Assessment of Vitamin B-6 Estimated Average Requirement and Recommended Dietary Allowance for Adolescents Aged 13–15 Years Using Vitamin B-6 Intake, Nutritional Status and Anthropometry[1]

(Manuscript received 30 January 2003. Initial review completed 19 March 2003. Revision accepted 23 July 2003.)

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ABSTRACT Because of limited available information, the Estimated Average Requirement (EAR) and Recommended Dietary Allowance (RDA) of vitamin B-6 for adolescents were recently estimated by extrapolation from adult values. To determine vitamin B-6 requirements and to provide recommendations for intakes, vitamin B-6 intake, nutritional status and anthropometry were studied in 134 healthy adolescents (63 boys and 71 girls) aged 13–15 y in Tainan, Taiwan. Direct and indirect vitamin B-6 indicators were measured in plasma, erythrocytes and urine. The anthropometric data of the adolescents in this study were similar to those of the first Nutrition and Health Survey in Taiwan (NAHSIT), conducted from 1993 to 1996, showing the normal growth and development of this adolescent group. All subjects had plasma pyridoxal-5′-phosphate (PLP) concentrations ≥ 20 nmol/L, indicating an adequate vitamin B-6 status. The mean dietary vitamin B-6 intakes of boys and girls were 1.04 ± 0.24 and 0.83 ± 0.26 mg/d, respectively. Vitamin B-6 status indicators, including plasma PLP, erythrocyte alanine activity coefficient (EALT-AC), aspartate aminotransferase activity coefficient (EAST-AC) and urinary 4-pyridoxic acid (4-PA), were correlated with vitamin B-6 intake (r = 0.84, −0.84, −0.77 and 0.86, respectively, $P < 0.01$). Adequate values of plasma PLP (≥20 nmol/L), EALT-AC (<1.25), EAST-AC (<1.8) and urinary 4-PA (>3.0 μmol/d) were used to determine the EAR according to the Dietary Reference Intake committee methodology. The present study suggests that vitamin B-6 EAR (RDA) for adolescent boys and girls aged 13–15 y are 1.07 (1.28) and 0.90 (1.08) mg/d, respectively. J. Nutr. 133: 3191–3194, 2003.

KEY WORDS: • vitamin B-6 • nutritional status • Estimated Average Requirement • Recommended Dietary Allowance • adolescent

In 1998, because of limited information, the Dietary Reference Intake (DRI)[3] committee extrapolated the vitamin B-6 Estimated Average Requirement (EAR) and Recommended Dietary Allowance (RDA) for children and adolescents aged 1–18 y from adult values (1). The results from the first Nutrition and Health Survey in Taiwan 1993–1996 (NAHSIT) showed that mean concentrations of plasma pyridoxal-5′-phosphate (PLP) and total aldehyde B-6 are lowest for children in the 7–12- and 13–18-y-old groups (2). Therefore, these age groups were selected for the study of vitamin B-6 requirements because the status of this nutrient is possibly marginal among various population groups. We have reported the vitamin B-6 EAR and RDA for children aged 7–12 y (3), which are comparable with those of the DRI committee (1). Here, the EAR and RDA of vitamin B-6 for adolescents aged 13–15 y were studied continuously in Taiwan and those for the 16–18-y age group are currently ongoing in our laboratory. The study of dietary intake, nutritional status and functional consequences of a certain range of intake is essential in determining nutrient requirements (4). This study was undertaken to determine the recommendation of vitamin B-6 intake for adolescents aged 13–15 y. We evaluated the effect of vitamin B-6 intake on adequate vitamin B-6 status indicators in plasma, erythrocyte and urine of adolescents determined by anthropometric measurements to be healthy. The EAR was determined and the RDA was calculated according to the DRI committee methodology (1).

SUBJECTS AND METHODS

Subjects. Healthy adolescents from 2 out of 27 public junior middle schools in Tainan, the southern part of Taiwan, voluntarily participated in this study during the fall of 2000. 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 the study. A total of 134 adolescents participated and 103 (45 boys and 58 girls) completed the study. The rest of the adolescents withdrew and had only anthropometric measurements taken. The study protocol including the use of human subjects was approved by the Department of Biology, National Cheng Kung University, Tainan, Taiwan, and was explained to the subjects' parents before submission of informed consent. All measurements, including dietary intake, anthropometry and vitamin B-6

[1] Supported in part by the Department of Health, (DOH89-TD-1055) and the National Science Council (NSC89–2320-B-006–114), Taiwan.

