Changes in Plasma Pyridoxal 5'-Phosphate Concentration during Pregnancy Stages in Japanese Women

Katsumi Shibata, Akiko Tachiki, Kana Mukaeda, Tsutomu Fukuwatari, Satoshi Sasaki and Yoshiki Jinno

1 Department of Nutrition, School of Human Cultures, The University of Shiga Prefecture, Hikone, Shiga 522–8533, Japan
2 Department of Social and Preventive Epidemiology, School of Public Health, The University of Tokyo, Bunkyo-ku, Tokyo 113–0033, Japan
3 Jinno Ladies Clinic, Hikone, Shiga 522–0063, Japan

(Received February 21, 2013)

Summary  Most Japanese women do not consume the estimated average requirement of vitamin B6 (1.7 mg/d) during pregnancy. Nevertheless, these deficiencies are not reported. We investigated a nutritional biomarker of vitamin B6 in pregnant Japanese women as well as their vitamin B6 intakes. Vitamin B6 intakes in the first, second, and third trimesters of pregnancy, and 1 mo after delivery were 0.79±0.61 (n=56), 0.81±0.29 (n=71), 0.90±0.35 (n=92), and 1.00±0.31 (n=44) mg/d, respectively. Plasma pyridoxal 5'-phosphate (PLP) concentrations in the first, second, and third trimesters of pregnancy, and 1 mo after delivery were 57.1±27.6 (n=56), 23.3±16.7 (n=71), 18.3±12.5 (n=92), and 43.9±33.4 (n=44) nmol/L, respectively. The plasma concentrations significantly decreased in the second and third trimesters of pregnancy compared to values from the first trimester (p<0.05), and these concentrations returned to the values of the first trimester of pregnancy 1 mo after birth.

Key Words  vitamin B6, pyridoxal 5'-phosphate, pregnant women, blood, requirement

Note

Animal and human studies have shown that plasma pyridoxal 5'-phosphate (PLP) concentrations decrease progressively during pregnancy, and large doses of vitamin B6 supplementation are required to maintain plasma PLP at early or pre-pregnancy levels (1–14). From this background, an additional amount of vitamin B6 is recommended for pregnant women in the Dietary Reference Intakes (DRIs) for Japan (15): the estimated average requirement (EAR) of vitamin B6 is set at 1.7 mg/d for the pregnant women. However, this value might be higher than what is truly necessary for pregnant Japanese women because nutrient intake surveys reported that intake of vitamin B6, 0.5 mg/1,000 kcal (16) and 1.0 mg/d (17), is far lower than the EAR. Nevertheless, there are no reports in the literature concerning deficiencies of vitamin B6 in pregnant Japanese women, such as abnormal metabolism of L-tryptophan (18) or abnormal electroencephalograms (19).

We recently reported that the urinary excretion of 4-pyridoxic acid, a major catabolite of vitamin B6, during pregnancy was not lower compared with the pre-pregnancy period (17). In our opinion, the present EAR for vitamin B6 for pregnant women is higher than the necessary amount. Thus, we measured plasma concentrations of PLP during pregnancy and 1 mo after delivery in Japanese women.

This study was reviewed and approved by the Ethical Committee of the University of Shiga Prefecture and was conducted according to the guidelines set forth in the Declaration of Helsinki.

The purpose and protocol of this study were explained to all participants. Before joining the study, each participant provided written informed consent. We excluded participants who had taken multi-vitamin supplements at least once during the previous month. Healthy pregnant Japanese women, all of whom were married, were recruited from a private obstetric hospital in Hikone, Japan, between May 2011 and December 2012. A total of 406 married Japanese women (99, 127, 127, and 53 women in the first, second, and third trimesters of pregnancy, and 1 mo after delivery, respectively) took part in the survey. To investigate the PLP concentrations in the plasma, whole blood samples were collected during a pregnancy checkup. Further, to determine the habitual intake of energy and nutrients by the participants, we gave a self-administered, comprehensive dietary history questionnaire (DHQ) (20–22) to the participants. However, 143 of the participants did not return the DHQ (43, 56, 35, and 9 women in the first, second, and third trimesters of pregnancy, and 1 mo after delivery, respectively). As a result, the number of the participants with the blood and DHQ data was 263 (56, 71, 92, and 44 women in the first, second, and third trimesters, and 1 mo after delivery, respectively).

