Vitamin B-6 Inadequacy Is Prevalent in Rural and Urban Indonesian Children

Budi Setiawan, David W. Giraud and Judy A. Driskell

Department of Nutritional Science and Dietetics, University of Nebraska, Lincoln, NE 68583

ABSTRACT The vitamin B-6 status of Indonesian children was evaluated by determining their dietary vitamin B-6 intakes, erythrocyte alanine aminotransferase activity coefficients and plasma pyridoxal phosphate (PLP) concentrations. Thirty-eight third-grade elementary school children (ages 8–9 y) in rural and 39 in urban areas of Bogor, West Java, Indonesia, voluntarily served as subjects. The subjects included 39 male and 38 female students. The mean vitamin B-6 intake of the subjects was 0.57 mg/d. Fifty-five percent of the children reported consuming <0.5 mg/d of vitamin B-6 (the 1998 Estimated Average Requirement for those 4–8 y). Erythrocyte alanine aminotransferase activity coefficients >1.25 were observed in 30%, and plasma PLP concentrations <30 nmol/L were observed in 25%; these values are considered indicative of vitamin B-6 inadequacy. Similar percentages of male and female subjects had inadequate vitamin B-6 status. Significantly more (P < 0.05) rural children than urban had inadequate vitamin B-6 status as assessed by the three indices. Vitamin B-6 inadequacy was found to be prevalent among these Indonesian children, especially those living in rural areas.


KEY WORDS: vitamin B-6 status • alanine aminotransferase • plasma PLP • Indonesian children

Nutritional deficiencies are frequently observed in children in developing countries (Pollitt 1990). Food and nutrition intervention programs often are designed to help eliminate predominant nutrition problems. A food supplementation program was implemented in 1996 in Indonesia (Syarief et al. 1996) for this reason. Information regarding the vitamin B-6 status of Indonesian children does not exist, nor are data available on their intakes of the vitamin.

Currently, no single index of vitamin B-6 status is recommended. Most investigators utilize two or more assessment indices in their studies (Driskell 1994, Leklem 1990). Reynolds (1990) indicates that the most fundamental, but not necessarily the best, index of vitamin B-6 status of an individual may be the amount of the vitamin in the typically consumed diet (Reynolds 1990). Erythrocyte alanine aminotransferase (EALAT) activity coefficient is frequently utilized in the assessment of vitamin B-6 status and is considered to be a long-term status indicator because of the life span of erythrocytes (Driskell 1994, Leklem 1990). Plasma pyridoxal phosphate (PLP) level has been the most acceptable and most widely used vitamin B-6 status index in the last decade (Driskell 1994). Leklem (1990) suggested PLP as an appropriate additional index of vitamin B-6 status, because it is the primary form of vitamin B-6 and it crosses all membranes under postprandial conditions. In humans, plasma PLP concentration has been correlated with dietary vitamin B-6 intake (Leklem 1991).

The objectives of the present study were to evaluate the vitamin B-6 status of third-grade elementary school children (ages 8–9 y) in Bogor, West Java, Indonesia, by three commonly used status criteria: vitamin B-6 dietary intake, EALAT activity coefficient and plasma PLP concentration. Comparisons were made among data obtained by the three methodologies as well as between genders and rural vs. urban residential areas.

MATERIALS AND METHODS

Study sites. The study was conducted in elementary schools located in rural and urban residential areas in Bogor, West Java, Indonesia, during Fall 1997. The study was approved by the Institute of Research of Institut Pertanian Bogor (Bogor Agricultural University) in Indonesia and the Institutional Review Board of the University of Nebraska (Lincoln, NE).

Subjects. Thirty-eight third-grade elementary school children in rural and 39 in urban areas voluntarily participated in this study. The subjects included male and female students. These subjects came from three rural and two urban randomly selected schools in Bogor, Indonesia; all students eligible volunteered as subjects. Informed consent was obtained from both the students and their parents. Subjects were measured for height and weight while wearing light clothing but no shoes (Gibson 1990). Their parents were asked if they perceived that their children were in good health; also, the parents were asked whether their children took medications or supplements; students taking these were not included in the study. Demographic informa-
tion (number of children, household size, father’s occupation, educational level of each parent and family income) was also obtained from the parents.

