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Intrauterine Vitamin B_2 Uptake of Preterm and Full-Term Infants

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ABSTRACT

Intrauterine uptake of vitamin B_2 in preterm and full-term infants was examined. Factors of influence on vitamin supply were considered. Forty-four women and their infants were included in the study. Fetal vitamin uptake was calculated as arteriovenous concentration gradient in cord plasma times umbilical plasma flow. Concentration of vitamin B_2 (free riboflavin and flavocoenzymes) was determined by high performance liquid chromatography of placental tissue and blood plasma (maternal vein, umbilical artery, umbilical vein). Flavocoenzymes were analyzed as flavin mononucleotide after acid hydrolysis of flavin adenine dinucleotide. Umbilical plasma flow was measured using pulsed Doppler sonography. Both free riboflavin and flavocoenzymes were transferred from the maternal plasma to the umbilical vein, but only free riboflavin was accumulated (~1:4 for preterm and full-term infants, respectively). Flavocoenzyme concentration was higher in the umbilical vein than in the umbilical artery ($p < 0.05$). This indicated a median uptake of flavocoenzymes of 1.5 nmol/min·kg in preterm infants and 0.4 nmol/min·kg in full-term infants (preterm *versus* full-term, $p < 0.01$). Fetal vitamin supply depended on umbilical plasma flow and on maternal vitamin status (the latter was shown only in full-term infants). No dependence on placental vitamin concentration was observed ($p > 0.05$). Concentration of free riboflavin was higher in umbilical artery than in umbilical vein ($p < 0.05$). This indicated a release of free riboflavin from fetal tissues independent of gestational age (0.4 nmol/min·kg, preterm; 0.2 nmol/min·kg, full-term; $p > 0.05$). (***Pediatr Res* 38: 585–591, 1995**)

Abbreviations
FAD, flavin adenine dinucleotide
FMN, flavin mononucleotide

Arteriovenous concentration differences in umbilical blood have been reported for a variety of nutrients, *e.g.* amino acids or glucose (1, 2). In general, concentrations of these nutrients have been found to be more elevated in plasma from the umbilical vein than the umbilical artery. Such differences suggest a fetal uptake of nutrients (Fig. 1); however, they do not allow any quantitative assessment. To be able to quantify fetal uptake, it is not only necessary to determine arteriovenous gradients, but also plasma flow in cord vessels (4).

Reports about arteriovenous gradients of water-soluble vitamins in cord plasma are scarce (5). A description of arteriovenous gradients with simultaneous measurement of umbilical plasma flow is not available. In the case of vitamin B_2, an effective fetal extraction from blood plasma gains importance in view of earlier observations. A reduced transplacental transfer of vitamin B_2 was demonstrated in comparison to other water-soluble vitamins (5). Free riboflavin was accumulated in fetal circulation, but concentration of the flavocoenzymes, FAD and FMN, was higher in maternal plasma. The sums of maternal and fetal vitamin B_2 concentrations (free riboflavin and flavocoenzymes) did not differ. However, the necessity for sufficient riboflavin availability in the neonate organism is demonstrated by the enhanced requirement during treatment of hyperbilirubinemia with phototherapy (6, 7).

Several factors may influence intrauterine vitamin B_2 uptake, *i.e.* maternal vitamin status, placental transfer process, and umbilical plasma flow. Maternal vitamin B_2 status, assessed by the erythrocyte glutathione reductase coefficient, often becomes inadequate in the course of pregnancy (8, 9). In such instances a reduced transplacental transfer is expected to occur (10).

The placenta's role is threefold: to sequester free riboflavin and flavocoenzymes, to hydrolyze the majority of the flavocoenzymes to yield free riboflavin, and to release both free riboflavin and its coenzyme forms into fetal circulation (5, 11). The build-up of a riboflavin concentration gradient was also shown by *in vitro* placenta perfusion experiments (12, 13). A reduced activity of placental metabolism will diminish fetal vitamin supply.

