Nutritional Sciences 5(1), February, 2002, pp. 20-25

Evaluation of Vitamin B_6 Status and Korean RDA in Korean College Students Following a Uncontrolled Diet*

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ABSTRACT

The vitamin B_6 status of 49 healthy college student (women, aged 20 – 26 y) was estimated for evaluation of vitamin B_6 status and the Korean Recommended Dietary Allowance (RDA) for vitamin B_6. The average daily vitamin B_6 intake of the subjects was 0.86 ± 0.289 mg/d or 61.43 ± 24.10% of Korean RDA. The average ratio of vitamin B_6 intake to daily protein intake was 0.014 ± 0.003 mg/g protein. Foods from animal and plant sources provided 34.25 ± 18.62% and 65.78 ± 18.72%, respectively, of total vitamin B_6. Plasma pyridoxal 5'-phosphate (PLP) concentration was significantly ($p < .01$ – $p < .001$) positively correlated to intakes of all other nutrients except vitamin C. However, no significant correlation was found between plasma PLP and nutrient intake. Vitamin B_6 intake only tended to have a positive correlation with plasma PLP concentration. Plasma total cholesterol was correlated to plasma PLP concentration ($p < .05$). Plasma PLP had no correlation with levels of glucose, triglyceride, and albumin. These results confirm that the present Korean RDA for vitamin B_6 of 1.4 mg/d based on 0.02 mg/g protein is adequate.

KEY WORDS: vitamin B_6 status, plasma pyridoxal 5'-phosphate, recommended dietary allowance, blood biochemical indices.

INTRODUCTION

Vitamin B_6 is frequently identified as a nutrient of which those even in Western countries[1-5] get an inadequate supply. Nevertheless, vitamin B_6 deficiencies are rare in Korea. Subclinical vitamin B_6 deficiencies may influence the rate of mental and physical functional deterioration that normally becomes more severe with advancing age. Because vitamin B_6 may be associated with cardiovascular diseases[6] and may contribute to some neurological disorders[7] and impaired immune function,[8,9] the prevention of marginal vitamin B_6 intake and biochemical deficiencies are important for favorable health outcomes.

The present Korean RDA for vitamin B_6 is based on a dietary vitamin B_6 to protein ratio of 0.02 mg/g.[10] Because there have been few studies of vitamin B_6 status among Koreans, the Korean RDA for vitamin B_6 had to be established on the basis of Western data, which is grounded in Western dietary habits and intake. However, Korean dietary habits and intake are different from those in the West and nutrient requirements are affected by physio-cultural factors including race. Also, it is reported that the bioavailability of vitamin B_6 from meat is higher than that from plant-based foods because the bioavailability of vitamin B_6 is incomplete when it is conjugated with β-glucosides.[11] The major food sources of vitamin B_6 in Western countries[1-5] are meat, whereas the major food sources of vitamin B_6 in Korea are plant-based foods.[12] Therefore, there might be potential limitations to establishing the Korean RDA on the basis of Western data.

Plasma pyridoxal 5'-phosphate (PLP) concentration is the most frequently used index of vitamin B_6 status in humans and generally reflects vitamin B_6 intake and tissue concentration[13] because it is the primary form of vitamin B_6 and crosses all membranes under postprandial conditions.[14] The most fundamental, but not necessarily the best, index of vitamin B_6 status may be the amount of the vitamin in a person's diet.[15]

The aim of this study, therefore, was to assess the vitamin B_6 status of Koreans, to evaluate the present Korean Recommended Dietary Allowance (RDA) for vitamin B_6 and observe any interrelation between plasma PLP concentration and blood biochemical indices.

*This study was supported by 2001 Research fund of Institute of Natural Science Research, Duksung Women's University.