Dietary intake assessments. A trained dietary interviewer obtained food intake information from the subjects via two 24-h food recalls. One recall was for a weekday and the other, a weekend day. Food models were used in estimating portion sizes (Gibson 1990). A pilot study was conducted by observing 12 students characteristic of the study population while they ate a meal, and the next day a trained dietary interviewer had these students recall what and how much they ate at this meal. Basically, these students accurately described what they had eaten. The decision was made to interview the children to obtain dietary intake information. The dietary energy and protein intakes were calculated using the Indonesian Food Composition Table (Departemen Kesehatan Republik Indonesia 1995). The dietary vitamin B-6 intakes were calculated using analyzed vitamin B-6 values (Setiawan et al. 1999). The estimated daily energy and protein intakes were compared with the Indonesian Recommended Dietary Allowances (RDA) for the appropriate age group (Muhilal et al. 1996) and the Indonesian Estimated Average Requirements (EAR) intended for use in the United States and Canada (Institute of Medicine 1998).

Blood collection. Venous blood (−20 mL) was collected from fasting subjects in two EDTA-containing vacutainer tubes by a qualified phlebotomist between 0800 and 1000 h. The samples were kept in crushed ice and protected from light. Blood samples were centrifuged at 3000 × g and 5°C for 10 min. Plasma was frozen at −20°C for plasma PLP analyses. Erythrocytes were treated as described by Heddle et al. (1963) utilizing saline and phosphate buffer, and frozen at −20°C for EALAT analyses (Driskell and Moak 1986).

EALAT and plasma PLP analyses. EALAT activities were determined by the method of Tonhazy et al. (1950) as modified by Heddle et al. (1963) utilizing saline and phosphate buffer, and frozen at −20°C for EALAT analyses (Driskell and Moak 1986). Erythrocyte hemolysates were spiked with pyruvate and alanine prior to analyses. Pilot studies indicated that no loss in enzymatic activity was observed when aliquots of 0.5 mL of hemolysates were frozen for 1 mo. Plasma PLP concentrations were measured using radioenzymatic assay by the method described by Chabner and Livingston (1970) as modified by Fries et al. (1981). This method is based on the measurement of 14CO2 evolved during the decarboxylation of L-tyrosine-1-14C by PLP-dependent tyrosine aminotransferase, EC 4.1.1.25, using a Scintillation Analyzer (Tri-Carb Liquid Scintillation Analyzer, Model 1900 TR; Packard Instrument Company, Meriden, CT). A recovery of 94% was obtained when plasma samples were spiked with PLP before analyses. Pilot studies indicated that no loss of plasma PLP concentration occurred when samples were frozen for 1 mo.

Statistical analyses. Height and weight values were converted to height-for-age, weight-for-age and weight-for-height Z-scores (WHO 1983). Data were analyzed using the general linear model, Duncan’s multiple range test, Chi-square and Pearson correlation procedures of SAS (version 6.12; SAS Institute, Cary, NC). Differences were considered significant at $P < 0.05$. The percentages of subjects having low (Z-scores of −2) height-for-age (stunted), weight-for-age (underweight) and weight-for-height (wasted) values (WHO 1995) were calculated.