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[3] Abbreviations used: DRI, Dietary Reference Intake; E-ALAT, erythrocyte alanine aminotransferase; EALT-AC, erythrocyte alanine aminotransferase activity coefficient; EAR, Estimated Average Requirement; EAST-TC, erythrocyte aspartate aminotransferase activity coefficient; 4-PA, 4-pyridoxic acid; MAC, midarm circumference; NAHSIT, Nutrition and Health Survey in Taiwan; PLP, pyridoxal-5′-phosphate; RDA, Recommended Dietary Allowance; TSF, tricep skindfold thickness.

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status, were made at the beginning and end of the semester and averaged to determine the semester data for each subject.

Dietary intake assessment. A trained dietary interviewer obtained food intake information from the subjects using three 24-h dietary recalls. One recall was for a weekend day and the other two for weekdays. A computer program, Nutritional IV (N-squared, Salem, OR), was used to calculate the daily energy and nutrient intakes.

Anthropometric measurements. Anthropometric measurements including height, weight, midarm circumference (MAC), tricep skinfold thickness (TSF) and the percentage of 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 of body fat were measured simultaneously using a Body Fat Monitor/Scale (TBF-531; Tanita, Tokyo, Japan). Weight and height measurements were used to calculate the BMI as weight/height^2 (kg/m^2). Midarm muscle circumference was calculated from the MAC. TSF was measured by skinfold calipers (Lafayette Instrument, Lafayette, IN). Anthropometric data were compared with that of the NAHSIT 1993–1996 (5) for the appropriate age group.

Sample collection. Venous blood was collected from fasting subjects in vacutainer tubes containing EDTA between 0800 and 0900 h, 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) according to the method of Woodring and Storvick (6). A 24-h urine collection was obtained on the same day of blood sample collection using toluene as a 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 (7). The recovery (mean ± SD) of added PLP from plasma was 102.3 ± 3.3%. Within- and between-day reproducibilities were 1.46 and 2.46%, respectively. Erythrocyte alanine and aspartate aminotransferase activities were measured with and without added PLP (6) on the same day blood was drawn. The EALT-AC and EAST-AC were calculated as the ratio of stimulated (PLP added) to unstimulated (no PLP added) activities. Urinary 4-PA was analyzed by HPLC with fluorometric detection (8). The recovery (mean ± SD) of added 4-PA from urine was 90.5 ± 0.3%. Within- and between-day reproducibilities were 4.13 and 4.13%, respectively.

Statistical analyses. Data were analyzed using SAS statistical analysis computer program (version 6.12; SAS Institute, Cary, NC) and expressed as means ± SD 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 adolescents 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 $P < 0.01$. The percentages of subjects with adequate plasma PLP, EALT-AC, EAST-AC and urinary 4-PA were calculated (9). Adequate status indicators were used to calculate estimates and 95% CI of vitamin B-6 EAR (1,9). The RDA was calculated using the formula RDA = 1.2 × EAR, which assumes an EAR CV of 10% (1).

RESULTS

Anthropometric measurement. Anthropometric measurements were similar to those of NAHSIT, 1993–1996 (5), indicating that the growth of adolescents was within normal ranges and that the subjects were healthy.

Dietary intake. The majority of adolescents reported that these three 24-h dietary recalls were typical of their usual intakes. Energy, protein and vitamin B-6 intakes did not differ between boys and girls or from the beginning to the end of the semester.

Biochemical status of vitamin B-6. Plasma PLP concentrations, EALT-AC, EAST-AC and urinary 4-PA excretions did not differ between boys and girls or from the beginning to the end of the semester. Plasma PLP concentrations for all adolescents were ≥20 nmol/L **(Table 1)**, indicating an adequate vitamin B-6 status (9) and meeting the cutoff of 20 nmol/L for EAR (1). Mean intakes of vitamin B-6 for the adolescents, who had adequate vitamin B-6 status evaluated by EALT-AC < 1.25, EAST-AC < 1.8 and urinary 4-PA excretion ≥3.0 μmol/d (9), were similar among boys or girls, although the percentages for those having adequate status indicator values were different.