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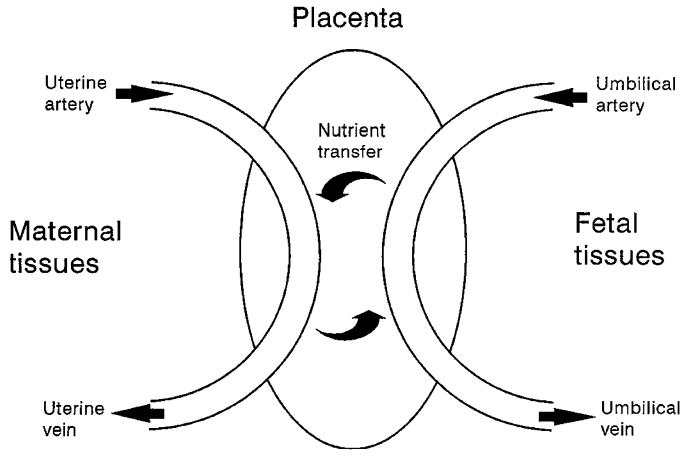

Figure 1. Diagram of the blood flow through the placenta (*arrows* indicate direction of blood flow). A simplified model of a concurrent exchanger is shown (3).

Once the vitamin has entered the umbilical vein plasma, umbilical blood flow becomes important for fetal supply, *i.e.* the transport of plasma to tissues. There is a marked increase in umbilical blood flow up to the 36th/37th week of gestation; afterward only a small increase is observed until term (14). Therefore, differences in vitamin supply may also depend on gestational age.

Our aim was to assess the intrauterine uptake of vitamin B_2 in preterm and full-term infants: *1*) the quantification of fetal tissue uptake (arteriovenous vitamin gradient times umbilical plasma flow) and *2*) the assessment of factors influencing this process (placental vitamin concentration, umbilical plasma flow, maternal vitamin status).

METHODS

Subjects. Forty-four pregnant women staying in the gynecologic ward of the University Clinic Giessen participated in our study. Depending on the gestational age at delivery participants were divided into two study groups. Infants born at ≤36 wk gestation were referred to as preterm (n = 13), whereas infants born >36 wk of gestation were referred to as full-term (n = 31). Characteristics of the two groups are given in Table 1. The smallest infant in the full-term group had a body weight of 2440 g. None of the infants attracted attention in postnatal routine check-up. Premature delivery was caused by early onset of labor or by rupture of the amniotic sac. Eight preterm infants (61.5%) and five full-term infants (16.1%) were delivered by cesarean section. Two risk pregnancies (diabetes mellitus, adiposis) were included in the full-term group.

Table 1. *Anthropometric characteristics of subjects**

	Preterm infants	Full-term infants
Maternal age (y)	28 ± 6	26 ± 5
Gestational age (wk)	34 ± 2	39 ± 2
Birth weight (g)	2141 ± 577	3361 ± 485
Birth length (cm)	44 ± 4	52 ± 2

* Means ± SD are reported; preterm infants (n = 13), full-term infants (n = 31).

The study design as outlined below was accepted by the Ethic Committee of the Justus-Liebig-University Giessen. Informed consent was obtained from each volunteer before participation.

Study design. Blood samples from a maternal vein, umbilical artery (a. umbilicalis) and umbilical vein (v. umbilicalis) were obtained from all subjects. Maternal blood as well as samples of cord blood were taken immediately after delivery. Vitamin concentration (blood plasma), hematocrit, pH, Po_2, and Pco_2 were determined in all blood samples. Analysis of acid base status was performed to ascertain the correct sampling procedure of arterial and venous blood withdrawal.

The umbilical blood flow in the umbilical vein was determined by pulsed Doppler sonography. The median time for blood flow determination was 8 h before delivery for both groups—between minimum and maximum ranges of 2–126 h for the preterm group and 1–60 h for the full-term group, respectively ($p > 0.05$). Blood flow was converted into plasma flow as described below.

In 35 cases (10 preterm and 25 full-term infants) placenta was taken from afterbirth and prepared for vitamin analysis as described below.

Data concerning maternal age and week of gestation and birth weight and length of infant were recorded for each case. Previous use of alcohol and vitamin supplements was not protocolled.

Sample preparation. Blood samples were collected in heparinized tubes. A small part of whole blood was used for determining hematocrit and for gas analysis, respectively. The majority of whole blood was centrifuged immediately to separate blood plasma (15 min, 2500 × g, 4°C). Plasma was immediately frozen at −80°C. Within 7 d it was transported to the Institute of Nutritional Science for analysis (working group of W. Kübler); transport was done in liquid nitrogen and took 15 min. Plasma was stored at −80°C until analysis. Samples were analyzed within 3 wk after withdrawal.

