89
Girls	16	25	160·14	5·01	56·97	13·25	22·08	4·21	28·20	6·88	19·42	5·59	24·74	3·83	186·38	23·24
	17	26	158·09	5·06	53·50	7·06	21·45	3·11	27·81	5·17	19·40	5·16	24·44	2·66	183·48	14·95
	18	23	159·94	5·20	52·39	6·35	20·47	2·20	25·43	4·50	16·28	3·77	23·55	2·46	184·39	16·38
	Total	74	159·36	5·09	54·33	8·93	21·36	3·20	27·20	5·54	18·44	4·87	24·26	2·99	184·74	18·20

* For details of subjects and procedures, see Materials and methods.
† Values measured at the beginning and end of the semester for each student were averaged and used to calculate the mean.
MAC, midarm circumference; MAMC, midarm muscle circumference TSF, triceps skin-fold thickness.

(SD 0·29) to 1·16 (SD 0·26) mg/d in boys and from 0·96 (SD 0·27) to 1·06 (SD 0·27) mg/d in girls.

Correlations among vitamin B_6 status indicators

Vitamin B_6 intake was positively correlated with plasma PLP and urinary 4-PA and negatively correlated with EALT-AC and EAST-AC (Table 5). Plasma PLP was positively correlated with urinary 4-PA and negatively correlated with both EALT-AC and EAST-AC. Urinary 4-PA excretion was also negatively correlated with both EALT-AC and EAST-AC. EALT-AC was positively correlated with EAST-AC.

Vitamin B_6 intake

The median intakes of vitamin B_6 were determined from those who had adequate plasma PLP concentration, EALT-AC, EAST-AC and urinary 4-PA excretion (Table 6). The mean of median intakes of four vitamin B_6 status indicators were 1·08 and 1·01 mg/d for boys and girls, respectively. The vitamin B_6 requirements for high school boys and girls aged 16–18 years were suggested to be approximately 1·1 and 1·0 mg/d, respectively.

Discussion

In the present study, median intakes of vitamin B_6 at 1·08 and 1·01 mg/d for high school boys and girls aged 16–18 years, respectively, were determined on the basis of adequacy of PLP (>35 nmol/l), urinary 4-PA excretion (>3·0 mol/d), EALT-AC (<1·25) and EAST-AC (<1·8). The vitamin B_6 requirements for these boys and girls should be approximately less than 1·1 and 1·0 mg/d, respectively, which are the levels set by the Dietary Reference Intake committee (USA). Monge-Rojas (2001) reported that 50 percentiles of vitamin B_6 intakes from prospective 3-d diet records were 1·3 and 1·2 mg/d for Costa Rican adolescents aged 12–19 years.

In the Navajo Health and Nutrition Survey, mean intakes of vitamin B_6 were reported to be 1·5 (SD 0·1) mg/d obtained from a single 24-h diet recall for both sexes of adolescents aged 12–19 years, whereas the median intakes of vitamin B_6 were 1·0 mg/d and below the RDA (Ballew *et al.* 1997).

Table 2. Daily energy, protein and vitamin B_6 intakes and the B_6:protein ratios of boys and girls aged 16–18 years†
(Mean values and standard deviations)

	Semester					
	Beginning		End		Average‡	
	Mean	SD	Mean	SD	Mean	SD
Boys (*n* 65)						
Energy (kJ/d)	9323	3198	8514	2372	8919	2043
Protein (g/d)	80	30	70	20	75	20
Vitamin B_6 (mg/d)	1·03	0·32	1·04	0·38	1·04	0·29
Vitamin B_6:protein (mg/g)	0·014	0·006	0·017	0·009	0·015	0·005
Girls (*n* 62)						
Energy (kJ/d)	8640	2043	7774	2295	8207*	1932
Protein (g/d)	70	18	63	21	67	15
Vitamin B_6 (mg/d)	0·94	0·35	0·97	0·34	0·95	0·28
Vitamin B_6:protein (mg/g)	0·014	0·006	0·016	0·006	0·015	0·004

Mean values are significantly different from those of the boys; *$P<0·05$.
† For details of subjects and procedures, see Materials and methods.
‡ Average of two values measured at the beginning and end of the semester for each student was used to calculate the mean.

Table 3. Plasma, urinary and erythrocyte vitamin B_6 status measures of boys and girls aged 16–18 years†

(Mean values and standard deviations)

	Semester					
	Beginning		End		Average*	
Vitamin B_6 status indicator	Mean	SD	Mean	SD	Mean	SD
Boys (*n* 65)						
Plasma PLP (nmol/l)	55·5	16·3	60·7	26·8	58·1	17·6
Urinary 4-PA (μmol/d)	6·7	3·4	6·0	3·2	6·3	2·8
EALT-AC	1·05	0·36	0·99	0·29	1·02	0·27
EAST-AC	1·68	0·35	1·63	0·36	1·65	0·29
Girls (*n* 62)						
Plasma PLP (nmol/l)	55·5	24·4	58·8	28·4	57·2	19·3
Urinary 4-PA (μmol/d)	6·6	4·0	5·6	2·3	6·1	2·6
EALT-AC	1·14	0·14	1·08	0·22	1·11	0·13
EAST-AC	1·66	0·39	1·69	0·31	1·68	0·26

* Average of two values measured at the beginning and end of the semester for each student was used to calculate the mean. No significant differences were detected by general linear model.

