Intrauterine elimination of pyridoxal 5′-phosphate in full-term and preterm infants

Gerold Link and Janos Zempleni

ABSTRACT This study addressed the intrauterine elimination of pyridoxal 5′-phosphate (PLP) in 15 preterm and 31 full-term infants, thereby providing estimates of fetal vitamin consumption as well as maternal vitamin requirements during pregnancy. Elimination was calculated as the difference in the plasma PLP concentration between umbilical vein and umbilical artery times the umbilical plasma flow. Plasma flow in the umbilical vein was calculated from pulsed Doppler ultrasonographic determination of blood flow and from the hematocrit value. Plasma PLP concentrations were assayed in maternal and umbilical veins and the umbilical artery; PLP concentrations were similar in preterm and full-term infants (P > 0.05). In both groups of infants the PLP concentration in the umbilical vein (preterm: 100.3 nmol/L; full-term: 63.9 nmol/L) was ninefold higher than in maternal circulation (P < 0.001). In full-term infants, PLP concentrations in maternal and umbilical veins correlated weakly (r = 0.358, P < 0.05), but no significant correlation was found in the preterm group (P > 0.05). The arteriovenous concentration gradient of PLP in cord vessels was higher in preterm infants (15.0 nmol/L) than in full-term infants (2.1 nmol/L), but the difference between groups was not significant (P > 0.05). Preterm infants eliminated 1.7 nmol PLP · kg⁻¹ · min⁻¹ in utero, whereas full-term infants eliminated 0.2 nmol PLP · kg⁻¹ · min⁻¹ (P < 0.05). The significantly higher plasma flow in preterm infants (116 mL · min⁻¹ · kg⁻¹) compared with full-term infants (78 mL · min⁻¹ · kg⁻¹) contributed to the higher PLP elimination in preterm infants. Am J Clin Nutr 1996;64:184-9.

KEY WORDS Pyridoxal 5′-phosphate, intrauterine supply and elimination, full-term infants, preterm infants

INTRODUCTION

The term vitamin B-6 includes the compounds pyridoxine, pyridoxal, and pyridoxamine as well as the 5′-phosphates of each of these; 4-pyridoxic acid is the principal urinary catabolite of vitamin B-6 in humans. Pyridoxal, pyridoxal 5′-phosphate (PLP), and 4-pyridoxic acid are quantitatively the most important vitamin B-6 compounds in plasma (1). Infants show higher blood vitamin B-6 concentrations than their mothers because of elevated PLP and pyridoxamine 5′-phosphate concentrations in cord blood (2, 3). Vitamin B-6 concentrations in blood are similar in preterm and full-term infants (4). Loading experiments with pyridoxine in pregnant women (5) and [¹⁴C]pyridoxine in pregnant rats (6) have suggested that PLP is quantitatively the most important placental transport form of vitamin B-6. PLP is the metabolite that is released into fetal circulation against a concentration gradient. However, transmembrane movement of PLP is controversial (7, 8).

The placental nutrient transfer is the sole vitamin source for the human fetus. Vitamins are transported to the maternal surface of the placenta via uterine artery. After uptake by the placenta, vitamins are released at the fetal surface of the placenta into the umbilical vein. The fetal metabolic waste is transported to the placenta via the two umbilical arteries (9). A higher compound concentration in the umbilical vein compared with the umbilical arteries indicates that the compound under consideration is incorporated into fetal tissues, metabolized, or excreted. In a previous report on mostly full-term infants, we showed that such an arteriovenous gradient exists for fetal PLP (10). However, no data on fetal plasma flow in cord vessels were obtained in the previous study, although determination of the arteriovenous concentration gradient in conjunction with assessment of umbilical plasma flow would allow one to calculate fetal PLP elimination. This approach was used previously to estimate fetal consumption of a variety of nutrients, eg, amino acids (11), glucose (12), and riboflavin (13). The aim of the present study was to assess PLP elimination in preterm and full-term infants, thereby providing estimates of fetal vitamin consumption as well as maternal vitamin requirements during pregnancy. Gestational age, maternal vitamin status, and umbilical plasma flow may influence intrauterine PLP turnover and were considered for analysis.