Accepted : January 18, 2002

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SUBJECTS AND METHODS

1. Subjects

Forty-nine young women, all college students with no

Table 1. Anthropometric measurements of the subjects

Category	Mean ± SD
Age (yrs)	21.94 ± 0.58
Height (cm)	161.86 ± 3.81
Weight (kg)	51.11 ± 5.71
BMI[1)] (kg/m^2)	19.49 ± 1.78
PIBW[2)] (%)	90.13 ± 10.32

1) Body mass index = Weight (kg)/Height (m)^2
2) Percent ideal body weight = Body weight/Ideal body weight × 100

health problems, participated in this study. The subjects were taught and tested on the exact food portions to be taken under this study. The ages of the subjects ranged from 20 to 26. Characteristics of the subjects are given in Table 1.

2. Dietary intake and anthropometric data

Three-day recall method was used to record the standard dietary intake of the subjects: two days during the week, one day on the weekend. Food portion sizes used were based on published average portion sizes.[16)] Dietary intake was converted using a computerized dietary analysis program[17)] and was based on data pertaining to the vitamin B_6 content of foods cited in Korean Food values[10)] and USDA agriculture handbooks.[18)] Intakes of vitamin B_6 and nutrients were evaluated in accordance with the Korean RDA for the appropriate age group.[10)] On the day's worth of dietary data, weights and heights were reported and body mass index (BMI) was calculated.

3. Biochemical measurements

After a 12-hour fast, venous blood was used for the assessment of vitamin B_6 status and biochemical indices. Immediately following blood drawing in heparinized vacutainer, blood was centrifuged at 3000 rpm for 15 min to separate the plasma. The plasma was stored at −20℃ in aliquots until analyzed.

Plasma PLP concentration was measured using the HPLC method,[19)] which was modified as follows: Mobile phase (0.1M potassium dihydrogen phosphate containing 0.1M sodium perchlorate, 0.5 g/L sodium bisulfite, pH 3) was pumped at a flow rate of 1.0 ml/min into the column (Bondapack ODS column, 3.9 300 mm, 10 μm porus packing, C18, Waters). Plasma was added to perchrolic acid (0.8M) and allowed to sit for one hour to release PLP from protein. This mixture was then centrifuged (18000 rpm, 4℃, 15 min) and supernatent removed. One hundred μl of supernatant was loaded in the sample loop and then injected into the column. Samples for vitamin B_6 analysis were prepared under yellow fluorescent lighting to prevent photodegradation of the vitamers.[20)]

Table 2. Daily nutrient intake of the subjects

Nutrient	Mean ± SD	% of Korean RDA[1)]
Energy (Kcal)	1675.98 ± 334.22	83.80%
Carbohydrate (g)	248.57 ± 50.21	
Protein (g)	62.04 ± 16.07	112.78%
Animal protein	30.58 ± 11.14	
Plant protein	31.45 ± 7.76	
Fat (g)	48.22 ± 12.99	
Animal fat	26.78 ± 8.07	
Plant fat	25.60 ± 11.47	
Cholesterol (mg)	252.47 ± 115.87	
Calcium (mg)	434.42 ± 117.89	62.06%
Phosphorus (mg)	927.59 ± 222.33	132.51%
Iron (mg)	9.37 ± 2.54	58.56%
Sodium (mg)	3828.42 ± 1176.61	
Potassium (mg)	2123.37 ± 565.66	
Vitamin A (RE)	623.89 ± 238.02	89.13%
Vitamin B_1 (mg)	1.12 ± 0.36	112%
Vitamin B_2 (mg)	1.04 ± 0.30	86.67%
Vitamin B_6 (mg)	0.86 ± 0.29	61.43%
Niacin (NE)	13.13 ± 4.27	101%
Vitamin C (mg)	93.96 ± 67.27	134.23%

1) Recommended Dietary Allowances for Koreans, 7th Revision, 2000

The levels of plasma total protein, albumin, total cholesterol, triglyceride, and glucose were measured using an autoanalyzer (Vitalab Eclipse Plus, MERCK).

4. Statistical analysis

The statistical analysis was carried out using the Statistical Analysis System. Pearson's correlation coefficient was used to determine possible relationships among the indices of vitamin B_6 status and blood biochemical indices. Values for vitamin B_6 status were regressed based on vitamin B_6 intake and dietary vitamin B_6 to protein ratio.

