

## Assessment of vitamin B<sub>6</sub> status in Korean patients with newly diagnosed type 2 diabetes

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### Abstract

The purpose of this study was to assess vitamin B<sub>6</sub> intake and status in Korean patients with newly diagnosed type 2 diabetes. Sixty-four patients with newly diagnosed type 2 diabetes and 8-11% glycosylated hemoglobin (A1C), along with 28 age-matched non-diabetic subjects, participated. Dietary vitamin B<sub>6</sub> intake was estimated by the 24 hour recall method and plasma pyridoxal 5'-phosphate (PLP) was measured. There was a significant difference in daily total calorie intake between the diabetic and non-diabetic groups (1,917 ± 376 vs 2,093 ± 311 kcal). There were no differences in intake of total vitamin B<sub>6</sub> (2.51 ± 0.91 vs 2.53 ± 0.81 mg/d) or vitamin B<sub>6</sub>/1,000 kcal (1.31 ± 0.42 vs 1.20 ± 0.32 mg) between the diabetic and non-diabetic groups, and intakes of total vitamin B<sub>6</sub> were above the Korean RDA in both groups (180.0 ± 57.9 vs 179.0 ± 65.4). There was a higher percentage of diabetic subjects whose plasma PLP concentration was <30 nmol/L compared to non-diabetic group. Plasma PLP levels tended to be lower in the diabetic subjects than in the non-diabetic subjects, although the difference was not statistically significant due to a large standard deviation (80.0 ± 61.2 nmol/L vs 68.2 ± 38.5 nmol/L). Nevertheless, plasma PLP levels should be monitored in pre-diabetic patients with diabetic risk factors as well as in newly diagnosed diabetic patients for long-term management of diabetes, even though this factor is not a major risk factor that contributes to the development of degenerative complications in certain patients.

**Key Words:** Vitamin B<sub>6</sub> intake, vitamin B<sub>6</sub> status, plasma pyridoxal 5'-phosphate, type 2 diabetes

### Introduction

In developed countries, classical vitamin-deficiency syndromes such as scurvy, beriberi, and pellagra are now uncommon, but specific population subgroups remain at risk for modest vitamin insufficiencies. This is notably the case in diabetes patients who frequently display inadequate vitamin status, including many from the B group. Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and insufficiency of secretion or action of endogenous insulin. Although the etiology of diabetes is not well defined, aging is a major factor in the development and progression of diabetes. Due to functional insufficiency, the vitamin B<sub>6</sub> status of elderly individuals may be subject to higher variability than that of younger adults who are frequently taken as references. Moreover, it is reported that plasma PLP level is affected by age and gender [1].

It has been reported that vitamin B<sub>6</sub> may be involved in the metabolism of carbohydrate. Pyridoxal 5'-phosphate (PLP), the active form of vitamin B<sub>6</sub>, acts as an integral part of glycogen phosphorylase (EC 2.4.1.1.) which catalyzes the breakdown of glycogen [2]. A deficiency of vitamin B<sub>6</sub> is associated with impaired gluconeogenesis [3] and impaired glucose tolerance [4]. It has also been reported that vitamin B<sub>6</sub> deficiency can lead to a decrease in the level of circulating insulin as well as pancreas

function and relates to increased risk of coronary artery disease in animal models [5,6]. Thus, proper vitamin B<sub>6</sub> status can be deemed an important factor in the care of type 2 diabetes for the prevention of degenerative complications. However, there have been few reports on vitamin B<sub>6</sub> status in the Korean elderly population or in those with diabetes.

Therefore, the aim of the present study was to assess vitamin B<sub>6</sub> status using plasma PLP levels and vitamin B<sub>6</sub> intakes of Korean patients with newly diagnosed type 2 diabetes since plasma PLP level [7,8] and vitamin B<sub>6</sub> intake have been the most accepted and widely used indices of vitamin B<sub>6</sub> status in the last decade. In addition, these status indices were compared to those of age and gender-matched healthy non-diabetic subjects to exclude variance from age, as well as gender effects.