TABLE 1

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Height-for-age</th>
<th>Weight-for-age</th>
<th>Weight-for-height</th>
<th>Stunted2</th>
<th>Underweight2</th>
<th>Wasted2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rural females</td>
<td>18</td>
<td>–1.38 ± 0.57ab</td>
<td>–1.31 ± 0.59a</td>
<td>–0.43 ± 0.71a</td>
<td>11</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>Rural males</td>
<td>20</td>
<td>–1.67 ± 0.84a</td>
<td>–1.52 ± 0.80a</td>
<td>–0.48 ± 0.79a</td>
<td>0</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>Urban females</td>
<td>20</td>
<td>–0.83 ± 0.88bc</td>
<td>–1.03 ± 0.86b</td>
<td>–0.53 ± 1.02b</td>
<td>15</td>
<td>16</td>
<td>5</td>
</tr>
<tr>
<td>Urban males</td>
<td>19</td>
<td>–0.46 ± 1.16bc</td>
<td>0.01 ± 1.40b</td>
<td>0.57 ± 1.76b</td>
<td>11</td>
<td>16</td>
<td>5</td>
</tr>
<tr>
<td>All rural subjects</td>
<td>38</td>
<td>–1.53 ± 0.73a</td>
<td>–1.42 ± 0.71a</td>
<td>–0.46 ± 0.74</td>
<td>7</td>
<td>16</td>
<td>3</td>
</tr>
<tr>
<td>All urban subjects</td>
<td>39</td>
<td>–0.65 ± 1.03b</td>
<td>–0.53 ± 1.25b</td>
<td>0.01 ± 1.52</td>
<td>13</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>All females</td>
<td>38</td>
<td>–1.09 ± 0.79</td>
<td>–1.16 ± 0.75</td>
<td>–0.48 ± 0.88</td>
<td>13</td>
<td>13</td>
<td>3</td>
</tr>
<tr>
<td>All males</td>
<td>39</td>
<td>–1.08 ± 1.17</td>
<td>–0.78 ± 1.36</td>
<td>0.03 ± 1.44</td>
<td>5</td>
<td>16</td>
<td>5</td>
</tr>
<tr>
<td>All subjects</td>
<td>77</td>
<td>–1.09 ± 0.99</td>
<td>–0.97 ± 1.11</td>
<td>–0.22 ± 1.21</td>
<td>6</td>
<td>14</td>
<td>4</td>
</tr>
</tbody>
</table>

1 Means ± sd, ab Values with the same superscript in the column in each subgrouping were not significantly different, $P > 0.05$.
2 Using −2 Z-score definitions (WHO 1995).

RESULTS

Demographic information. The urban groups had significantly lower ($P < 0.05$) numbers of children in the family and more fathers having professional rather than laborer positions, greater family incomes and higher levels of fathers’ and mothers’ education than the rural groups. However, household sizes of rural and urban groups were not different ($P > 0.05$). In the urban groups, most of the households had two children (62%), fathers worked as professionals (74%) and the level of fathers’ education (51%) and mothers’ education (49%) were senior high school graduate. In contrast, in rural groups the highest percentage (32%) had five children, fathers worked as laborers (50%) and the levels of fathers’ education (68%) and mothers’ education (82%) were elementary school graduates. The males were 103.6 ± 3.4 mo old and the females, 102.4 ± 3.8 mo (means ± sd).

Anthropometric measurements. The height-for-age, weight-for-age and weight-for-height Z-scores of the four groups of subjects were significantly different ($P < 0.0005$)(Table 1); however, these scores did not differ ($P > 0.05$) due to residential area (rural vs. urban) or gender. Between 0 and 17% of the subjects in each group had Z-score values indicative of their being stunted, underweight or wasted. Height-for-age and weight-for-age Z-scores ($r = 0.76, P < 0.0001$), and weight-for-age and weight-for-height Z-scores ($r = 0.78, P < 0.0001$) were correlated.

Dietary intake measurements. Dietary information obtained from the weekday 24-h recall was not significantly different ($P > 0.05$) from that of the weekend, so the data were combined. The majority of the subjects also reported that these were typical of their usual intakes. There were significant
The mean vitamin B-6 contributions by food groups utilized in Indonesia (Departemen Kesehatan Republik Indonesia 1995) indicated that subjects in urban areas received significantly more (P < 0.05) of the vitamin from meat, milk and fruit groups and not starch, fish, egg, legume and vegetable groups (Table 3).

**EALAT and plasma PLP measurements.** EALAT activity coefficients did not differ (P ≥ 0.05) between residential areas or genders (Table 4). However, the mean EALAT activity coefficient of rural females was significantly higher (P < 0.05) than that of urban females.

Plasma PLP concentrations of the subjects were not significantly different (P ≥ 0.05) due to residential area or gender. However, the plasma PLP concentration of rural females was significantly lower (P < 0.05) than those of other groups.