RESULTS

Table 2 shows the nutrient intake expressed as a percentage of Korean RDA. The nutrient intakes of the subjects were 58.56−134.23% of Korean RDA. The estimated average daily intake of vitamin B_6 for the subjects was 0.860 ± 0.289 mg/d (mean ± SD), ranged from 0.384 to 1.732 mg/d and was 61.43 ± 24.10% of Korean RDA. Table 3 lists the total daily vitamin B_6, mg vitamin B_6/1000 kcal, mg vitamin B_6/g dietary protein intake and percentage of Korean RDA of vitamin B_6 for the subjects. The daily vitamin B_6 intake from plant and animal sources for the subjects expressed as a percentage of the total is also shown in Table 3. Foods from animal and plant sources provided 34.25 ± 18.62% and 65.78 ± 18.72%, respectively, of total vitamin B_6 intake.

Table 3. Dietary vitamin B_6 intake of the subjects

	Mean ± SD	Range
Vitamin B_6 per day (mg/d)	0.860 ± 0.289	0.384 – 1.732
Vitamin B_6 per 1000 kcal (mg/1000 kcal)	0.509 ± 0.115	0.291 – 0.830
Vitamin B_6 per g protein (mg/g protein)	0.014 ± 0.003	0.008 – 0.024
Percent of RDA (%)[1]	61.43 ± 24.10	27. 42 – 123. 71
Vitamin B_6 form plant foods (%)	65.78 ± 18.72	41. 66 – 87. 16
Vitamin B_6 form animal foods (%)	34.25 ± 18.62	12. 84 – 58. 34

1) Recommended Dietary Allowances for Koreans, 7th Revision, 2000

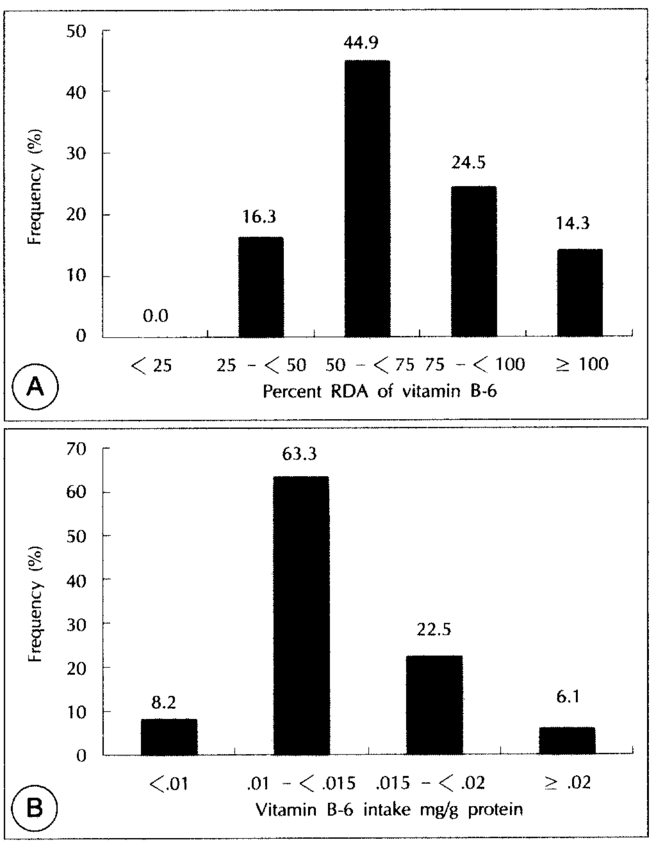

Fig. 1. Weighted frequency distribution of vitamin B-6 intakes: % of RDA (A), mg/g protein (B).