Placenta was weighed after delivery; aliquots were frozen immediately at −80°C. Transport in liquid nitrogen to the location of analysis where it was stored at −81°C took 5 min. Analysis was performed within 4 wk after collection. All samples were protected against light during preparation.

Vitamin analysis. Vitamin B_2 in blood plasma was analyzed by high performance liquid chromatography. The method used is described in detail elsewhere (5). In brief, FAD was converted to FMN by acid hydrolysis, flavocoenzymes were then determined as FMN. Free riboflavin and flavocoenzymes (as FMN) were separated by reversed-phase chromatography and detected fluorimetrically. Unless otherwise indicated in the following sections, the term *vitamin* B_2 is understood to comprise free riboflavin and flavocoenzymes (FAD, FMN), and the term *flavocoenzymes* means the sum of FAD and FMN.

The method used to determine placental vitamin concentration has been described elsewhere (15, 16). In brief, deep frozen (−81°C) pieces of placenta were homogenized with a Potter-Elvehjem grinder. The homogenates were deproteinated with 5% (wt/vol) trichloroacetic acid and centrifuged (6 min, 2000 × g). An acid hydrolysis of FAD to FMN was performed at 85°C in a water bath as with plasma analysis (5). The

solution was injected directly into the chromatographic system. Chromatographic conditions were the same as described for plasma analysis, except for some small modifications (column's particle size: 5 μm; flow rate of mobile phase: 0.8 mL/min; wavelengths on fluorescence detector set at 450/565 nm).

The size of placental vitamin B_2 pool was calculated as placental weight times vitamin concentration.

Umbilical blood flow. Umbilical blood flow was determined using pulsed Doppler sonography. Venous frequency shift was recorded at the placental origin of the umbilical vein. The diameter of the investigated segment was determined by measuring the distance between the proximal wall at the external surface and the distal wall at the internal surface of the vessel. The method has been described in detail elsewhere (14). The volume of umbilical blood flow was calculated according to equation 1.

$$Q = V \cdot d^2 \cdot \pi \cdot 0.15 \quad (1)$$

where Q = volume of umbilical blood flow (mL/min), V = mean velocity (cm/sec), and d = diameter of umbilical vein (mm).

All measurements of mean velocity of blood flow and diameter of umbilical vein were performed by the same physician to avoid interobserver variation. Each measurement was repeated three times (altogether four measurements). The blood flow in the umbilical vein was calculated from the means of consecutive measurements (coefficient of variation = 47.4%). Values obtained for blood flow were moderately higher than data previously recorded for the same working group (the earlier measurements were conducted by different physicians) (14). Data recorded in this study follow the values from the literature (17, 18) (see Discussion).

Umbilical plasma flow (QP, mL/min) was obtained from blood flow by correction for hematocrit of umbilical venous blood (equation 2).

$$QP = Q \cdot (1 - hematocrit/100) \quad (2)$$

Calculation of fetal vitamin uptake and release. Vitamin uptake by the fetus was calculated according to equation 3, fetal tissues being supplied by the umbilical vein. Release of vitamin from fetal tissues was calculated according to equation 4.

$$\text{Uptake} = (C_{vein} - C_{artery}) \times QP \quad (3)$$

$$\text{Release} = (C_{artery} - C_{vein}) \times QP \quad (4)$$

where C_{vein} = vitamin plasma concentration in umbilical vein (nmol/L), C_{artery} = vitamin plasma concentration in umbilical artery (nmol/L), and QP = plasma flow in umbilical vein (L/min). Vitamin uptake and release were calculated per kg of body weight.

Blood gas and pH analysis in umbilical vein and umbilical artery. Po_2, Pco_2 and pH were determined using a Corning 178 pH/blood gas analyzer (Ciba-Corning Diagnostics GmbH, Neuss, FRG).

Statistics. Unless otherwise indicated median values (minimum − maximum) are reported. Differences were assessed for significance by the Mann-Whitney U test (unpaired data, *e.g.* preterm *versus* full-term), and Wilcoxon-test (paired data, *e.g.* umbilical artery *versus* umbilical vein within the same study group). Differences were described as significant when $p < 0.05$. Tests of significance and linear regression analysis were computed using SPSS/PC+ (version 4.0; SPSS Inc., Chicago IL).