SUBJECTS AND METHODS

Subjects

Forty-six white women with uncomplicated pregnancies and their infants participated in the study (Table 1). Fifteen of these women delivered preterm infants (gestational age ≤ 36 wk) and 31 women delivered full-term infants (gestational age ≥ 36 wk).

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Received January 31, 1996.

Accepted for publication April 4, 1996.

TABLE 1
Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Preterm infants (n = 15)</th>
<th>Full-term infants (n = 31)</th>
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<tbody>
<tr>
<td>Maternal age (yr)</td>
<td>29 ± 5</td>
<td>26 ± 5</td>
</tr>
<tr>
<td>Gestational age (wk)</td>
<td>33 ± 3(^2)</td>
<td>40 ± 2</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>1963 ± 62(^2)</td>
<td>3415 ± 464</td>
</tr>
<tr>
<td>Birth length (cm)</td>
<td>43 ± 4(^2)</td>
<td>52 ± 2</td>
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\(^{1}\) ± SD.

\(^{2}\) Significantly different from full-term infants, \(P < 0.001\).

> 36 wk). Eleven preterm infants (73%) and six full-term infants (19%) were delivered by cesarean section. The body weight of all infants was above the 10th percentile according to the growth charts of Lubchenco et al (14). None of the infants showed chromosomal or congenital abnormalities. Mothers who used drugs known to interfere with vitamin B-6 metabolism were excluded from participation. No information was obtained about maternal dietary and smoking habits or use of vitamin supplements during pregnancy. The study design as outlined below was approved by the Ethics Committee of the Justus-Liebig-University Giessen and informed consent was obtained from all women before participation.

Blood samples

Three blood samples were collected from each mother-infant pair; one maternal blood sample was withdrawn from the antecubital vein within 1 h after delivery, and two fetal blood samples (from the umbilical artery and umbilical vein) were collected immediately after delivery before dissection of the cord. Blood samples (2 mL each) were collected into brown tubes to avoid light decomposition of PLP. Small portions of all blood samples were used to determine hematocrit values and to analyze blood gases (Corning 178 pH/blood gas analyzer; Ciba-Corning Diagnostics GmbH, Neuss, Germany).

Hematocrit determination was needed for calculation of umbilical plasma flow, which was derived from blood flow (see below). Blood gas analysis was necessary to confirm correct assignment of blood samples from cord vessels to the arterial and venous system. Correct sampling procedures were absolutely essential in our study. Blood gas analysis showed that blood withdrawals were done correctly, ie, pH and partial pressure of oxygen were higher in samples from umbilical vein than in samples from the artery, whereas the partial pressure of carbon dioxide was higher in arterial samples (Table 2). Blood not needed for determination of hematocrit and blood gases was centrifuged at 2500 \(\times\) g, and the plasma was frozen at \(-80\ °C\) until analyzed for PLP.

Sample analysis

The PLP concentration in plasma was determined by HPLC (10). No other vitamin B-6 compounds were measured. Plasma samples were protected against exposure to light by work under subdued light and by using brown tubes. Blood flow in the umbilical vein was assessed by pulsed Doppler ultrasonography as described previously (4). All flow determinations were made in quadruplicate by the same experienced investigator because the use of ultrasonography is subject to errors in precision and interobserver variation (15). The means of these quadruplicate analyses were used for further calculations. Values recorded for blood flow in the present study agreed well with data reported in the literature (see below). Flow determinations were made 11.3 ± 2.2 h (full-term infants) and 17.8 ± 32.5 h (preterm infants) before delivery. Times of flow determination were not significantly different between groups (\(P > 0.05\)). Blood flow was transformed into plasma flow by using the values obtained for hematocrit in the umbilical vein. Fetal PLP elimination was calculated as follows:

\[
\text{Elim}_{\text{PLP}} = (C_{\text{vein}} - C_{\text{artery}}) \times \text{flow} \quad (J)
\]

where \(\text{Elim}_{\text{PLP}}\) is fetal PLP elimination (nmol/min); \(C_{\text{vein}}\) is the PLP concentration in the umbilical vein (nmol/L); \(C_{\text{artery}}\) is the PLP concentration in the umbilical artery (nmol/L); and flow is the plasma flow in the umbilical vein (L/min).

Statistics

Means ± SDs are reported. Differences were assessed for significance by the Mann-Whitney \(U\) test (unpaired data, ie, preterm compared with full-term infants) and the Wilcoxon test (paired data, eg, umbilical artery compared with umbilical vein within the same study group) (16). Values were tested for normality by the Kolmogorov-Smirnov test. Differences were considered significant if \(P\) values were < 0.05. Tests of significance and linear-regression analysis were computed by using SPSS/PC+ (version 5.0.1; SPSS Inc, Chicago).

RESULTS

The blood flow in umbilical vein was higher in full-term infants than in preterm infants (Table 3). However, the plasma flow per kilogram body weight was higher in preterm infants because of their lower hematocrit and lower body weight. Values recorded for blood flow in this study agree well with previous studies in which umbilical vein blood flow (\(J \pm \text{SEM}\)) was reported to be 125 ± 7.5 (15) and from 117 ± 7.5 to 130 ± 8.2 mL/min/kg body wt\(^{-1}\) (17).

PLP concentrations in infants and their mothers (Table 4) were similar in the full-term and preterm groups (\(P > 0.05\)). In both groups, PLP concentrations were about ninefold higher in umbilical vein than in maternal vein. PLP concentrations in maternal plasma and in fetal plasma (full-term infants, umbilical vein) correlated significantly; no significant correlation was observed for the preterm group (Figure 1). However, even
in the full-term group the correlation was rather weak \((r = 0.358)\). We show only the regression analysis between PLP concentrations in umbilical vein and maternal circulation because fetal vitamin uptake is into the umbilical vein. In both infant groups, the correlation between maternal vein and umbilical arteries was not significant \((P > 0.05)\). As expected, there was a significant correlation between PLP concentrations in umbilical arteries and umbilical veins \((P < 0.001)\). Correlation coefficients of \(r = 0.965\) (preterm infants) and \(r = 0.920\) (full-term infants) were calculated for the two fetal compartments. For both groups of infants under study, the venous PLP concentration was significantly higher than that in umbilical arteries (Table 4). The significance of arteriovenous PLP concentration gradients in full-term infants depended on which statistical test was used. The Wilcoxon test showed a significant difference \((P < 0.05)\). The nonparametric Wilcoxon test was used in this situation because the PLP concentrations in umbilical vessels were not normally distributed. Arteriovenous PLP concentration gradients were \(15.0 \pm 20.4\) nmol/L in preterm infants and \(2.1 \pm 15.5\) nmol/L in full-term infants. However, the influence of gestational age on the concentration gradient was not significant because of the large interindividual variation \((P = 0.07)\). Fetal PLP elimination was about 10-fold higher in preterm infants than in full-term infants \((P < 0.05)\) when expressed per kilogram body weight (Table 5).

### DISCUSSION

Preterm and full-term infants had higher plasma PLP concentrations than their mothers, ie, both infant groups were capable of intrauterine PLP sequestration. Similar findings were reported for mostly full-term infants in previous studies when blood or plasma was analyzed \((2, 3, 10)\). The correlation between maternal and fetal plasma PLP concentrations in the present study was rather weak or not significant (preterm infants). The maternal plasma PLP concentrations were within the same range as reported previously for women supplemented with \(\approx 12.2\) \(\mu\)mol pyridoxine hydrochloride/d \((\leq 2.5\) mg/d) during pregnancy \((18)\). Presumably, a wider range of maternal plasma PLP concentrations needs to be investigated to see a physiologically relevant correlation between maternal and fetal vitamin status. In addition, the activity of plasma alkaline phosphatase is a major factor in the regulation of plasma PLP concentration, as indicated by high PLP concentrations in patients with low phosphatase activity \((19)\).