Correlations among vitamin B-6 status indicators. Vitamin B-6 intake was positively correlated with plasma PLP (r = 0.84) and urinary 4-PA (r = 0.86), and negatively correlated with EALT-AC (r = −0.84) and EAST-AC (r = −0.77). Plasma PLP was positively correlated with urinary 4-PA (r = 0.82) and negatively correlated with both EALT-AC (r = −0.65) and EAST-AC (r = −0.59). Urinary 4-PA excretion was also negatively correlated with both EALT-AC (r = −0.74) and EAST-AC (r = −0.61). EAST-AC was positively correlated with EALT-AC (r = 0.66).

Vitamin B-6 requirement. The EAR of vitamin B-6 was calculated from the daily intake of one-half of the healthy adolescents who had adequate plasma PLP concentrations, EALT-AC, EAST-AC and urinary 4-PA excretions **(Table 2)**. The mean intake of four vitamin B-6 status indicators resulted in an EAR of 1.07 and 0.90 mg/d for boys and girls, respectively. The RDA of 1.28 and 1.08 mg/d for boys and girls, respectively, were calculated according to the DRI committee methodology (1).

DISCUSSION

In this study, a vitamin B-6 EAR of 1.07 and 0.90 mg/d for adolescent boys and girls aged 13–15 y, respectively, was first determined on the basis of the adequacy of plasma PLP (≥20 nmol/L), urinary 4-PA excretion (>3.0 μmol/d), EALT-AC

TABLE 1

Vitamin B-6 intakes and the dietary vitamin B-6 to protein ratios of boys and girls (aged 13–15 y) of adequate vitamin B-6 status[1]

Vitamin B-6 status indicator	*n*	%	B-6 intake	B-6/protein
			mg/d	*mg/g*
			Boys (n = 45)	
Plasma PLP[2] ≥ 20 nmol/L	45	100	1.04 ± 0.24	0.013 ± 0.005
Urinary 4-PA ≥ 3.0 μmol/d	43	96	1.06 ± 0.24	0.013 ± 0.005
EALT-AC < 1.25	38	84	1.10 ± 0.21	0.014 ± 0.004
EAST-AC < 1.8	38	84	1.10 ± 0.21	0.014 ± 0.004
			Girls (n = 58)	
Plasma PLP ≥ 20 nmol/L	58	100	0.83 ± 0.26	0.014 ± 0.004
Urinary 4-PA ≥ 3.0 μmol/d	48	83	0.87 ± 0.26	0.014 ± 0.004
EALT-AC < 1.25	32	55	0.97 ± 0.26	0.015 ± 0.003
EAST-AC < 1.8	28	48	1.00 ± 0.26	0.015 ± 0.003

[1] Values are means ± SD.

[2] Abbreviations: EALT-AC, erythrocyte alanine aminotransferase activity coefficient; EAST-TC, erythrocyte aspartate aminotransferase activity coefficient; 4-PA, 4-pyridoxic acid; PLP, pyridoxal-5′-phosphate.

TABLE 2

Vitamin B-6 requirement calculated by daily intake of one-half of the healthy boys and girls (aged 13–15 y) with adequate vitamin B-6 status indicators

Vitamin B-6 status indicator	Boys (EAR)[1]	Girls (EAR)
	mg/d (95% CI)	
Plasma PLP ≥ 20 nmol/L	1.03 (0.97–1.11)	0.77 (0.76–0.90)
Urinary 4-PA ≥ 3.0 μmol/d	1.06 (0.99–1.13)	0.83 (0.80–0.94)
EALT-AC < 1.25	1.10 (1.03–1.17)	0.94 (0.88–1.06)
EAST-AC < 1.8	1.10 (1.03–1.17)	1.07 (0.90–1.10)
Mean	1.07	0.90

[1] Abbreviations: EALT-AC, erythrocyte alanine aminotransferase activity coefficient; EAR, Estimated Average Requirement; EAST-TC, erythrocyte aspartate aminotransferase activity coefficient; 4-PA, 4-pyridoxic acid; PLP, pyridoxal-5′-phosphate.

(<1.25) and EAST-AC (<1.8). The vitamin B-6 RDA of 1.28 and 1.08 mg/d for adolescent boys and girls aged 13–15 y, respectively, was calculated from the EAR according to the DRI committee methodology (1). These values are comparable with those of the DRI committee, which determined a vitamin B-6 EAR (RDA) of 1.1 (1.3) and 1.0 (1.2) mg/d for adolescent boys and girls aged 14–18 y. These values were extrapolated from adult values because of the limited information on adolescents (1).