RESULTS

Blood gas and pH analysis. Due to the differences in vitamin concentration between the umbilical artery and the umbilical vein (see below), a reliable procedure for blood withdrawal was essential. We confirmed the correct allocation of arterial and venous blood by blood gas analysis and by measurement of pH. The pH was significantly higher in the umbilical vein compared with the umbilical artery ($p < 0.005$, preterm; $p < 0.001$, full-term; Table 2). Po_2 and Pco_2 of all samples was also used to show correct allocation: *i.e.* Po_2 was significantly higher in umbilical vein than in umbilical artery ($p < 0.005$, preterm; $p < 0.001$, full-term); Pco_2 was significantly lower in umbilical vein than in umbilical artery ($p < 0.005$, preterm; $p < 0.001$, full-term), respectively (Table 2).

Vitamin concentrations in maternal vein, umbilical artery, and umbilical vein. Vitamin concentrations in blood plasma of the maternal vein, umbilical artery, and umbilical vein did not differ significantly between both study groups (Table 3). Within the groups the levels of free riboflavin were significantly higher in the umbilical vein and umbilical artery compared with the maternal vein ($p < 0.005$ and $p < 0.01$, respectively). This means that levels were 4.2 times higher in the preterm group and 4.3 times higher in the full-term group (umbilical vein *versus* maternal vein). In both groups of infants we found slightly increased concentrations of free riboflavin in umbilical artery compared with umbilical vein ($p < 0.05$). These differences amounted to 1.1 nmol/L (preterm infants) and 2.0 nmol/L (full-term infants).

In the full-term group flavocoenzyme concentration was significantly higher in the blood plasma of the maternal vein compared with the umbilical vein and artery ($p < 0.005$ and $p < 0.01$, respectively; Table 3). Concentration was 1.6 times higher in the maternal circulation than in the umbilical vein. No such gradient was observed in the preterm group in which

Table 2. *Blood gas and pH analysis of umbilical vein and umbilical artery (whole blood)**

	Preterm infants		Full-term infants	
	Umbilical artery	Umbilical vein	Umbilical artery	Umbilical vein
pH	7.32 ± 0.06†	7.36 ± 0.07	7.32 ± 0.06‡	7.35 ± 0.07
Po_2 (atm)	0.030 ± 0.006†	0.037 ± 0.010	0.025 ± 0.007‡	0.032 ± 0.009
Pco_2 (atm)	0.061 ± 0.011†	0.054 ± 0.012	0.064 ± 0.009‡	0.055 ± 0.009

* Means ± SD are reported; preterm infants (n = 13), full-term infants (n = 31).

† $p < 0.005$ (differences between umbilical artery and umbilical vein of preterm infants).

‡ $p < 0.001$ (differences between umbilical artery and umbilical vein of full-term infants).

Table 3. *Concentrations of free riboflavin and flavocoenzymes in maternal vein, umbilical vein, and umbilical artery (blood plasma)**

	Preterm infants			Full-term infants		
	Maternal (nmol/L)	Umbilical artery (nmol/L)	Umbilical vein (nmol/L)	Maternal (nmol/L)	Umbilical artery (nmol/L)	Umbilical vein (nmol/L)
Free riboflavin	5.9†‡ (2.5–17.4)	26.1§ (13.4–63.6)	25.0 (13.3–54.1)	4.7†‡ (1.4–22.7)	22.2§ (10.6–62.0)	20.2 (7.4–59.7)
Flavocoenzymes	70.9† (36.5–174.5)	47.5§ (29.4–145.9)	71.8 (36.3–161.2)	90.7†‡ (61.0–156.4)	49.3§ (28.0–134.6)	55.8 (27.4–148.6)
Vitamin B_2	85.8 (49.3–177.6)	79.4§ (44.2–209.5)	92.6 (49.6–215.1)	94.9†‡ (62.4–159.1)	76.3 (49.8–165.6)	77.2 (45.8–158.3)

* Preterm (≤36 wk of gestation, $n = 13$), full-term (>36 wk of gestation, $n = 31$); median concentrations (minimum – maximum) are reported. FMN and FAD were analyzed as (total) flavocoenzymes. Vitamin B_2 comprises riboflavin, FMN, and FAD. No significant differences between preterm and full-term groups were observed ($p > 0.05$).