### Subjects and Methods

#### Subjects

Sixty-four patients with newly diagnosed type 2 diabetes and 8-11% glycosylated hemoglobin (A1C), which is used primarily to identify average plasma glucose concentrations over a prolonged period of time, and 28 age-matched healthy non-diabetic subjects

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were recruited consecutively from the diabetes clinic at E Hospital between February and April, 2005. Subjects were excluded before recruitment if they were taking supplemental vitamins, including multivitamins.

#### Dietary intake and anthropometric measurements

Dietary vitamin B<sub>6</sub> intake and food sources were estimated with the 24 hour recall method. The subjects each reported three consecutive daily dietary intakes with the help of a trained interviewer. Food portion sizes were estimated by using standard household measures and published average portion sizes [9]. Nutrient intakes were estimated using a computer-aided nutritional analysis program developed by the Korean Nutrition Society [10]. When information was unavailable for a particular food, a value was assigned based on values for similar foods. The foods in the vitamin B<sub>6</sub> database were categorized into those of animal or plant origin, and the amount of daily vitamin B<sub>6</sub> provided by these two categories was calculated for each person. On the day of dietary data collection, weights and heights were measured and body mass index (BMI) was calculated from the measurements of body weight and height.

#### Biochemical and statistical analysis

After a 12-hour fast, venous blood was used for the assessment of vitamin B<sub>6</sub> status and biochemical indices. Immediately following blood draw into heparinized vacutainers, the blood was centrifuged at 3,000 rpm for 15 min to separate the plasma. The plasma was stored at -20°C in aliquots until analyzed. Plasma PLP concentration was measured using the HPLC method [11], which was modified as follows: Mobile phase (0.1 M potassium dihydrogen phosphate containing 0.1 M 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). The plasma was added to perchloric acid (0.8 M) and allowed to sit for one hour to release PLP from the protein. This mixture was then centrifuged (18,000 rpm, 4°C, 15 min) and the supernatant removed. One hundred microliters of supernatant was loaded in the sample loop and then injected into the column.

Statistical analyses were carried out using the Statistical

Analysis System (SAS). Pearson's correlation coefficient was used to determine possible relationships among the indices of vitamin B<sub>6</sub> status and blood biochemical indices. Values for vitamin B<sub>6</sub> status were regressed based on vitamin B<sub>6</sub> intake and dietary vitamin B<sub>6</sub> to protein ratio.

## Results

Because aging is one of the major factors affecting both plasma PLP levels and the development and progression of diabetes, comparisons of plasma PLP concentrations of diabetic patients to those of age-matched healthy non-diabetic subjects are important. Sixty-four diabetic patients and 28 age-matched non-diabetic subjects participated in this study, with mean age being 50 years for the diabetic patients and 51.5 years for the non-diabetic subjects. The characteristics of the subjects are given in Table 1. Table 2 shows the nutrient intakes of the diabetic patients

