Adequacy of Maternal Pyridoxine Supplementation during Pregnancy in Relation to the Vitamin B₆ Status and Growth of Neonates at Birth

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Summary To evaluate the adequacy of maternal pyridoxine supplementation during pregnancy for both maternal and neonatal status at birth, vitamin B₆ status, assessed by plasma pyridoxal phosphate (PLP), pyridoxal (PL) and total aldehyde vitamer (PLP+PL) concentrations, and the growth of neonates, including weight, length, head and chest circumferences, were examined for 209 neonates whose mothers were supplemented with 0, 1, 2 or 3 mg pyridoxine·HCl (PN·HCl)/d during pregnancy. Maternal PN·HCl supplementations were positively correlated to both maternal (r=0.62) and cord (r=0.78) plasma PLP concentrations (p<0.0001). Mothers supplemented with 2 or 3 mg/d PN·HCl had significantly higher plasma concentrations of PLP and total B₆ aldehyde vitamer in maternal and cord blood compared with those receiving 0 or 1 mg PN·HCl/d. A growth benefit for neonates whose mothers had maternal and cord plasma PLP concentrations ≥40 nm was revealed by the maternal supplementation of 2 mg PN·HCl/d during pregnancy. Thus, in healthy pregnant women, according to our study, a daily supplement of 2 mg PN·HCl provides the adequacy of maternal and neonatal vitamin B₆ status and the satisfactory growth of neonates at birth.

Key Words pyridoxine supplement, B₆ vitamers, nutritional status, pregnancy, neonate

Maternal vitamin B₆ supplementation has been studied extensively, yet information concerning adequacy for the vitamin during pregnancy is equivocal. The current U.S.RDA was based on the additional vitamin B₆ needed for the increased protein allowance for pregnancy (1). No allowance was made to compensate for either increased fetal and maternal metabolic needs for vitamin B₆ (2) or hormonal induction of vitamin B₆ dependent enzyme (3–6). Studies show that pregnant women have significantly lower levels of both vitamin B₆ and pyridoxal-5-phosphate (PLP) in plasma and decreased activities of erythrocyte

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alanine aminotransferase (ALAT), as well as decreased PLP saturation of the enzyme compared to values for nonpregnant women (7–9). PLP levels in maternal plasma in late pregnancy and in cord plasma were particularly low in women who did not take vitamin B₆ supplements during pregnancy.

Several studies show that pregnant women require at least 4 mg/d of vitamin B₆. Levels up to 10 mg/d may be needed by some women during pregnancy to normalize both maternal and fetal biochemical indices of vitamin B₆ status (2, 7, 10–13). The essentiality of vitamin B₆ for normal development of the central nervous system of rat pups is well documented (14–16). In human studies, a multiple-regression analysis of nutrient and non-nutrient variables showed that the vitamin B₆ status of the mother was the strongest factor affecting neurobehavioral development of infants and maternal–infant interactions (17). Additionally, Heiskanen et al. (18) suggested that gestationally accumulated stores were important for the maintenance of adequate vitamin B₆ status of infants during the early months of infancy.

The importance of vitamin B₆ sufficiency for the pregnant women is evident. Hence, this study was designed to evaluate the adequacy of maternal vitamin B₆ supplementation in relation to the vitamin B₆ status of both mother and neonate, and the general well-being of neonates at birth. Both plasma PLP and PL measurements were suggested requirements for an accurate assessment of vitamin B₆ nutritional status and a proper understanding of vitamin B₆ physiology, especially during pregnancy (19–21). Maternal vitamin B₆ supplementation and nutritional status data, in conjunction with that for vitamin intake, plasma PLP and PL concentrations and growth of neonates were used to determine both the effect of maternal vitamin B₆ supplementation on the vitamin B₆ status of mother and neonate and on the growth of the neonate.