Plasma PLP concentrations and EALAT activity coefficients were negatively correlated (r = -0.54, P < 0.0001). A negative correlation (r = -0.47, P < 0.0001) was observed between daily vitamin B-6 intakes and EALAT activity coefficients. Daily vitamin B-6 intakes and plasma PLP concentrations were positively correlated (r = 0.54, P < 0.0001).

**DISCUSSION**

The socioeconomic status of the parents of the urban subjects was generally higher than that of the parents of the rural subjects. The height and weight values of the subjects in the current study, particularly the urban groups, were comparable to the standards for height and weight for 7–9- y-old healthy Indonesian children of 120 cm and 24 kg (Muhifal et al. 1994). Anthropometric indices, expressed in terms of Z-scores, should be used in evaluating anthropometric data from less industrialized countries (WHO 1995). This method measures the deviation of anthropometric measurements from reference medians in terms of Z-scores. Mean Z-score values for height-for-age, weight-for-age and weight-for-height of the children in the current study were indicative of normalcy; however, some of the subjects did have Z-score values (WHO 1995) indicative of their being stunted, underweight or wasted. The vast majority of Indonesian children that served as subjects in the current study were not malnourished as most of these Z-score values were within two standard deviations of the median for well-nourished populations. However, the prevalence of stunting, underweight and wasting may have increased due to the recent monetary crisis in Indonesia. The anthropometric measurements presented in the current study may be useful in the establishment of normal ranges for Indonesian children.

The Indonesian RDA for energy and protein for children 7–9- y-old are 7.95 MJ/d and 37 g/d, respectively (Lembaga Ilmu Pengetahuan Indonesia 1999). Nutrient intakes below recommended levels do not necessarily indicate that the intakes are inadequate to meet the individual’s requirements. However, it is appropriate to consider levels well below the RDA to indicate risks to dietary adequacy; the existence of deficiencies must be confirmed or rejected on the basis of differences (P < 0.05) between residential areas (rural vs. urban) in food energy, protein and vitamin B-6 intakes (Table 2). However, intakes by gender did not differ (P ≥ 0.05).

The mean vitamin B-6 contributions by food groups utilized in Indonesia (Departemen Kesehatan Republik Indonesia 1995) indicated that subjects in urban areas received significantly more (P < 0.05) of the vitamin from meat, milk and fruit groups and not starch, fish, egg, legume and vegetable groups (Table 3).

**EALAT and plasma PLP measurements.** EALAT activity coefficients did not differ (P ≥ 0.05) between residential areas or genders (Table 4). However, the mean EALAT activity coefficient of rural females was significantly higher (P < 0.05) than that of urban females.

Plasma PLP concentrations of the subjects were not significantly different (P ≥ 0.05) due to residential area or gender. However, the plasma PLP concentration of rural females was significantly lower (P < 0.05) than those of other groups.

Plasma PLP concentrations and EALAT activity coefficients were negatively correlated (r = -0.54, P < 0.0001). A negative correlation (r = -0.47, P < 0.0001) was observed between daily vitamin B-6 intakes and EALAT activity coefficients. Daily vitamin B-6 intakes and plasma PLP concentrations were positively correlated (r = 0.54, P < 0.0001).

**DISCUSSION**

The socioeconomic status of the parents of the urban subjects was generally higher than that of the parents of the rural subjects. The height and weight values of the subjects in the current study, particularly the urban groups, were comparable to the standards for height and weight for 7–9-y-old healthy Indonesian children of 120 cm and 24 kg (Muhifal et al. 1994). Anthropometric indices, expressed in terms of Z-scores, should be used in evaluating anthropometric data from less industrialized countries (WHO 1995). This method measures the deviation of anthropometric measurements from reference medians in terms of Z-scores. Mean Z-score values for height-for-age, weight-for-age and weight-for-height of the children in the current study were indicative of normalcy; however, some of the subjects did have Z-score values (WHO 1995) indicative of their being stunted, underweight or wasted. The vast majority of Indonesian children that served as subjects in the current study were not malnourished as most of these Z-score values were within two standard deviations of the median for well-nourished populations. However, the prevalence of stunting, underweight and wasting may have increased due to the recent monetary crisis in Indonesia. The anthropometric measurements presented in the current study may be useful in the establishment of normal ranges for Indonesian children.