### TABLE 3

<table>
<thead>
<tr>
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<th>Preterm infants ((n = 15))</th>
<th>Full-term infants ((n = 31))</th>
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<tbody>
<tr>
<td>Blood flow (mL/min)</td>
<td>406 (\pm 126)</td>
<td>514 (\pm 129)</td>
</tr>
<tr>
<td>Blood flow (mL \cdot min(^{-1}) \cdot kg(^{-1}))</td>
<td>211 (\pm 33)</td>
<td>151 (\pm 34)</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.45 (\pm 0.06)</td>
<td>0.49 (\pm 0.05)</td>
</tr>
<tr>
<td>Plasma flow (mL/min)</td>
<td>224 (\pm 52)</td>
<td>265 (\pm 78)</td>
</tr>
<tr>
<td>Plasma flow (mL \cdot min(^{-1}) \cdot kg(^{-1}))</td>
<td>116 (\pm 26)</td>
<td>78 (\pm 21)</td>
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\(^1 x \pm SD.\)

\(^{2-4}\) Significantly different from full-term infants: \(^2 P < 0.01, ^3 P < 0.001, ^4 P < 0.05.\)

Our data agree with those of previous reports, which suggested that PLP is transported into fetal circulation \((5, 6)\). Otherwise, the higher PLP concentration in umbilical vein compared with that in arteries cannot be explained. When the placental perfusion technique and radiolabeled pyridoxal were used, Schenker et al \((8)\) showed that pyridoxal crosses the placenta readily in both directions but that transport is significantly greater in the direction of the fetus. Their data are most suggestive of passive transport of pyridoxal across the placenta. The pyridoxal fetal-to-maternal ratio never exceeded unity whereas PLP accounted for the accumulation of vitamin B-6 in the fetal circulation. The authors suggested that PLP is transported bound to protein analogous to the release of hepatic PLP into plasma.

In this study, preterm infants eliminated 1.7 nmol PLP \(\cdot \) kg\(^{-1}\) \cdot min\(^{-1}\) (2506 nmol PLP \(\cdot \) kg\(^{-1}\) \cdot d\(^{-1}\)) from plasma whereas full-term infants eliminated \(\approx 0.2\) nmol PLP \(\cdot \) kg\(^{-1}\) \cdot min\(^{-1}\) (245 nmol PLP \(\cdot \) kg\(^{-1}\) \cdot d\(^{-1}\)). The dependency of fetal PLP elimination on gestational age was not caused by differences in vitamin supply because the PLP concentration in maternal and umbilical veins was similar in the full-term and preterm groups. The greater intrauterine PLP elimination in preterm infants compared with their full-term counterparts resulted from both greater umbilical plasma flow and a greater arteriovenous concentration gradient of PLP. Values calculated for intrauterine PLP elimination showed large interindividual variation. However, the nonparametric tests used for significance testing reduced the influence of extreme values substantially. Nevertheless, we suggest a careful interpretation of the findings made for PLP elimination in full-term infants. The arteriovenous PLP concentration gradient in full-term infants was small and was significant only when a nonparametric test was used.