Fig. 1 shows the frequency distribution of vitamin B_6 intake as a percentage of Korean RDA (A) and relative to daily protein intake (B), respectively. 14.3% of all subjects had intakes 100% of RDA for vitamin B_6. Approximately 16.3% of subjects had estimated intakes < 50% of RDA. As for vitamin B_6 to protein ratio, 63.3% of subjects fell within a range of 0.01 – 0.015 mg vitamin B_6/g protein, 22.5% of subjects were within 0.015 – 0.02, 8.2% of subjects showed less than 0.01 and only 6.1% of subjects showed more than 0.02 on the survey day.

There were correlations among the indices of vitamin B_6 status and nutrient intake (Table 4). As expected, vi-

Table 4. Correlation among the indices of vitamin B_6 status and nutrient intake per day of the subjects

Nutrients	Vitamin B_6 intake (mg)	Plasma PLP (nmol/L)
Energy (Kcal)	0.74349*** (0.0001)[1]	0.10221 (0.4847)
Total protein (g)		
Animal (g)	0.63830*** (0.0001)	0.09539 (0.5144)
Vegetable (g)	0.54347*** (0.0001)	0.11380 (0.4362)
Total fat (g)		
Animal (g)	0.49714*** (0.0003)	0.00193 (0.9895)
Vegetable (g)	0.44230** (0.0015)	0.14688 (0.3139)
Carbohydrate (g)	0.65395*** (0.0001)	0.11148 (0.4457)
Ca (mg)	0.37305** (0.0083)	0.15971 (0.2730)
P (mg)	0.66892*** (0.0001)	0.12619 (0.3879)
Fe (mg)	0.68129*** (0.0001)	0.20951 (0.1485)
Na (mg)	0.58189*** (0.0001)	0.06473 (0.6586)
K (mg)	0.67308*** (0.0001)	0.10187 (0.4861)
Vitamin A (RE)	0.38494** (0.0063)	0.07851 (0.5918)
Vitamin B_1 (mg)	0.69591*** (0.0001)	0.12383 (0.3966)
Vitamin B_2 (mg)	0.50014*** (0.0001)	0.08339 (0.5689)
Vitamin B_6		0.21111 (0.1454)
Niacin (NE)	0.79845*** (0.0001)	0.06944 (0.6354)
Vitamin C (mg)	0.20944 (0.1486)	0.00618 (0.9664)
Cholesterol (mg)	0.37201** (0.0085)	0.04741 (0.7463)

1) p-value
*: significant at $p < 0.05$, **: significant at $p < 0.01$
***: significant at $p < 0.001$

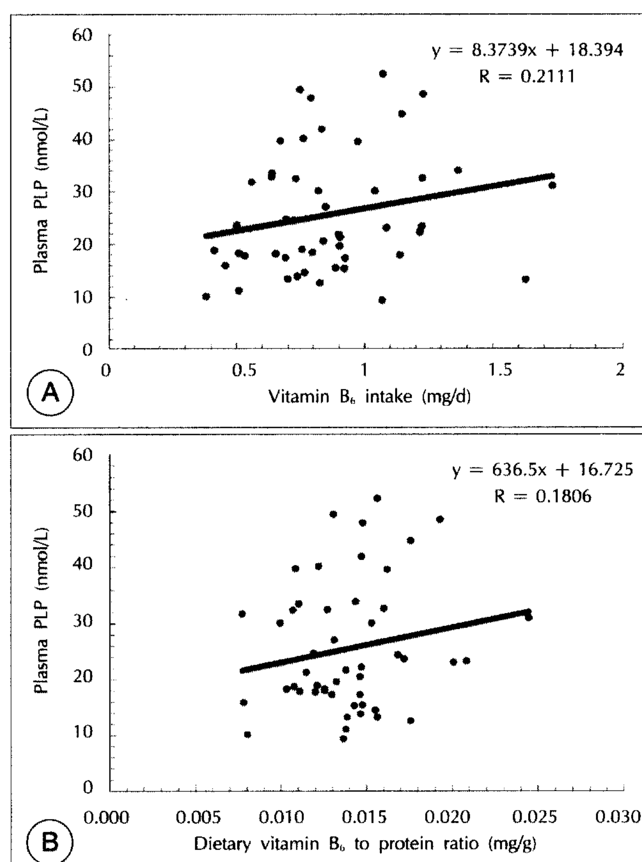

Fig. 2. Linear regression analysis of plasma pyridoxal 5'-phosphate (PLP) concentration vs. vitamin B_6 intake (A) and the dietary vitamin B_6 to protein ratio (B) for the subjects.