† $p < 0.01$ (differences between maternal vein and umbilical artery).

‡ $p < 0.005$ (differences between maternal vein and umbilical vein).

§ $p < 0.05$ (differences between umbilical artery and umbilical vein).

we detected equal concentrations of flavocoenzymes in the maternal vein and umbilical vein ($p > 0.05$), but significantly lower concentrations in the umbilical artery compared with the maternal vein ($p < 0.01$). Both groups of infants exhibited significantly higher concentrations of flavocoenzymes in the umbilical vein plasma than in the umbilical artery ($p < 0.05$). The difference amounted to 24.3 nmol/L (preterm infants) and 6.5 nmol/L (full-term infants). The difference between the two groups was not quite significant ($p = 0.05$).

In the preterm group, plasma concentration of (total) vitamin B_2 did not differ significantly between the maternal vein and the cord vessels ($p > 0.05$), but was higher in the umbilical vein than in the umbilical artery ($p < 0.05$). In the full-term group, concentration of vitamin B_2 was higher in the maternal vein than in the umbilical vein and artery ($p < 0.005$ and $p < 0.01$, respectively).

A linear regression analysis was performed to investigate the influence of maternal vitamin status on fetal supply. For this purpose we chose the values obtained for total vitamin B_2 instead of its single metabolites. By doing this we were able to circumvent the complex interrelationships of metabolites during transplacental transfer. Only subjects for whom complete sets of parameters were obtained (blood plasma and placenta) were considered for regression analysis (see below). Statistical significance of regression analysis for preterm infants (umbilical vein *versus* maternal vein) depended on one extreme data point (215.1 *versus* 126.7 nmol/L). Exclusion of this data point from analysis resulted in a failure to detect a statistical significance ($p > 0.05$; Fig. 2). Due to this observation and the low number of available cases ($n = 10$), we decided not to show a regression line. Contrary to the preterm infants, a strong dependence of fetal vitamin concentration (umbilical vein) on maternal status was found for full-term infants ($p < 0.005$; Fig. 3).

Vitamin concentrations in placental tissue. In placental tissue, flavocoenzymes were more abundant than free riboflavin. In the preterm group, placental concentration of free riboflavin and flavocoenzymes was 0.24 nmol/g wet weight (0.01–0.79) and 3.36 nmol/g wet weight (0.12–16.99), respectively. In the full-term group, placental concentration of free riboflavin and flavocoenzymes reached 0.27 nmol/g wet weight

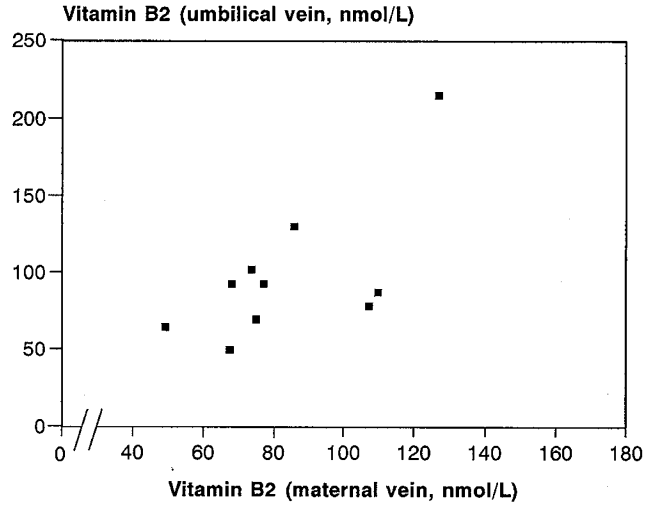

Figure 2. Concentration of vitamin B_2 (free riboflavin + flavocoenzymes) in blood plasma of umbilical vein *vs* maternal vein in preterm infants ($n = 10$). Statistical significance of regression analysis depended on one extreme data point (215.1 *vs* 126.7 nmol/L). Exclusion of this data point from analysis resulted in a failure to detect a statistical significance ($p > 0.05$). Therefore no regression line is shown (see text).