**MATERIALS AND METHODS**

Two-hundred-and-nine pregnant women in this study were clients attending the National Cheng Kung University Hospital, Tainan Municipal Hospital and the private H. J. Cheng Obstetrics and Gynecology Hospital located in Tainan, Taiwan. They volunteered to participate in the study during their initial clinic visit. This study was reviewed and approved by the Committee on Clinical Research of the Department of Health, Executive Yuan, R.O.C. (Project No. DOH 81-TD-101), and written informed consent was obtained from all participants at the beginning of the study. The subjects were in good health and none of them were known to suffer from diseases such as diabetes, chronic hypertension, or kidney disease. None had used oral contraceptives or drugs known to interfere with vitamin B₆ metabolism within 1 yr of the current pregnancy.

The women were divided into four groups according to their use of pyridoxine·HCl (PN·HCl) supplements during their pregnancies. Groups I–IV were comprised of prospective mothers who received supplements of 0, 1, 2, or 3 mg/d,
respectively; the number of mother-infant pairs were 83, 63, 43 and 20 per group, respectively.

As we could not analyze the dietary vitamin B₆ intake of the mothers, it was estimated from a food frequency table. Mothers were asked to record their vitamin B₆ intake including amounts of PN·HCl supplementation and the intake frequency of high-vitamin B₆ content food (>0.1 mg B₆/100 g food). Vitamin B₆ content of food in the frequency table was calculated via a Food Composition Table for use in East Asia (22).

A 10 mL sample of cord blood and 5 mL sample of maternal venous blood were collected from mothers and placed in heparinized tubes at the time of delivery. Blood samples were centrifuged immediately and the plasma was stored at -30°C in a vial protected from light until analysis. Plasma samples were deproteinized by 0.5 M perchloric acid and used for determining plasma PLP and PL concentrations by chromatographic separation modified from Edwards et al (23). The HPLC system (Hitachi, Japan) consisted of the following components: a model L-7100 solvent delivery system (Hitachi), a model F-1050 fluorescence detector (Hitachi), and a model SISC-LAB data processor. The mobile phase consisted of a mixture of phosphate buffer (pH 3.0, 0.1 M): methanol = 80:20 and 0.5 g/L sodium bisulfite. The flow rate was 1.0 mL/min in an experimental environment. The mobile phase solvents were degassed by vacuum filtration through a 0.22 μm cellulose acetate filter (Sartorius GmbH, W. Germany). Fluorescence detector excitation and emission wavelengths were 300 and 400 nm, respectively. A 100 μL sample was injected onto a reversed-phase HPLC column (Mightsil RP-18GP, 5 μm particle size, 250 × 4.6 mm, Kanto Chemical, Tokyo, Japan). Plasma PLP and PL concentrations were identified and quantified by comparing retention times and peak areas with standards.

Recoveries of B₆ vitamers were determined by spiking plasma samples with vitamers (final concentration 100 nM) before the deproteinization step and comparing the increase in vitamer concentration in relation to that added. Recoveries of PLP and PL added to plasma by the HPLC method averaged 94 ± 3% and 95 ± 4% (± SD), respectively.

Within-assay reproducibility was determined by analysis of six replicate plasma samples. The precision of PLP and PL, expressed as coefficients of variation (CV), was 3.0 and 3.1%, respectively. The CV was calculated by dividing the SD by the mean × 100.

Length, weight, and head and chest circumference measurements of the neonates were obtained at birth. Apgar scores of neonates at 1 and 5 min after birth were also collected. All measurements were made by one individual, a trained pediatric nurse. An electronic balance (New-series, New-20K, Japan) was used to obtain neonatal weights. Lengths were measured with a portable tape board equipped with a head and foot board. Pediatric tapes were used for head and chest circumference measurements.

A computer program from the Statistical Analysis System (SAS) (24) was used.
One-way ANOVA was performed on plasma concentrations of B₆ vitamers in cord and maternal blood, anthropometric measurements and Apgar scores at delivery. Student’s t-test was used to distinguish differences (p<0.05) among individual means. Pearson γ correlation coefficients were determined from a regression analysis to evaluate the relationships among certain variables.