PLP elimination is not necessarily equivalent to utilization, ie, coenzyme incorporation into fetal tissues. Nevertheless, the fetal vitamin supply is obviously sufficient to guarantee a high degree of coenzyme (PLP) saturation in erythrocyte alanine aminotransferase \((20)\) and aspartate aminotransferase \((4)\) in preterm and full-term infants. Plasma alkaline phosphatase activity is \(\approx 30\)% higher in preterm infants than in full-term infants \((20)\). Correspondingly, the concentration of pyridoxal in cord plasma is \(\approx 50\)% higher in preterm infants and the ratio of pyridoxal to PLP is increased. High activity of plasma alkaline

### TABLE 4

<table>
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<th>Preterm infants ((n = 15))</th>
<th>Full-term infants ((n = 31))</th>
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<tbody>
<tr>
<td>Pyridoxal 5'-phosphate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal vein</td>
<td>10.7 (\pm 9.2)</td>
<td>7.3 (\pm 3.8)</td>
</tr>
<tr>
<td>Umbilical vein</td>
<td>100.3 (\pm 77.3)</td>
<td>63.9 (\pm 38.5)</td>
</tr>
<tr>
<td>Umbilical artery</td>
<td>85.3 (\pm 71.8)</td>
<td>61.8 (\pm 38.7)</td>
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</table>

\(^1 x \pm SD.\) No significant differences were found between full-term and preterm infants, \(P > 0.05\) (Mann-Whitney U test).

\(^2\) Significantly different from umbilical vein and umbilical artery, \(P < 0.001\) (Wilcoxon test).

\(^3-4\) Significantly different from umbilical artery: \(^3 P < 0.01, ^4 P < 0.05\) (Wilcoxon test).


**FIGURE 1.** Regression analysis of pyridoxal 5'-phosphate (PLP) concentrations in the plasma of maternal and umbilical veins (●, preterm infants; ■, full-term infants). In full-term infants (solid line) the regression line was $y = 3.64x + 37.2$ ($r = 0.358$, $P < 0.05$). In preterm infants the correlation between maternal and fetal PLP concentrations was not significant ($P > 0.05$). For completeness, the regression line for the preterm group (dashed line) was $y = 2.68x + 71.9$ ($r = 0.317$).

**TABLE 5**

<table>
<thead>
<tr>
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<th>Preterm infants ($n = 15$)</th>
<th>Full-term infants ($n = 31$)</th>
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<tr>
<td>Elim&lt;sub&gt;PLP&lt;/sub&gt; (nmol/min)</td>
<td>3.4 ± 4.2</td>
<td>0.5 ± 4.7</td>
</tr>
<tr>
<td>Elim&lt;sub&gt;PLP&lt;/sub&gt; (nmol·kg&lt;sup&gt;-1&lt;/sup&gt;·min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>1.7 ± 2.3&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.2 ± 1.3</td>
</tr>
<tr>
<td>Elim&lt;sub&gt;PLP&lt;/sub&gt; (nmol·kg&lt;sup&gt;-1&lt;/sup&gt;·d&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>2506 ± 3253&lt;sup&gt;2&lt;/sup&gt;</td>
<td>245 ± 1840</td>
</tr>
</tbody>
</table>

<sup>1</sup> ± SD. Elim<sub>PLP</sub>, fetal elimination of PLP.  
<sup>2</sup> Significantly different from full-term infants, $P < 0.05$.

Phosphatase in preterm infants may explain the increased PLP elimination observed in the present study. Presumably, PLP elimination is caused by hydrolysis to pyridoxal, followed by 1) uptake into tissues, 2) excretion into amniotic fluid, or 3) back-transport into maternal circulation. For most tissues, hydrolysis of PLP to pyridoxal is needed before transport from plasma into cells and subsequent intracellular phosphorylation by pyridoxine kinase (21, 22). The finding that PLP crosses erythrocyte membranes without prior hydrolysis (23) is apparently restricted to incubation in the absence of plasma proteins (7). The failure of premature infants (≤ 29 wk gestation) to produce increased plasma PLP concentrations after intravenous pyridoxine administration led to the initial assumption that PLP synthesis is impaired in these infants (24, 25). However, more extensive studies by the same researchers showed that very-low-birth-weight infants can, indeed, synthesize PLP as evidenced by the high concentration of PLP in erythrocytes (26) and by the high degree of coenzyme saturation in erythrocyte aspartate aminotransferase (4).