(0.04–0.73) and 10.78 nmol/g wet weight (0.29–18.47), respectively. Placental concentration of free riboflavin, flavocoenzymes, and total vitamin B_2 did not differ significantly between preterm and full-term infants ($p > 0.05$). Regression analysis of the (total) vitamin B_2 data revealed no significant dependence of the placental concentration on the maternal concentration or of the fetal concentration (umbilical vein) on the placental concentration ($p > 0.05$). The same was true for the single metabolites (data not shown).

Median placental weight was 420 g (350–630) in the preterm group, and 600 g (350–740) in the full-term group ($p < 0.005$). Placental vitamin B_2 pool in the preterm group amounted to 113.25 nmol of free riboflavin (5.50–284.40) and 1524.75 nmol of flavocoenzymes (42.00–7305.70). In the full-term group it was 148.20 nmol of free riboflavin (24.00–362.60) and 6124.10 nmol of flavocoenzymes (179.80–12190.20). Differences in pool size between both groups were not significant ($p > 0.05$) except for the total vitamin B_2 pool,

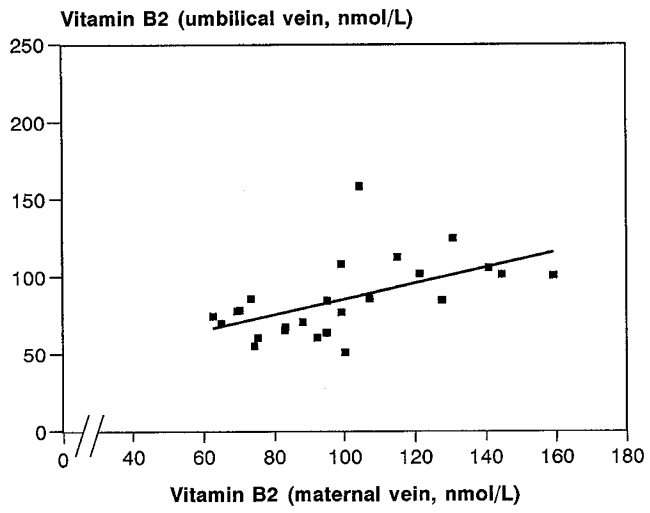

Figure 3. Regression analysis of vitamin B_2 concentration (free riboflavin + flavocoenzymes) in blood plasma of umbilical vein *vs* maternal vein in full-term infants. Regression was $y = 0.509x + 34.89$; $r = 0.549$; $p < 0.005$ ($n = 25$).

which was significantly larger in full-term infants ($p < 0.05$) because of their higher placental weight.

Blood flow in umbilical vein. Table 4 gives the values for umbilical blood flow, hematocrit and umbilical plasma flow. Hematocrit was significantly higher in full-term infants compared with their preterm counterparts ($p < 0.05$). Umbilical plasma flow was higher in preterm than in full-term infants after correction for body weight ($p < 0.001$). A detailed publication of these results is in preparation (Link G, Künzel W, in preparation).

Fetal vitamin uptake and release. As indicated above, the flavocoenzyme concentration was elevated in the umbilical vein plasma compared with the umbilical artery, *i.e.* fetal vitamin uptake took place. Preterm infants exhibited a significantly higher flavocoenzyme retention per kg of body weight than their full-term counterparts ($p < 0.01$; Table 5). In contrast to flavocoenzymes, free riboflavin concentration was higher in the umbilical artery plasma than in the umbilical vein. Fetal release of free riboflavin did not differ significantly between both groups of infants under investigation ($p > 0.05$; Table 5).

Table 4. *Blood flow, hematocrit (umbilical vein) and plasma flow (total and per kg of body weight) of preterm and full-term infants**

	Preterm infants	Full-term infants
Blood flow (mL/min)	416	481
	(284–663)	(289–741)
Hematocrit (%)	44†	47
	(38–55)	(41–60)
Plasma flow (mL/min)	233.0	256.6
	(130.6–377.9)	(155.9–377.9)
Plasma flow (mL/min·kg)	118.1‡	77.9
	(82.7–149.2)	(39.0–108.0)

* Median values (minimum – maximum) are reported (preterm, $n = 13$; full-term, $n = 31$).

† $p < 0.05$ (differences preterm *vs* full-term).

‡ $p < 0.001$ (differences preterm *vs* full-term).