**Table 2.** Daily nutrient intakes of non-diabetic and diabetic<sup>1)</sup> subjects

Nutrient	Non-diabetic (n = 28)	Diabetic (n = 64)
Energy (kcal)	1,917 ± 376	2,093 ± 311 <sup>*2)</sup>
Carbohydrate (g)	290 ± 43.7	323 ± 56.3 <sup>ns</sup>
Total protein (g)	83.9 ± 19.3	91.9 ± 26.7 <sup>ns</sup>
Animal protein (g)	39.7 ± 18.7	47.8 ± 24.0 <sup>ns</sup>
Plant protein (g)	44.1 ± 10.1	44.1 ± 11.7 <sup>ns</sup>
Total fat (g)	46.2 ± 16.4	52.0 ± 26.2 <sup>ns</sup>
Animal fat (g)	23.9 ± 15.6	27.7 ± 16.9 <sup>ns</sup>
Plant fat (g)	22.3 ± 9.79	24.3 ± 16.1 <sup>ns</sup>
Cholesterol (mg)	217 ± 131	267 ± 196 <sup>ns</sup>
Calcium (mg)	621 ± 227	684 ± 317 <sup>ns</sup>
Phosphorus (mg)	1,108 ± 214	1,205 ± 357 <sup>ns</sup>
Iron (mg)	17.0 ± 4.76	19.0 ± 5.91 <sup>ns</sup>
Sodium (mg)	5,478 ± 1674	5,159 ± 1,909 <sup>ns</sup>
Potassium (mg)	3,236 ± 717	3,339 ± 923 <sup>ns</sup>
Vitamin A (RE)	924 ± 627	83.6 ± 460 <sup>ns</sup>
Vitamin B <sub>1</sub> (mg)	1.32 ± 0.41	1.39 ± 0.49 <sup>ns</sup>
Vitamin B <sub>2</sub> (mg)	1.24 ± 0.33	1.24 ± 0.38 <sup>ns</sup>
Vitamin B <sub>6</sub> (mg)	2.51 ± 0.91	2.53 ± 0.81 <sup>ns</sup>
Niacin (NE)	19.1 ± 6.65	19.0 ± 6.37 <sup>ns</sup>
Vitamin C (mg)	114 ± 55.3	141 ± 97.7 <sup>ns</sup>

<sup>1)</sup> Diabetic subjects had newly diagnosed type 2 diabetes with 8-11% A1C and non diabetic subjects were age-matched.

<sup>2)</sup> P-value, ns: not significant at P<0,05

**Table 1.** General characteristics of non-diabetic and diabetic<sup>1)</sup> subjects

Category	Non-diabetic			Diabetic		
	Men (n = 15)	Women (n = 13)	Total (n = 28)	Men (n = 36)	Women (n = 28)	Total (n = 64)
Age (yrs)	50.6 ± 12.3	49.5 ± 9.8	50.0 ± 10.8	47.9 ± 11.7	55.4 ± 13.6	51.5 ± 12.9
Height (cm)	167.1 ± 4.6	157.0 ± 7.0	161.0 ± 7.9	170.6 ± 5.9	156.3 ± 6.2	164.0 ± 9.5
Weight (kg)	66.1 ± 8.9	52.4 ± 8.5	59.2 ± 11.0	72.7 ± 10.0	63.7 ± 6.5	68.7 ± 10.3 <sup>**3)</sup>
BMI <sup>2)</sup>	23.6 ± 2.6	21.2 ± 2.6	22.5 ± 3.1	24.9 ± 2.8	26.1 ± 2.2	25.5 ± 2.6 <sup>**</sup>

<sup>1)</sup> Diabetic subjects had newly diagnosed type 2 diabetes with 8-11% A1C and non diabetic subjects were age-matched.

<sup>2)</sup> BMI: Body mass index = weight(kg)/height(m<sup>2</sup>)

<sup>3)</sup> \*\* Significant difference between non-diabetic and diabetic subjects at P<0,01

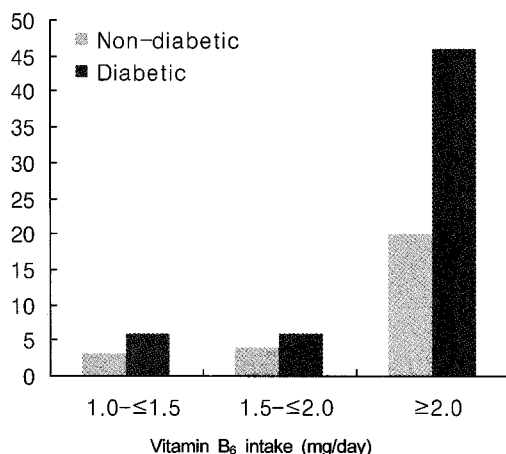
**Table 3.** Dietary vitamin B<sub>6</sub> intake and plasma PLP levels of non-diabetic and diabetic<sup>1)</sup> subjects