RESULTS

Pregnancy outcome

The mean age of pregnant women in this study was 29.1±3.9 yr. Body weight and height were 50.1±5.7 kg and 157.6±4.4 cm, respectively. Body mass index (BMI) was 20.2±2.2 kg/m². Average weight gain during pregnancy was 14.5±4.0 kg. No significant differences were found between maternal weight gain during pregnancy and the growth of infant at birth, including Apgar score, weight, length, and head and chest circumferences. General information of the pregnant women supplemented with different levels of PN·HCl is listed in Table 1. The lengths of prenatal supplement for the four groups were similar and ranged from 6.7 to 7.7 mo. There were no significant differences for age, length of gestation, parity, weight, height, BMI and weight gain during pregnancy among the four groups.

Vitamin B₆ intakes of mothers

The mean dietary vitamin B₆ intake of the pregnant women was not significantly different among the groups, and approximately 1.0 mg/d. Among these, 0.75 mg/d of vitamin B₆ was ingested from rice which was the main dish of these women. The differences of total vitamin B₆ intake among the groups were attributed to the
Maternal Vitamin B₆ Supplement and Neonatal Condition

Table 2. Mean concentrations of PLP, PL and total B₆ aldehyde vitamers in maternal plasma at delivery.¹

<table>
<thead>
<tr>
<th>PN·HCl supplement (mg/d)</th>
<th>PLP (nm)</th>
<th>PL (nm)</th>
<th>Total B₆ aldehyde vitamer (nm)</th>
<th>PL/PLP ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>18 ± 6ᵃ</td>
<td>13 ± 6ᵃ</td>
<td>30 ± 9ᵃ</td>
<td>0.65</td>
</tr>
<tr>
<td>1</td>
<td>20 ± 9ᵇ</td>
<td>22 ± 10ᵇ</td>
<td>43 ± 10ᵃ</td>
<td>1.09</td>
</tr>
<tr>
<td>2</td>
<td>43 ± 10ᵇ</td>
<td>26 ± 6ᵇ</td>
<td>68 ± 6ᵇ</td>
<td>0.59</td>
</tr>
<tr>
<td>3</td>
<td>58 ± 8ᵇ</td>
<td>33 ± 11ᵇ</td>
<td>91 ± 17ᵇ</td>
<td>0.56</td>
</tr>
</tbody>
</table>

¹ Mean ± SD.
Means within a column which do not have a common superscript letter are significantly different from each other (p < 0.001).

Table 3. Mean concentrations of PLP, PL and total B₆ aldehyde vitamers in cord plasma at delivery.¹

<table>
<thead>
<tr>
<th>PN·HCl supplement (mg/d)</th>
<th>PLP (nm)</th>
<th>PL (nm)</th>
<th>Total B₆ aldehyde vitamer (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>29 ± 6ᵃ</td>
<td>15 ± 8</td>
<td>44 ± 11ᵃ</td>
</tr>
<tr>
<td>1</td>
<td>40 ± 8ᵃ</td>
<td>14 ± 4</td>
<td>55 ± 9ᵃ</td>
</tr>
<tr>
<td>2</td>
<td>78 ± 2ᵇ</td>
<td>14 ± 2</td>
<td>91 ± 2ᵇ</td>
</tr>
<tr>
<td>3</td>
<td>90 ± 7ᵇ</td>
<td>16 ± 6</td>
<td>106 ± 6ᵇ</td>
</tr>
</tbody>
</table>

¹ Mean ± SD.
Means within a column which do not have a common superscript letter are significantly different from each other (p < 0.001).

amounts of supplementation.