The vitamin B-6 concentration in amniotic fluid is ~6100 ng/L (27), i.e., 37 nmol/L if all the vitamin were present as pyridoxal. Excretion into amniotic fluid is probably not as PLP but as pyridoxal and 4-pyridoxic acid. Renal excretion of PLP is negligible even during intravenous administration of pharmacologic doses of pyridoxine hydrochloride into adults, associated with peak plasma PLP concentrations of ~900 nmol/L (1). Vitamin B-6 may be reabsorbed from amniotic fluid because mature fetuses near full term swallow ~500 mL amniotic fluid/d (28); the volume of amniotic fluid swallowed by immature fetuses ranges from 7 mL/d at 16 wk gestation to 120 mL/d at 28 wk gestation.

The back-transfer of vitamin B-6 metabolites from fetal plasma into maternal circulation should be considered a factor that contributes to vitamin elimination in fetuses (8). In previous studies of riboflavin, the placental ability of transporting riboflavin from the fetal surface into the maternal circulation was shown (29). However, riboflavin transport from fetal to maternal circulation was by passive diffusion whereas transport in the opposite direction was an active process.

Intrauterine elimination data may be useful for estimating optimal vitamin intake during pregnancy because the PLP eliminated by fetuses has been sequestered from maternal circulation. The data may also be useful to optimize dose regimens in enteral or parenteral infant nutrition. For full-term
infants, vitamin B-6 intake with breast milk and intrauterine PLP elimination are within the same order of magnitude. Breast milk contains \(\approx 770 \text{ nmol vitamin B-6/L} \) in women with adequate vitamin B-6 status (30). Approximately 60% of the vitamin in milk is present as pyridoxal (31). Assuming a milk intake of 800 mL/d and a body weight of 3 kg, breast milk provides \(\approx 0.14 \text{ nmol vitamin B-6·kg}^{-1} \cdot \text{min}^{-1} \) (205 nmol vitamin B-6·kg\(^{-1}\)·d\(^{-1}\)). PLP elimination in full-term infants is less than the suggested vitamin B-6 intake in infants receiving total parenteral nutrition (5900 nmol pyridoxine/d), whereas intrauterine elimination in preterm infants is substantially higher than the suggested parenteral dose (1100 nmol pyridoxine·kg\(^{-1}\)·d\(^{-1}\)) (32).

Some factors need consideration when deriving recommendations for vitamin intake from intrauterine vitamin elimination:

1. In utero, nutrients pass the metabolically active liver before reaching other organs whereas in parenteral nutrition the liver is bypassed by 75% of blood flow (33). Therefore, intrauterine vitamin metabolism resembles oral rather than parenteral vitamin B-6 administration (34).

2. Different vitamin B-6 analogs are utilized to a different extent, eg, pyridoxal is transformed more rapidly than is pyridoxine into the inactive 4-pyridoxic acid (35).

3. Intrauterine vitamin turnover does not provide information regarding adequate vitamin intake under special circumstances (disease, drug-nutrient interactions).

4. The amount of vitamin B-6 required for optimal development postpartum is not necessarily equal to the intrauterine elimination. Normal blood vitamin concentrations change during infancy with a peak erythrocyte PLP concentration and erythrocyte aspartate aminotransferase activity observed at the age of 4 mo (36).

5. Alterations in protein intake may influence vitamin B-6 requirements (37).

We conclude that the calculation of intrauterine PLP elimination provides useful information about fetal vitamin consumption and maternal vitamin B-6 requirements. Limitations of this approach are as noted above. The elimination of PLP is substantially greater in preterm than in full-term infants and suggests an important role of plasma alkaline phosphatase. The dependence of intrauterine vitamin B-6 turnover on gestational age justifies the different recommendations for vitamin B-6 intake for full-term and preterm infants.

REFERENCES

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