Non-fasting blood samples (2 mL) were collected in a Venoject II (code no. VP-D052K; Terumo Corporation,
Tokyo, Japan) from a cubital vein during a pregnancy checkup. The collected blood samples were centrifuged at room temperature for 30 min at 1,500 \times g to separate plasma and particles. The resulting supernatants were retained and stored at \( -80^\circ C \) until analysis.

PLP monohydrate (C\(_{8}\)H\(_{10}\)NO\(_6\)P-H\(_2\)O \text{MW 265.16}) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemicals used were of the highest purity available from commercial sources. For measuring plasma PLP, 0.1 mL of thawed plasma was added to 0.1 mL of 5% metaphosphoric acid and the suspension was mixed well for 2 min. The mixture was centrifuged for 15 min at 18,000 \times g (4 \text{ C}). The resulting supernatant was retained. The acidified supernatant (0.1 mL) was added to 0.1 mL of methylene chloride and the suspension was mixed well for 2 min. The mixture was centrifuged for 15 min at 18,000 \times g (4 \text{ C}). The water fraction was retained and filtered through a 0.45-\mu m microfilter. The filtrate (20 \mu L) was directly injected into the HPLC system. The analytical method was based on the report by Rybak and Pfeiffer (23).

The non-parametric Kruskal-Wallis test following Dunn’s post test was used to analyze statistical differences among all life stages and \( p<0.05 \) was considered statistically significant. Statistical analysis was performed using GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA).

Subject characteristics are shown in Table 1. The average age of the sample was 30 y and the average height was 160 cm. Body weight and energy intake increased depending on the progress of pregnancy. Fat intake, in terms of percent energy, also increased throughout pregnancy. The intake of vitamin B\(_6\) stores is saturated because a PLP-albumin complex is released from the liver to the blood when the capacity of liver vitamin B\(_6\) stores is saturated (24). The cut-off value is 30 nmol/L (14). Table 2 shows the plasma PLP concentrations in the first, second, and third trimesters of pregnancy and 1 mo after delivery. The plasma PLP concentrations decreased in the second and third trimesters of pregnancy in the present experiment and returned to the values observed during the first trimester in the month after delivery.

Some of the subjects did not return the food survey questionnaire, the DHQ. Therefore, we could not analyze the correlation between plasma PLP concentrations and the intake of vitamin B\(_6\) for all participants.

**Table 1. Basic sample characteristics of pregnant Japanese women.**

<table>
<thead>
<tr>
<th>n</th>
<th>First trimester</th>
<th>Second trimester</th>
<th>Third trimester</th>
<th>1 mo after delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>56</td>
<td>71</td>
<td>92</td>
<td>44</td>
</tr>
<tr>
<td>Age (y)</td>
<td>30.3±4.8</td>
<td>29.3±4.3</td>
<td>30.3±4.9</td>
<td>31.2±5.9</td>
</tr>
<tr>
<td>Pregnancy wk</td>
<td>10.6±1.4( ^a )</td>
<td>27.2±3.0( ^b )</td>
<td>35.8±1.4( ^c )</td>
<td>—</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>159.7±5.3</td>
<td>158.8±5.8</td>
<td>159.9±5.8</td>
<td>158.8±7.4</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>53.5±9.5( ^a )</td>
<td>57.8±7.3( ^b )</td>
<td>61.6±7.5( ^c )</td>
<td>55.5±7.4( ^b )</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>21.0±3.8( ^a )</td>
<td>22.9±2.6( ^b )</td>
<td>24.1±2.3( ^b )</td>
<td>22.8±5.6( ^a )</td>
</tr>
<tr>
<td>Energy intake (kcal/d)</td>
<td>1.498±56.2( ^a )</td>
<td>1.579±410( ^b )</td>
<td>1.714±442( ^a )</td>
<td>2.112±132( ^a )</td>
</tr>
<tr>
<td>Protein intake (% E)</td>
<td>12.4±2.2</td>
<td>13.0±2.0</td>
<td>13.2±1.9</td>
<td>12.8±1.7</td>
</tr>
<tr>
<td>Fat intake (% E)</td>
<td>26.2±6.4( ^a )</td>
<td>30.2±6.0( ^b )</td>
<td>30.4±5.3( ^b )</td>
<td>29.2±5.5( ^a )</td>
</tr>
<tr>
<td>Carbohydrate intake (% E)</td>
<td>60.5±7.5( ^a )</td>
<td>55.6±6.4( ^b )</td>
<td>55.5±6.1( ^b )</td>
<td>57.0±6.3( ^b )</td>
</tr>
<tr>
<td>Vitamin B(_6) intake (mg/d)</td>
<td>0.79±0.61( ^a )</td>
<td>0.81±0.29( ^b )</td>
<td>0.90±0.33( ^b )</td>
<td>1.00±0.31( ^c )</td>
</tr>
<tr>
<td>Vitamin B(_6) intake (mg/1,000 kcal)</td>
<td>0.50±0.18</td>
<td>0.51±0.12</td>
<td>0.52±0.13</td>
<td>0.53±0.12</td>
</tr>
</tbody>
</table>