Vitamin B₆ status of mother and infant

The mean concentrations of PLP in the maternal plasma taken at delivery from mothers supplemented with 0 or 1 mg PN·HCl/d during pregnancy were statistically similar (18 ± 6 and 20 ± 9 nm, respectively) but significantly lower than those of mothers supplemented with 2 or 3 mg PN·HCl (43 ± 10 and 58 ± 8 nm, respectively) (Table 2). Similarly, the mean concentrations of PLP in the cord plasma of mothers who received 0 or 1 mg PN·HCl/d during pregnancy were 29 ± 6 and 40 ± 8 nm, respectively. These values were significantly lower than those of mothers who received 2 or 3 mg PN·HCl (78 ± 2 and 90 ± 7 nm, respectively) (Table 3).

Maternal PN·HCl supplements during pregnancy were significantly correlated to the PLP and total B₆ aldehyde vitamer of the maternal and cord plasmas (Table 4). PLP and total B₆ aldehyde vitamer concentrations in the cord and maternal plasmas were likewise correlated (Table 5).

Vol 45, No 4, 1999
Table 4. Correlation of maternal PN·HCl supplements to plasma PLP, PL and total B₆ aldehyde vitamers in maternal and cord blood at delivery.

<table>
<thead>
<tr>
<th>PN·HCl supplements (mg/d)</th>
<th>Pearson correlation coefficient (r)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlated with maternal measurements</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma PLP (nm)</td>
<td>0.62</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Plasma PL (nm)</td>
<td>-0.07</td>
<td>NS</td>
</tr>
<tr>
<td>Total B₆ aldehyde (nm)</td>
<td>0.41</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td>Correlated with cord measurements</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma PLP (nm)</td>
<td>0.78</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Plasma PL (nm)</td>
<td>-0.14</td>
<td>NS</td>
</tr>
<tr>
<td>Total B₆ aldehyde (nm)</td>
<td>0.61</td>
<td>&lt;0.0002</td>
</tr>
</tbody>
</table>

NS means not significantly different.

Table 5. Correlation of maternal vitamin B₆ status to measurements of cord vitamin B₆ status at delivery.

<table>
<thead>
<tr>
<th>Cord vitamin B₆ status</th>
<th>Maternal vitamin B₆ status</th>
<th>PLP</th>
<th>PL</th>
<th>Total B₆ aldehyde</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLP</td>
<td>r = 0.85</td>
<td>NS</td>
<td></td>
<td>r = 0.66</td>
</tr>
<tr>
<td></td>
<td>p &lt; 0.0001</td>
<td></td>
<td></td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>PL</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Total B₆ aldehyde</td>
<td>r = 0.71</td>
<td>NS</td>
<td></td>
<td>r = 0.57</td>
</tr>
<tr>
<td></td>
<td>p &lt; 0.0001</td>
<td></td>
<td></td>
<td>p &lt; 0.001</td>
</tr>
</tbody>
</table>

NS means not significantly different.

Table 6. Growth of neonate with various concentrations of plasma PLP in cord blood at birth.¹

<table>
<thead>
<tr>
<th>Plasma PLP in cord (nm)</th>
<th>Weight (kg)</th>
<th>Length (cm)</th>
<th>Head circumference (cm)</th>
<th>Chest circumference (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;40</td>
<td>3.0 ± 0.3ᵃ</td>
<td>48.7 ± 1.6</td>
<td>33.7 ± 1.2</td>
<td>32.4 ± 1.3</td>
</tr>
<tr>
<td>40–50</td>
<td>3.3 ± 0.4ᵇ</td>
<td>50.2 ± 2.1</td>
<td>33.7 ± 1.4</td>
<td>32.7 ± 2.1</td>
</tr>
<tr>
<td>≥50</td>
<td>3.4 ± 0.3ᵇ</td>
<td>50.7 ± 2.4</td>
<td>34.8 ± 1.1</td>
<td>34.2 ± 1.6</td>
</tr>
</tbody>
</table>

Mean ± SD.
Means within a column which do not have a common superscript letter are significantly different from each other (p < 0.001).
Growth of neonates

The growth measurements at birth including weight, length, and head and chest circumferences were not significantly different among neonates whose mothers were supplemented with different levels of PN·HCl/d. However, the birth weights of neonates born with PLP concentrations of ≥40 nM in the cord plasma were significantly greater than those with <40 nM PLP at birth (Table 6). Length, head and chest circumferences of neonates with plasma PLP of ≥40 nM were greater than those with <40 nM PLP, although the difference was not statistically significant.

