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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.

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

| Vitamin $B_6$ status indicator | Median intake† (mg/d) 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.

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.

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