The Indonesian RDA for energy and protein for children 7–9-y-old are 7.95 MJ/d and 37 g/d, respectively (Lembaga Ilmu Pengetahuan Indonesia 1999). Nutrient intakes below recommended levels do not necessarily indicate that the intakes are inadequate to meet the individual’s requirements. However, it is appropriate to consider levels well below the RDA to indicate risks to dietary adequacy; the existence of deficiencies must be confirmed or rejected on the basis of
brieﬁed to compare the dietary intakes of these Indonesian children to the 1998 EAR and RDA for vitamin B-6.

The EAR (Institute of Medicine 1998) may be used to estimate the prevalence of inadequate nutrient intake. The 1998 EAR for vitamin B-6 is 0.5 mg for children 4–8-y-old and 0.8 mg for those 9–13-y-old. In that 66 of the 77 subjects were 8-y-old, the EAR for 4–8-y-old (0.5 mg/d) was utilized for comparisons. Utilizing the method of Guenther et al. (1997) in calculating usual intakes, the percentages of subjects in the current study consuming less than the EAR were as follows: rural females (72.2%), rural males (65.0%), urban females (45.0%), urban males (36.8%), all rural subjects (68.4%), all urban subjects (38.5%), all females (57.9%), all males (51.3%) and all subjects (54.5%). Signiﬁcantly more (P < 0.05) rural subjects than urban had inadequate vitamin B-6 intakes as assessed using this variable. However, similar percentages of male and female subjects had inadequate vitamin B-6 intakes.

Requirements of children for vitamin B-6 are based on relatively few studies. There are no well-designed feeding studies that have evaluated vitamin B-6 requirements, and no experimental studies have been conducted to directly determine requirements as related to protein intake. Published data are available for dietary vitamin B-6 intakes and status (Driskell 1994, Leklem 1991). Hence, the requirement for vitamin B-6 for children may be estimated assuming that a direct relationship between protein intake and vitamin B-6 requirement exists (Leklem 1991, Driskell 1994), or that a proportional relationship exists between vitamin B-6 requirements and the protein requirement for growth (Institute of Medicine 1998).

The dietary vitamin B-6/protein intake ratio has been expressed as 0.020 mg/g (Leklem 1991). Ninety-nine percent of the subjects in the present study reported consuming <0.020 mg of vitamin B-6/g protein. A study with black and white girls (ages = 12–16 y) living in Virginia found that 58% of the 186 subjects reported consuming <0.020 mg vitamin B-6/g protein (Driskell and Moak 1986). Another study with younger children (ages = 2–9 y) reported that 59% of the 225 subjects consumed <0.020 mg of vitamin B-6/g protein (Lewis and Nunn 1977). Using 2/3 of the vitamin B-6/protein ratio value (or <0.015 mg vitamin B-6/g protein), the percentage of the subjects in the current study having ratios less than the cutoff was 74%.

Protein requirements of Southeast Asian countries, including Indonesia, have been adjusted by the net protein utilization of 60–70 for the protein quality of the diet (Tee 1998). Studies by the Nutrition Research and Development Center, Bogor, Indonesia, reported that the protein digestibility of the typical Indonesian diet was about 85%. Moreover, based on the consumption survey, the amino acid score for proteins consumed by Indonesian school children was 76 (Muhilal et al. 1994). Adjustment for the Indonesian dietary pattern may be made by deriving a weighted digestibility factor based on the digestibilities of the protein sources consumed and the amino acid score. The vitamin B-6/protein ratio of the subjects in the current study may also be adjusted, taking into account the protein digestibility (85%) and the amino acid score (76) of typical Indonesian diet. The mean ratio of 0.011 mg of vitamin B-6/g protein becomes 0.018 mg of vitamin B-6/g protein following adjustment. Hence, after adjustment, 74% of the subjects in the current study reported consuming <0.020 mg of vitamin B-6/g protein, the recommended ratio (Leklem 1991).