	Non-diabetic (n = 28)	Diabetic (n = 64)
Total vitamin B <sub>6</sub> (mg/day)	2.51 ± 0.91	2.53 ± 0.81 <sup>ns2)</sup>
Vitamin B <sub>6</sub> /1,000 kcal (mg)	1.31 ± 0.42	1.20 ± 0.32 <sup>ns</sup>
Percent of Korean RDA (%) <sup>1)</sup>	179.0 ± 65.4	180.0 ± 57.9 <sup>ns</sup>
Mean Plasma PLP (nmol/L)	80.0 ± 61.2	69.2 ± 38.5 <sup>ns</sup>
Subjects plasma PLP < 30 nmol/L (%)	8.3	9.7

<sup>1)</sup> Diabetic subjects had newly diagnosed type 2 diabetes with 8-11% A1C and non diabetic subjects were age-matched.

<sup>2)</sup> P-value, ns: not significant at P < 0.05

and non-diabetic subjects. There was a significant difference in daily total calorie intake between the diabetic and non-diabetic groups (1,917 ± 376 vs 2,093 ± 311 kcal/d) due to excess weight in the diabetic subjects. However, there were no differences in intakes of other nutrients including vitamin B<sub>6</sub>. Table 3 shows dietary vitamin B<sub>6</sub> intake and plasma PLP levels for the non-diabetic and diabetic subjects. There were no differences in intake of total vitamin B<sub>6</sub> (2.51 ± 0.91 vs 2.53 ± 0.81 mg/d) or vitamin B<sub>6</sub>/1,000 kcal (1.31 ± 0.42 vs 1.20 ± 0.32 mg) between the diabetic and non-diabetic groups. The intakes of total vitamin B<sub>6</sub> were above the Korean RDA for both groups. In the diabetic patients, the mean plasma PLP level was 15% lower as compared to the non-diabetic subjects (80.0 ± 61.2 vs 68.2 ± 38.5 nmol/L), but this difference was not statistically significant due to a large standard deviation. This indicates that the percentage of diabetic subjects whose plasma PLP concentration was < 30 nmol/L was higher than that of the non-diabetic group. Fig. 1 shows the frequency distribution of daily vitamin B<sub>6</sub> intake, and its relative intake to daily protein intake ratio. Daily vitamin B<sub>6</sub> intake was not normally distributed in either the non-diabetic or diabetic subjects, and the declination was steeper in the diabetic patients. Fig. 2 shows there was no significant correlation between plasma PLP level and intake of vitamin B<sub>6</sub> in either the non-diabetic subjects or diabetic patients. Also, plasma PLP levels and intakes of vitamin B<sub>6</sub> were more widely scattered in the diabetic patients



**Fig. 1.** Distribution of vitamin B<sub>6</sub> intake in non-diabetic and diabetic<sup>1)</sup> subjects. <sup>1)</sup> Diabetic subjects had newly diagnosed type 2 diabetes with 8-11% A1C and non diabetic subjects were age-matched.

**Table 4.** Correlations between plasma PLP level and biochemical values of the diabetic subjects<sup>1)</sup>

	Plasma PLP (nmol/L) <sup>3)</sup>	
	Non-diabetic (n = 28)	Diabetic (n = 64)
Glucose (mg/dl)	0.033 <sup>ns2)</sup>	0.211 <sup>ns</sup>
Postprandial glucose (mg/dl)	0.160 <sup>ns</sup>	0.243 <sup>ns</sup>
Triglyceride (mg/dl)	-0.125 <sup>ns</sup>	-0.138 <sup>ns</sup>
Albumin (g/dl)	-0.066 <sup>ns</sup>	0.075 <sup>ns</sup>
Total cholesterol (mg/dl)	-0.112 <sup>ns</sup>	0.048 <sup>ns</sup>

<sup>1)</sup> Diabetic subjects had newly diagnosed type 2 diabetes with 8-11% A1C and non diabetic subjects were age-matched.