Table 5. Correlation between plasma PLP concentration and biochemical values of the subjects

	Plasma PLP (nmol/L)	
Total protein (g/dl)	0.27728	(0.0621)[1]
Glucose (mg/dl)	0.02990	(0.8436)
Triglyceride (mg/dl)	0.19093	(0.2037)
Albumin (g/dl)	0.03542	(0.8152)
Total cholesterol (mg/dl)	0.32017*	(0.0301)

1) p-value
*: significant at $p < 0.05$

tamin B_6 intake had a positive correlation with all other nutrients except vitamin C. Among major nutrients, protein, fat, and carbohydrate intake had a strong positive correlation with vitamin B_6 intake ($p < 0.001$). Also, among micronutrients, phosphorous, iron, sodium, potassium, vitamin B_1, vitamin B_2 and niacin had strong positive correlations with vitamin B_6 intake ($p < 0.001$). However, no significant correlation was found between plasma PLP concentration and nutrient intake. Vitamin B_6 intake only tended to have a positive correlation with plasma PLP concentration ($r = 2.1111$, $p = 0.1454$). Linear regression analysis of plasma PLP concentration vs. vitamin B_6 intake (A) and dietary vitamin B_6 to protein ratio (B) are given in Fig. 2, respectively.

Table 5 shows the correlation between plasma PLP concentration and biochemical values. Plasma total cholesterol was only correlated to plasma PLP concentration ($p < 0.05$). There was a tendency toward a positive correlation between levels of plasma PLP and total protein. Plasma PLP had no correlation with levels of glucose, triglyceride, and albumin.

DISCUSSION

This study provides the data for evaluation of vitamin B_6 status and RDA in Korean women. Vitamin B_6 requirement studies being done in Western countries have some limitation to apply on the Korean requirement for vitamin B_6 because Korean dietary habits and intake are different from those of the Western and nutrient requirements are affected by physio-cultural factors including race. Vitamin B_6 intake and the status of the subjects in this study consumed uncontrolled, usual diet, whereas there are many previous studies[5)21 25)] about vitamin B_6 requirement and status of women in which subjects consumed a controlled diet containing various levels of vitamin B_6.

The Korean RDA for vitamin B_6, based on a dietary vitamin B_6 to protein ratio of 0.02 mg/g,[10)] for women aged 20-26 y (1.4 mg/d) is a little higher than those in other countries: 1.3 mg/d in the US,[26)] 1.2 mg/d in the UK,[27)] 1.2 mg/d in Japan,[28)] and 0.9-1.4 mg/d in Australia. [29)] In this study, the average vitamin B_6 intake of Korean women was 0.860 ± 0.289 mg/d, which was lower than 1.18 and 1.11 mg/d for young white and black women aged 12-34 y, respectively, in the NHANES Ⅱ.[2)] The average ratios of vitamin B_6 to daily protein intake were 0.014 ± 0.003 mg/g protein, which was lower than in the NHANES Ⅱ (0.019 and 0.018 mg/g dietary protein for young white and black women, respectively).