DISCUSSION

General information regarding the pregnant women who participated in this study as compared with those in a previous study (25) showed that BMI and weight gain during pregnancy increased from 18.5 kg/m² to 20.2 kg/m² and 13.9 kg to 14.5 kg, respectively. An improvement of the nutritional status found in this study was due to increased nutrient intake in Taiwan (26). Weight gain of the pregnant women during pregnancy in this study was 14.5 ± 4 kg, which was greater than that for those in the Taipei area studied during 1977–1978 (13.8 ± 3.3 kg) or those in Tokyo (12.4 ± 2.9 kg) (27). A relationship between weight gain during pregnancy and the growth of the infant at birth, including Apgar score, birth weight, length, chest and head circumferences, was not found in this study. Susser (27) reviewed studies in Germany (28), the USA (29), Canada (30), Colombia (31), Taiwan (32, 33), UK (34, 35) and Chile (36) and found that diet effects on birth weight apparently bypass maternal weight changes. Hence, he suggested that maternal diet appeared to deserve more attention than does weight gain in order to enhance the birth weight of neonates.

The mean dietary intake of vitamin B₆ of pregnant women who participated in this study was 1.0 mg/d. This value was representative and showed that dietary vitamin B₆ intake of the pregnant women in this study was low. This value was less than that of 1.48 ± 0.01 mg/d for a healthy adult (37) or 1.4 mg/d for lactating women (13) and 1.43 ± 1.58 mg/d for pregnant women (12). In the present study, 40% of the pregnant women did not take any vitamin B₆ supplement. Thirty percent of the pregnant women took 1.0 mg/d of vitamin B₆ supplement. Therefore, the daily vitamin B₆ intake of the majority of the women did not meet the 2.2 mg/d of the current U.S.RDA (1) or the 2.4 mg/d of RDNA (38) for pregnant women.

Maternal PN·HCl supplementations were positively correlated, not only to maternal PLP concentrations but also to the PLP concentration of cord plasma. This result indicated that maternal vitamin B₆ intake during pregnancy affected vitamin B₆ nutritional status of both the mother and neonate. Additionally, vitamin B₆ measurements, including total vitamin B₆, PLP and total B₆ aldehyde, in both maternal and cord plasmas were significantly correlated to each other for each parameter. These results indicated that vitamin B₆ intake of the mother during pregnancy was a strong indicator of neonatal vitamin B₆ status.

Vol 45, No 4, 1999
Mothers supplemented with 2 or 3 mg/d PN•HCl had significantly higher plasma concentrations of PLP and total B₆ aldehyde vitamer in the maternal and cord blood compared with those supplemented with 0 or 1 mg PN•HCl/d. If plasma PLP of <30 nM is used as a cut-off point for vitamin B₆ inadequacy (39), the results of the present study (Tables 2 and 3) suggest that 2 mg PN•HCl/d of maternal supplement resulted in relatively optimal levels of vitamin B₆ nutritional status in both the mother and neonate.

Growth measurements for neonates with plasma PLP concentrations of ≥40 nM were greater than those with <40 nM PLP (Table 6). Low vitamin B₆ status associated with failure to gain weight (40, 41) and reduced gain in length (41, 42) has been found in human infants. The Apgar scores at 1 and 5 min were also correlated with PLP concentrations in the cord plasma. The results of this study showed a growth benefit for neonates when the plasma PLP concentration in the placental cord was ≥40 nM, which was reflected by the maternal supplement of 2 mg PN•HCl/d during pregnancy.

In conclusion, maternal vitamin B₆ supplement affects the vitamin B₆ status of both the mother and neonate. A daily supplement of 2 mg PN•HCl during pregnancy was suggested to assure desirable nutritional status and growth of the neonate without compromising maternal status.

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REFERENCES