<sup>2)</sup> P-value, ns: not significant at P < 0.05

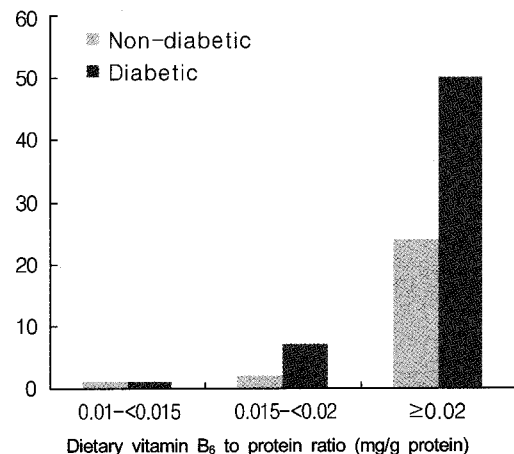
<sup>3)</sup> Plasma pyridoxal 5'-phosphate levels of non-diabetic and diabetic subjects

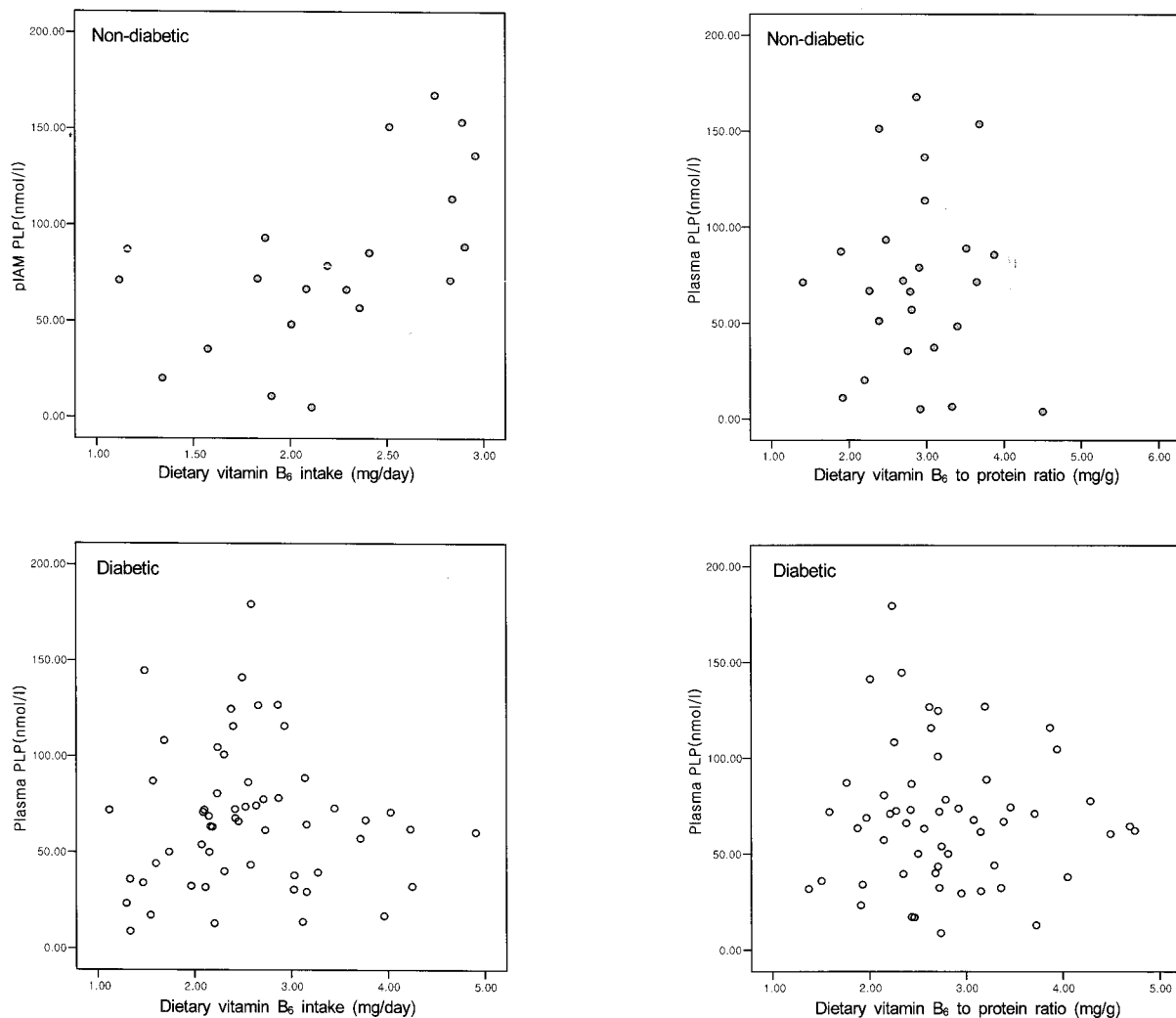
compared to the non-diabetic subjects. Table 4 shows that no significant correlations were found between plasma PLP level and biochemical risk indices including plasma levels of fasting glucose, postprandial glucose, triglyceride, albumin, and total cholesterol in either the non-diabetic subjects or diabetic patients.