Table 5. *Uptake of flavocoenzymes and release of free riboflavin in preterm and full-term infants**

	Preterm infants	Full-term infants
Flavocoenzyme uptake	1.5†	0.4
(nmol/min·kg)	(0.2–5.4)	(−5.7–6.6)
Free riboflavin release	0.4‡	0.2
(nmol/min·kg)	(−1.7–1.3)	(−0.3–1.4)

* Median values (minimum – maximum) are reported (preterm, $n = 13$; full-term, $n = 31$).

† $p < 0.01$ (differences preterm *vs* full-term).

‡ $p > 0.05$ (differences preterm *vs* full-term).

DISCUSSION

In our study we obtained two contrary arteriovenous concentration gradients in umbilical blood plasma. Flavocoenzymes were higher in the umbilical vein than in the artery, whereas free riboflavin was higher in the umbilical artery. This was the case in both groups under investigation, preterm and full-term infants. These concentration gradients indicate fetal flavocoenzyme consumption and release of free riboflavin, respectively. The gradient in free riboflavin concentration should be interpreted with care. Concentration differences between artery and vein were small (1–2 nmol/L), although significant, and we had not been able to demonstrate such a difference in a previous study (5). Referring to flavocoenzymes, preterm infants revealed a 4-fold higher uptake per kg of body weight than full-term infants. Consideration of the free riboflavin gradient leads to a reduction in the calculated uptake of total vitamin B_2 (flavocoenzyme uptake corrected by release of free riboflavin) in both groups of infants. Fetal uptake, calculated as free riboflavin, would then amount to 0.6 mg/kg·d in preterm infants and 0.1 mg/kg·d in full-term infants ($p < 0.05$). The lower vitamin uptake observed in full-term infants seems to remain without functional consequence. Correspondingly, coenzyme saturation of FAD-dependent enzymes such as glutathione reductase or acyl-CoA dehydrogenase was described to be sufficient for preterm and full-term infants, respectively (6, 19, 20).

In theory the arteriovenous concentration gradient in blood plasma could have been caused by vitamin uptake by erythrocytes, as these cells exhibit higher concentrations of flavocoenzymes than in blood plasma (21, 22). We assume that uptake by erythrocytes cannot fully explain the concentration gradient between the umbilical vein and artery. We found no significant venous-arterial difference in the FAD-dependent erythrocyte glutathione reductase activity coefficient in a different collective of 37 infants (40 wk median gestational age; minimum – maximum: 35–42 wk gestation). The activity coefficient (mean ± SD) was 1.17 ± 0.10 in the umbilical vein and 1.16 ± 0.10 in the umbilical artery ($p > 0.05$) (our unpublished observations). The concentrations of metabolites, other than FAD, have been shown to remain unchanged in erythrocytes, even when high oral doses of riboflavin were administered to healthy adult subjects (23).

The observed difference in total vitamin retention between preterm and full-term infants was accounted for by two facts: namely, the higher umbilical plasma flow per kg of body weight and the higher arteriovenous flavocoenzyme concentra-

tion difference in preterm than in full-term infants. The difference in the arteriovenous flavocoenzyme concentration between preterm and full-term infants was not quite significant ($p = 0.05$). This was presumably due to the small number of subjects in the preterm group and to the large variation in flavocoenzyme concentration. The latter might be accounted for by the fact that some of the mothers included in our study took vitamin supplements and others did not. On the other hand, release of free riboflavin did not differ significantly between both groups.

However, our results are significant in the formulation of recommendations for infant nutrition, particularly for neonates receiving total parenteral nutrition. The Subcommittee on Pediatric Parenteral Nutrient Requirement of the American Society for Clinical Nutrition recommended a supply of 1.4 mg/d of riboflavin for full-term infants receiving total parenteral nutrition; for preterm infants, the committee reduced its earlier recommendations from 0.56 mg/kg·d to 0.15 mg/kg·d (24). We were able to demonstrate vitamin B_2 uptake of preterm infants to be 4-fold higher than suggested by the supply recommendation in the current guidelines. This finding has to be interpreted carefully, because our results are not suitable for estimating requirement of infants. Requirement relates to prevention of a nutrient deficiency, which was not being addressed in our investigation. Furthermore, we did not differentiate vitamin uptake from utilization (see below). On the other hand our results permit more accurate estimates of maternal vitamin B_2 requirements during pregnancy. At present, an additional uptake of 0.3 mg/day is recommended during pregnancy (25). According to the calculation of fetal uptake in the present investigation, vitamin B_2 supplementation seems to be sufficient only in the last few weeks of pregnancy. Borderline deficiency of maternal riboflavin supply might occur in the early third trimester when fetal vitamin B_2 uptake is enhanced.