† For details of subjects and procedures, see Materials and methods.

EALT-AC, erythrocyte alaline aminotransferase activity coefficient; EAST-AC, erythrocyte aspartate aminotransferase activity coefficient; 4-PA, 4-pyridoxic acid; PLP, pyridoxal phosphate.

In the National Health and Nutrition Examination Survey, 1999–2000, mean intakes of vitamin B_6 estimated from one 24-h dietary recall interview were reported to be 2·0 (SD 0·08) and 1·6 (SD 0·14) mg/d for US adolescent boys and girls aged 12–19 years, and the median intakes of vitamin B_6 were 1·8 and 1·3 mg/d, respectively (Ervin *et al.* 2004). In their studies, only vitamin B_6 intake data were provided and the vitamin B_6 nutritional status was not determined. Therefore, the adequacy of vitamin B_6 intake could not be determined without the vitamin B_6 nutritional status from these studies.

Plasma PLP has been suggested to be the best single vitamin B_6 status indicator (Liu *et al.* 1985; Institute of Medicine, 1998) because it appears to reflect tissue stores. Plasma vitamin B_6 concentrations were found to be 199·6 (SD 57·9) and 186·0 (SD 47·1) nmol/l (<150 nmol/l is considered inadequate) in Nigerian adolescent boys and girls aged 16–18 years, respectively, with vitamin B_6 intakes of 1·84 (SD 0·49) and 1·36 (SD 0·23) mg/d, indicating that these values of vitamin B_6 intake may exceed the needs for these adolescents (Korede & Ajayi, 1991).

In the present study, plasma PLP concentration of every student except one girl (28·7 nmol/l) was above 36 and 35 nmol/l for boys and girls, respectively, indicating adequate status and meets the cut-off values of 20 nmol/l for estimated average requirement (Institute of Medicine, 1998), 28·3 nmol/l by Driskell & Moak (1986), 30 nmol/l by Leklem (1990) and 34·4 nmol/l by Rose *et al.* (1976). All of the students in the present study (except one girl) had plasma PLP concentrations higher than any of the levels suggested as being indicative of vitamin B_6 inadequacy by several researchers (Cleary *et al.* 1975; Rose *et al.* 1976; Shultz & Leklem, 1987; Liu *et al.* 1985; Driskell & Moak, 1986; Hunt *et al.* 1987; Institute of Medicine, 1998). The mean intakes of vitamin B_6 for total

Table 4. Percentages and means of vitamin B_6 intake and dietary vitamin B_6:protein ratio of boys and girls with adequate vitamin B_6 status*†

(Mean values and standard deviations)

			B_6 intake (mg/d)		B_6:protein (mg/g)	
Vitamin B_6 status indicator	*n*	%	Mean	SD	Mean	SD
Boys (*n* 65)						
Plasma PLP >35 nmol/l	65	100	1·04	0·29	0·014	0·004
Urinary 4-PA >3·0 μmol/d	62	95	1·05	0·29	0·014	0·004
EALT-AC <1·25	50	77	1·14	0·25	0·015	0·003
EAST-AC <1·8	46	71	1·16	0·26	0·015	0·003
Girls (*n* 62)						
Plasma PLP >35 nmol/l	61	98	0·96	0·27	0·014	0·004
Urinary 4-PA >3·0 μmol/d	58	94	0·97	0·29	0·014	0·004
EALT-AC <1·25	54	87	0·98	0·28	0·015	0·003
EAST-AC <1·8	44	71	1·06	0·27	0·015	0·003

* For details of subjects and procedures, see Materials and methods.

† Based on the average of two values measured at the beginning and end of the semester for each student.

EALT-AC, erythrocyte alanine aminotransferase activity coefficient; EAST-AC, erythrocyte aspartate aminotransferase activity coefficient; 4-PA, 4-pyridoxic acid; PLP, pyridoxal phosphate.