## Discussion

Because there were no differences in total vitamin B<sub>6</sub> intake and vitamin B<sub>6</sub>/1,000 kcal between the diabetic and non-diabetic groups, it is considered that the higher energy intake in diabetes patients did not affect intake of vitamin B<sub>6</sub>. A possible explanation for the tendency of lower vitamin B<sub>6</sub> status is based on plasma PLP level, which has been suggested as the best single status indicator because it appears to reflect tissue stores. In the diabetic subjects, the mean plasma PLP level was 15% lower than that of the non-diabetic subjects (80.0 ± 61.2 nmol/L vs 68.2 ± 38.5 nmol/L), but this difference was not statistically significant due to a large standard deviation.

However, even though there were no differences in total vitamin B<sub>6</sub> intake and vitamin B<sub>6</sub>/1,000 kcal between the diabetic and non-diabetic groups, and intakes of total vitamin B<sub>6</sub> were above the Korean RDA in both groups, the percentage of subjects





**Fig. 2.** Scatter plots of plasma PLP levels and vitamin B<sub>6</sub> intake in non-diabetic and diabetic<sup>1)</sup> subjects. <sup>1)</sup> Diabetic subjects had newly diagnosed type 2 diabetes with 8-11% A1C and non-diabetic subjects were age-matched.

with a plasma PLP concentration < 30 nmol/L (the proposed cutoff as an index of adequacy in diabetic patients) was higher in the diabetic subjects as compared to non-diabetic subjects. Also, plasma PLP levels and intakes of vitamin B<sub>6</sub> were more widely scattered in the non-diabetic group as compared to those of the diabetic group. Thus, if an eccentric value were considered, the 15% lower mean plasma PLP level in the diabetic subjects compared to that of the non-diabetic subjects is thought to be a meaningful decrease in a clinical situation, although this difference was not statistically significant. In addition, the results of this study are consistent with previous studies where circulating levels of plasma pyridoxal 5'-phosphate and total vitamin B<sub>6</sub> significantly decreased under conditions of hyperglycemia [12-14].