Because it is well established that the requirement for vitamin B_6 increases as dietary protein increases,[30)31)] the ratio of vitamin B_6 to protein was used to determine the vitamin B_6 requirement in this study. If the average intake is the amount needed to prevent clinical signs of vitamin B_6 deficiency and because the subjects showed no clinical signs of vitamin B_6, then based on the results of this study, a vitamin B_6 intake of 0.86 mg/d (0.014 mg/g protein) should be adequate for women. However, adding a margin of the safety to account for bioavailability concerns and to cover variance in the requirements of individuals would raise the vitamin B_6 RDA of young women. The Korean RDA for vitamin B_6 is based on the amount needed to satisfy 97.5% of the population. Adding two SD to the estimated average requirement (EAR) would mean 1.4 mg/d, 0.02 mg/g protein would be the intake required. Further support for adding two SD comes from the major food sources of vitamin B_6. The young women in this study obtained 65.78% of vitamin B_6 from plant sources, whereas in the NHANES Ⅱ approximately one-half of dietary vitamin B_6 was reported to come from meat and one-half from plant-based foods.

The Dietary Reference Intakes Committee based their recommendation on the fact that a PLP concentration of 20 nmol/L was chosen as the standard for adequate status.[26)] Also, Leklem *et al.*[14)23)] determined an acceptable value of 30 nmol/L for plasma PLP concentration, considering this adequate. In this study, linear regression calculation (Fig. 2) using the average of vitamin B_6 intakes and dietary vitamin B_6 to protein ratios predicted adequate plasma PLP concentration of 25 nmol/L. This line of support for 1.4 mg/d is provided by the results of the plasma PLP levels of the subjects. The amount of vitamin B_6 intake in subjects whose PLP levels were higher than 25 nmol/L was 0.944 mg/d, 0.014 mg/g protein. Thus, adding two SD (0.003 mg/g protein) to EAR(0.014 mg/g protein), 0.02 mg/g protein would be adequate for RDA. These results confirm that the dietary vitamin B_6 to protein ratio of 0.02 mg/g is sufficient to produce plasma PLP values indicative of adequate vitamin B_6 in young Korean women.

According to the results presented in Table 4, there were positive correlations between vitamin B_6 intake and those of other nutrients except vitamin C. There was only a tendency for a positive correlation between plasma PLP concentration and vitamin B_6 intake. As in other studies that have assessed the vitamin B_6 requirement, this study found a significant correlation between plasma PLP concentration and vitamin B_6 intake.[21-25] These studies were done using a controlled diet, in which a fixed menu was provided and there were various levels of vitamin B_6, whereas the present study was done using an uncontrolled, usual diet in which a relatively lower vitamin B_6 amount was provided. Thus, the bioavailability of vitamin B_6 might be higher with lower intakes of vitamin B_6 and effectively converted to PLP. The evidence for this hypothesis is drawn from the results of Huang JC *et al.*[23] which showed relatively higher plasma levels at a lower vitamin B_6 intake period. The lack of correlation between dietary intake and biochemical measurements may be the result of the sample size. Sharon M Madigan *et al.* ,[32] for example, showed no significant correlation between plasma PLP and vitamin B_6 intake in a sample of 62 elderly people, but van der Wielen *et al.*[33] reported a positive association between plasma PLP and vitamin B_6 intake in 546 elderly Europeans.

Between plasma PLP concentration and biochemical values, total cholesterol only had a significant correlation with plasma PLP concentration. In a study of the rhesus monkey,[34] there was a negative correlation between plasma PLP and total cholesterol level, whereas there was a positive correlation in this study. Meat may be a major source of vitamin B_6 and a major source of saturated fat and cholesterol at the same time, because the vitamin B_6 content of meat is higher than that of plant-based foods and the bioavailability of vitamin B_6 from animal sources is known to be better than that of vitamin B_6 from plant sources. Therefore, the vitamin B_6 status of subjects deriving more vitamin B_6 from animal foods may be good, but the subjects may ingest more cholesterol and animal fat. As a result, it may be presumed that there is a positive correlation between plasma PLP and total cholesterol. Thus, the population that is vulnerable to higher blood cholesterol levels needs to take EAR rather than the RDA.

In conclusion, the results of this study confirm that the present vitamin B_6 RDA of 1.4 mg/d (0.02 mg/g protein) for young Korean women is adequate, adding a margin of safety to account for bioavailability differences and variances in the requirements of individuals.

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