Table 5. Correlations among vitamin B_6 status indicators of high school students aged 16–18 years*†

	B_6 intake	Plasma PLP	Urinary 4-PA *r*	EALT-AC	EAST-AC
B_6 intake					
Plasma PLP	0·821				
Urinary 4-PA	0·723	0·770			
EALT-AC	−0·710	−0·535	−0·582		
EAST-AC	−0·793	−0·694	−0·639	0·058	

* For details of subjects and procedures, see Materials and methods.
† Based on the average of two values measured at the beginning and end of the semester for each student.
Pearson correlation coefficients (r) were all significant ($P<0·001$).
EALT-AC, erythrocyte alanine aminotransferase activity coefficient; EAST-AC, erythrocyte aspartate aminotransferase activity coefficient; 4-PA, 4-pyridoxic acid; PLP, pyridoxal phosphate.

boys and girls were 1·04 (SD 0·29) and 0·96 (SD 0·28) mg/d, respectively, which were similar to the median intakes of 1·08 and 1·01 mg/d as determined by the present study. A total of 95 % boys and 94 % girls had urinary 4-PA excretion of >3·0 μmol/d with vitamin B_6 intakes of 1·05 (SD 0·29) and 0·97 (SD 0·29) mg/d, respectively. These values were also comparable with those evaluated by the adequacy of plasma PLP reported in the present study.

Although urinary 4-PA excretion reflects current intake, it also provides complementary information in assessing vitamin B_6 status. EALT-AC and EAST-AC are commonly used as measures of long-term vitamin B_6 status. EALT-AC <1·25 and EAST-AC <1·8 are indicative of adequate status (Sauberlich *et al.* 1974; Leklem, 1990). In the present study, 23 % boy and 13 % girl students had inadequate B_6 status, indicated by EAST-AC >1·25. Other researchers have reported that teenage females frequently had EAST-AC >1·16 as well as >1·25 (Kirksey *et al.* 1978, Suter *et al.* 1984, Driskell *et al.* 1985, 1987, Driskell & Moak, 1986). For students having adequate EALT-AC (<1·25) and EAST-AC (<1·8), mean dietary vitamin B_6 intakes ranged from 0·98 (SD 0·28) to 1·16 (SD 0·26) mg/d, which were also comparable with those who had adequate plasma PLP.

Driskell *et al.* (1985) estimated the mean daily vitamin B_6 intake from food sources to be 1·20 (SD 0·06) mg for 583 adolescent girls aged 12, 14 and 16 years. Approximately 67 % of these adolescents had adequate vitamin B_6 status as indicated by coenzyme stimulation of erythrocyte alanine aminotransferase activity. The mean coenzyme stimulation of erythrocyte alanine aminotransferase activity and PLP values of the adolescent girls aged 12, 14 and 16 years was 13·5 % and 45·2 nmol/l, with the estimated daily vitamin B_6 intake of 1·25 (SD 0·04) mg (Driskell & Moak, 1986). Coenzyme stimulation values >25 % were observed in 18 % of these adolescents.

In the present study, mean plasma PLP levels of girls, being 57·2 (SD 19·3) nmol/l with mean B_6 intake of 0·95 (SD 0·28) mg/d, were comparable with 78·1 (SD 19·3) nmol/l plasma PLP for 1·48 mg/d B_6 intake in adolescent females reported by Chrisley *et al.* (1991) using HPLC, whereas Driskell & Moak (1986) used stimulation of tyrosine decarboxylase (L-tyrosine carboxylase) apoenzyme.

Recently, plasma PLP, urinary 4-PA, at least one indirect measure and the intakes of vitamin B_6 and protein have been recommended for proper assessment of vitamin B_6 status (Leklem, 1990). The present study determined the median intakes of vitamin B_6 by using a combination of plasma PLP, urinary 4-PA, EALT-AC and EAST-AC and derived the suggestion for the vitamin B_6 requirement. Vitamin B_6 intake and status indicators were correlated in the present study. In addition, direct biomarkers of vitamin B_6 intake (plasma PLP and urinary 4-PA excretion) were significantly related to functional indicators (EALT-AC and EAST-AC). Therefore, vitamin B_6 intakes resulting in the adequacies of these direct and functional indicators were used to suggest the vitamin B_6 requirement of high school students aged 16–18 years in the present study.

In conclusion, the median intakes of vitamin B_6 for senior high school boys and girls were determined on the basis of adequate levels of plasma PLP, urinary 4-PA, EALT-AC and EAST-AC. We combined the four indicators and suggested that the vitamin B_6 requirements were approximately 1·1 and 1·0 mg/d for high school boys and girls aged 16–18 years, respectively.

Table 6. Median intakes of vitamin B_6 for boys and girls who had adequate vitamin B_6 status indicators*

	Median intake† (mg/d)	
Vitamin B_6 status indicator	Boys	Girls
Plasma PLP >35 nmol/l	1·04	0·97
Urinary 4-PA >3·0 μmol/d	1·05	0·99
EALT-AC <1·25	1·11	0·99
EAST-AC <1·8	1·13	1·08
Mean	1·08	1·01

* For details of subjects and procedures, see Materials and methods.
† Based on the average of two values measured at the beginning and end of the semester for each student.
EALT-AC, erythrocyte alanine aminotransferase activity coefficient; EAST-AC, erythrocyte aspartate aminotransferase activity coefficient; 4-PA, 4-pyridoxic acid; PLP, pyridoxal phosphate.

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