Values are means±SD. The means in a row without a common superscripted letter differ significantly, \( p<0.05 \), as determined by the non-parametric Kruskal-Wallis test following Dunn’s post test.

**Table 2. Plasma PLP concentrations in women during the first, second, and third trimesters of pregnancy and 1 mo after delivery.**

<table>
<thead>
<tr>
<th>Number of all participants</th>
<th>First trimester</th>
<th>Second trimester</th>
<th>Third trimester</th>
<th>1 mo after delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma PLP (nmol/L)</td>
<td>55.9±32.2( ^a )</td>
<td>22.3±13.6( ^b )</td>
<td>19.3±12.9( ^b )</td>
<td>42.9±30.5( ^a )</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of participants returning DHQ</th>
<th>First trimester</th>
<th>Second trimester</th>
<th>Third trimester</th>
<th>1 mo after delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma PLP (nmol/L)</td>
<td>57.1±26.6( ^a )</td>
<td>23.3±16.7( ^b )</td>
<td>18.3±12.5( ^b )</td>
<td>43.9±33.4( ^a )</td>
</tr>
</tbody>
</table>

Values are expressed as means±SD. The means in a row without a common superscripted letter differ significantly, \( p<0.05 \), as determined by the non-parametric Kruskal-Wallis test following Dunn’s post test.
Consequently, we selected the data of the participants who returned the DHQ. The selected data were similar to those of all the participants (Table 2). We analyzed the relationship between the vitamin B6 intake and the plasma PLP concentrations. Plasma PLP concentrations were divided into three groups depending upon vitamin B6 intakes: bottom, middle, and upper tertile (Fig. 1). No significant relationships between the plasma PLP concentrations and the vitamin B6 intakes were observed in the first, second, or third trimesters of pregnancy, or 1 mo after delivery. Figure 1 also shows each value of plasma PLP of the participants. The percentages of the participants below the cut-off value were 16% (9/56), 82% (58/71), 84% (77/92), and 43% (19/44) in the first, second, third trimester for pregnancy and at 1 mo after delivery.

Previous studies from other laboratories have demonstrated a drop in plasma PLP to <15 nmol/L (2, 7, 8). In the present experiment, the plasma PLP concentrations decreased to 18 nmol/L during the third trimester. Maintenance of plasma PLP concentrations at non-pregnant values requires about 2 mg/d of supplemental pyridoxine during the first trimester and between 4 and 10 mg/d during the third trimester (2, 6, 11, 12, 25). Some investigators reported that umbilical cord plasma PLP concentrations were much higher than in maternal plasma PLP (6, 11, 12). This phenomenon might be attributed to the accelerated reaction of PLP to pyridoxal (PL) that is catalyzed by serum alkaline phosphatase (10). In fact, Barnard et al. (10) showed lower plasma PLP concentrations and higher PL concentrations in pregnant women compared to non-pregnant women. Therefore, the decrease in plasma PLP concentrations might be a physiological phenomenon during pregnancy rather than a vitamin B6 deficiency. An experiment using a mouse model shows that metabolism or utilization of vitamin B6 is altered in pregnancy (4). The determination of the optimal dose during pregnancy is still needed.

**Acknowledgments**

This investigation was part of the project Studies on the Dietary Reference Intakes for Japanese (principal investigator, Sinkan Tokudome), which was supported by a Research Grant for Comprehensive Research on Cardiovascular and Life-Style Related Diseases from the Ministry of Health, Labour and Welfare of Japan.

**REFERENCES**