Leklem (1990) suggested that an EALAT activity coefficient <1.25 was indicative of adequate vitamin B-6 status.
The EALAT activity coefficients of all subjects in the current study was 1.20 ± 0.16, mean ± SD. The percentages of subjects in the current study who had EALAT activity coefficients ≥ 1.25 were as follows: rural females (55.6%), rural males (30.0%), urban females (10.0%), urban males (26.3%), all rural subjects (42.1%), all urban subjects (17.9%), all females (31.6%), all males (28.2%) and all subjects (29.9%). Significantly more (P < 0.05) rural subjects than urban had inadequate vitamin B-6 status as assessed by the EALAT activity coefficient index. However, similar percentages of male and female subjects had inadequate vitamin B-6 status as indicated by this parameter.

The EALAT activity coefficients in the current study were higher than published values for children in the U.S. Driskell et al. (1985) reported that the EALAT activity coefficients of the adolescent girls were 1.14 ± 0.01, mean ± SEM, and 13% of the subjects had EALAT activity coefficients ≥ 1.25. Leklem (1990) also suggested that a plasma PLP >30 nmol/L be considered as being indicative of adequate vitamin B-6 status. Plasma PLP concentrations of the subjects in the current study were 54 ± 30 nmol/L (means ± SD). The percentages of subjects having plasma PLP concentrations ≤ 30 nmol/L were as follows: rural females (30.0%), rural males (30.0%), urban females (5.0%), urban males (15.8%), all rural subjects (39.5%), all urban subjects (10.3%), all females (26.3%), all males (23.1%) and all subjects (24.7%). Significantly more (P < 0.05) rural subjects than urban had inadequate vitamin B-6 status as assessed by plasma PLP concentrations. However, similar percentages of male and female subjects had inadequate vitamin B-6 status as assessed by this index. Other investigators have proposed that a plasma PLP concentration < 20 nmol/L is indicative of inadequacy (Institute of Medicine 1998, Liu et al. 1985). In the present study, only two rural males (10.0% of group) had plasma PLP < 20 nmol/L.

These two biochemical indices of vitamin B-6 deficiency were reflective of the vitamin B-6 intakes of children in the present study. The dietary vitamin B-6 intakes of subjects having adequate status as judged by EALAT activity coefficients (<1.25) and plasma PLP concentrations (>30 nmol/L) were as follows: (means ± SD) 9-y-old subjects, 0.66 ± 0.28 mg/d; 8-y-old subjects, 0.67 ± 0.22 mg/d; and 8–9-y-old subjects, 0.66 ± 0.23 mg/d.

A metabolic study on the effect of high protein diet (1.55 g protein/kg body weight) from either animal or plant sources on the vitamin B-6 requirement of young women was done by Kretsch and co-workers (1982). Most biochemical indices were normalized at an intake of 0.015 mg of vitamin B-6/g protein, with the rest being normalized at 0.020 mg of vitamin B-6/g protein with the exception of total urinary vitamin B-6 which was not normalized. Similar findings were observed for both plant protein and animal protein diets at this high protein intake. However, this may not be true if a diet lower in protein had been fed. The subjects in the current study reported consuming a diet high in protein.

Both rural and urban subjects consumed noodles providing vitamin B-6 (0.051 and 0.058 mg/d, respectively), and noodles are manufactured by large-scale producers; if the decision were made to fortify a food(s) in order to help students and others meet their vitamin B-6 requirements, noodles may be the ideal food to fortify.

All the three commonly used status criteria indicated that the percentages of subjects with vitamin B-6 inadequacy was significantly higher (P < 0.05) in rural than urban areas (Fig. 1). About 40% of rural and around 10% of urban children included in this study were found to have inadequate vitamin B-6 status, and almost 70 and 40% of rural and urban children, respectively, had inadequate intakes (<EAR) of the vitamin. Based on these data, elementary children in Indonesia need to be encouraged to consume vitamin B-6 dense foods that are inexpensive and locally available. These foods include plantain, banana, mango, water spinach and fish (Setiawan et al. 1999). Vitamin B-6 inadequacy appears to be a prevalent nutritional problem in Indonesian School children, particularly those in rural areas.

**LITERATURE CITED**