Recently, Monge-Rojas 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 y (10). Robson et al. (11) used the diet history method and indicated the median intakes of vitamin B-6 for adolescents aged 12 and 15 y were 1.45 and 1.86 mg/d for males ($n = 225$) and 1.28 and 1.29 mg/d for females ($n = 230$). Mean intakes of vitamin B-6 were reported to be 1.5 ± 0.1 mg/d for adolescents aged 12–19 y (12). 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 the vitamin B-6 intake couldn't 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 (1,13) because it appears to reflect tissue stores. Plasma PLP concentrations were found to be 190.5 ± 45.8 and 194.6 ± 39.6 nmol/L (<150 nmol/L is considered inadequate) in Nigerian adolescent boys and girls aged 13–15 y, respectively, with vitamin B-6 intakes of 1.6 ± 0.42 and 1.55 ± 0.36 mg/d, indicating that these values may exceed the needs for these adolescents (14). In the present study, plasma PLP concentration of every adolescent was above 20 nmol/L, indicating adequate status (9). The mean intakes of vitamin B-6 for total boys and girls were 1.04 ± 0.24 and 0.83 ± 0.26 mg/d, respectively, which were similar to EAR of 1.07 and 0.90 mg/d determined by four vitamin B-6 status indicators. The protein intake for the adolescents was similar to the Recommended Daily Nutrient Allowances (65 and 60 g/d) for boys and girls of this age group (15). In addition, the vitamin B-6 intake was reflected on the plasma PLP, which was indicated by a positive correlation between vitamin B-6 intake and plasma PLP ($r = 0.84$, $P < 0.01$). In combination with the finding of adequate plasma PLP (>20 nmol/L) for every adolescent in the present study, mean vitamin B-6 intakes of 1.04 ± 0.24 and 0.83 ± 0.26 mg/d for boys and girls, respectively, were considered to be adequate.

Urinary 4-PA excretion reflects vitamin B-6 intake (13) and is a short-term indicator of vitamin B-6 status, considered to be adequate at >3.0 μmol/d (9). In the present study, 96% of boys and 83% of girls had urinary 4-PA excretion of >3.0 μmol/d with vitamin B-6 intakes of 1.06 ± 0.24 and 0.87 ± 0.26 mg/d, respectively. These values were also comparable with those evaluated by the adequacy of plasma PLP reported in this 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. For subjects having adequate EALT-AC and EAST-AC, mean dietary vitamin B-6 intakes ranged from 0.97 ± 0.26 to 1.10 ± 0.21 mg/d, which were also comparable with those that had adequate plasma PLP. Driskell et al. (16) estimated the mean daily vitamin B-6 intake from food sources to be 1.20 ± 0.06 mg for 583 adolescent girls aged 12, 14 and 16 y. Approximately 67% of these adolescents had adequate vitamin B-6 status as indicated by coenzyme stimulation of erythrocyte alanine aminotransferase (E-ALAT) activity. The mean coenzyme stimulation of E-ALAT activity and PLP values of the adolescent girls aged 12, 14 and 16 y was 13.5% and 45.2 nmol/L, with the estimated daily vitamin B-6 intake of 1.25 ± 0.04 mg (17). Coenzyme stimulation values > 25% were observed in 18% of these adolescents. Kirksey et al. (18) reported that the mean daily intake of vitamin B-6 was 1.24 ± 0.07 mg for adolescent girls aged 12 and 14 y. Based on the stimulation of E-ALAT at ≥1.25 as an indicator of vitamin B-6 inadequacy, 13% of these adolescent girls were judged to have poor status. The information from these studies was limited to determining the EAR for the adolescents. 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 (9). The present study determined the vitamin B-6 EAR by using a combination of plasma PLP, urinary 4-PA, EALT-AC, EAST-AC and the intake of vitamin B-6.

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 determine the vitamin B-6 requirement of adolescents aged 13–15 y in this study.

In conclusion, EAR and RDA were calculated on the basis of adequate values of plasma PLP, urinary 4-PA, EALT-AC and EAST-AC using the DRI committee methodology (1). We combined the four indicators and determined an EAR of 1.07 and 0.90, and RDA of 1.28 and 1.08. mg/d for adolescent boys and girls aged 13–15 y, respectively.

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