However, recent epidemiology of plasma PLP in the U.S. population showed there was no difference between diabetes and non-diabetes subjects, regardless of using supplemental vitamin B<sub>6</sub> [15]. This discrepancy may be explained by differences in major vitamin B<sub>6</sub> sources, because several studies have indicated

that the utilization of vitamin B<sub>6</sub> from animal derived foods is more efficient than that from plant foods [16,17]. Furthermore, it was reported that Koreans obtain approximately 65% of vitamin B<sub>6</sub> from plant sources [18,19], whereas in the American diet, approximately 50% of dietary vitamin B<sub>6</sub> is from meat and the other 50% is from plant-based foods [20].

There was a previous review on age-associated B vitamin deficiency as a determinant of chronic diseases [21], and estimations of how much vitamin B<sub>6</sub> is required in diabetes must take into account all potential age-related alterations. By the comparison of vitamin B<sub>6</sub> status in diabetic patients to that of age-matched healthy non-diabetic subjects in this study, the tendency for a lower plasma PLP level in diabetic patients might not be due to age-related alterations.

This lowered level of plasma PLP in diabetic patients may be due to either lower intake of vitamin B<sub>6</sub> or altered distribution of PLP in diabetic patients. B vitamin intake is dependent on the food supply and many studies have demonstrated that

increased protein intake causes a relative decrease in B<sub>6</sub> status, as judged by a variety of B<sub>6</sub> status indicators [22-24]. Furthermore, in this study, vitamin B<sub>6</sub> intake was highly correlated to intakes of energy and protein. This has led some to define B<sub>6</sub> requirements in terms of protein intake or energy intake. However, there was no difference in vitamin B<sub>6</sub> intake when expressed as either a ratio of vitamin B<sub>6</sub> intake to daily protein intake or as the amount of vitamin B<sub>6</sub> intake to a 1,000 calorie intake in this study. These data show that plasma PLP levels tended to be lower in the diabetic subjects than in the non-diabetic subjects although there was no difference in vitamin B<sub>6</sub> intake. Thus, in this study, the lower plasma PLP levels of the diabetic patients might not have been due to insufficient intake of vitamin B<sub>6</sub>, but instead might be due to altered distribution of PLP in diabetic patients.

The mechanism involved in altered distribution of PLP in diabetes could be that elevated plasma glucose reduces plasma PLP levels and xanthurenic acid excretion due to abnormal tryptophan metabolism; xanthurenic acid forms xanthurenic acid-insulin complexes that reduce insulin sensitivity and result in elevated blood glucose levels [25]. Decreases of mitochondria pyridoxal phosphate and aspartate aminotransferase have been observed in diabetic animals [26]. It was also reported that urinary excretions of 3-hydroxykynurenine and 3-hydroxyanthranilic acid were increased in diabetic animals, and this might lead to the inhibition of islet insulin secretion [27].

In clinical situations, the question still remains whether altered distribution of PLP contributes to the development of degenerative complications, or results from those complications. Because no significant correlations were found between plasma PLP levels and any of the biochemical risk indices of degenerative diabetes complications, such as plasma levels of fasting glucose, postprandial glucose, triglyceride, albumin, and total cholesterol, in either the non-diabetic or diabetic subjects of this study, the cause-effect nature of this relationship could not be clarified. However, considering the major complications that occur in diabetic patients, maintaining normal vitamin B<sub>6</sub> status is an important diet strategy for diabetic patients since retrospective studies have suggested the association between risk of cardiovascular disease and vitamin B<sub>6</sub> status, although the cause-effect nature of this relationship has not been clarified [28-31]. Prospective studies also suggest that populations with higher PLP concentrations have lower risk of coronary heart disease [32,33].

Therefore, despite the many uncertainties regarding the mode of action, these results suggest that plasma PLP levels should be monitored in pre-diabetic patients with diabetic risk factors and in newly diagnosed diabetic patients for long-term management of diabetes, even though this factor is not a major risk factor that contributes to the development of degenerative complications in certain patients. The principal limitations of this study were the cross-sectional design and that a causative nature of the association could not be established.

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