We were not able to differentiate vitamin utilization from uptake in our study. This means that neither fetal urinary loss of vitamin nor tissue metabolism was considered. Intrauterine urine flow was reported to increase from 5 mL/h at 20 wk of gestation to 56 mL/h at 41 wk of gestation (26). Vitamin concentration in this urine is unknown. Amniocentesis performed in eight normal pregnancies indicated a riboflavin concentration in amniotic fluid of approximately 13 ± 7 ng/mL (~35 nmol/L) (27). This value is only slightly higher than the fetal plasma levels we observed. On the other hand, our calculation of fetal uptake is based mainly on differences in concentration of flavocoenzymes, as opposed to free riboflavin. Because renal elimination of metabolites, other than free riboflavin, occurs only in minor amounts (28), the arteriovenous concentration gradient should be produced predominantly by tissue uptake and not by renal elimination. As indicated above, sufficient coenzyme saturation of FAD-dependent enzymes with their cofactors was observed in preterm as well as in full-term infants (6, 19, 20). This also suggests successful utilization.

An arteriovenous gradient of flavocoenzymes does not rule out the possibility that free riboflavin is the metabolite which is accumulated in fetal tissue, followed by metabolic trapping. The drop in plasma concentration of flavocoenzymes as they pass fetal tissues could be due to *1*) hydrolysis of FAD and FMN and its subsequent cellular assimilation as free riboflavin and *2*) cellular uptake of intact coenzyme forms. We favor the former explanation. It was shown for enterocytes that coenzyme forms are hydrolyzed before penetrating the cell membrane (29). Riboflavin is subsequently accumulated in enterocytes by metabolic trapping *i.e.* FAD and FMN are built up. Metabolic trapping was also reported for hepatocytes and kidney cells (30, 31). We state explicitly that we measured coenzyme forms of vitamin B_2 as the total of FAD and FMN. FAD should be expected to be the predominant metabolite, as demonstrated by Lust *et al.* (11). They showed that FAD accounts for 82% of the coenzyme forms present in umbilical serum. Hydrolysis of these coenzyme forms, before entering the tissues, could explain the higher concentration of free riboflavin in the umbilical artery compared with the umbilical vein which we found. The small surplus of free riboflavin after coenzyme hydrolysis could be cleared from plasma while passing the placenta. Vitamin transfer from the fetus into placental tissue is possible, as shown by *in vitro* placental perfusion (13).

Our results demonstrate that free riboflavin was accumulated in fetal circulation at a 4-fold higher concentration than in maternal plasma. This was both the case in preterm and full-term infants. There is general agreement about elevated levels of free riboflavin in umbilical plasma compared with maternal plasma. Concentration gradients were found both *in vitro* and *in vivo* (5, 11–13, 32). Maternal-fetal ratios ranged from 1:1.45 to 1:4.7 in these studies. Dancis *et al.* (13) were not able to detect FAD or FMN released by placenta in their *in vitro* perfusion study. Findings from the present study suggest that a transplacental release of flavocoenzymes is the most probable explanation for the elevated flavocoenzyme concentration in the umbilical vein compared with the umbilical artery. Our results concerning transplacental transfer are in good agreement with our observations made in an earlier study in which we dealt with vitamin transfer in predominantly term infants (5). The concentration gradient of free riboflavin as well as the gradient of coenzyme forms in full-term infants were the same as described in this study. In our earlier investigation we found maternal flavocoenzyme plasma concentration to be 1.7-fold higher than fetal concentration (5). In the full-term group of the present study flavocoenzyme levels were 1.6 times higher in maternal plasma. In preterm infants flavocoenzymes reached the same concentration in the maternal vein and the umbilical vein. This suggests an easier placental transfer of FAD and FMN in the earlier stages of pregnancy.

In placenta flavocoenzymes were more abundant than free riboflavin. Presumably they were taken up as free riboflavin and by metabolic trapping the latter was converted to FMN and FAD and accumulated as described for other tissues (31). This is in agreement with the high percentage of vitamin B_2 composed of coenzyme forms that we observed. The vitamin would then be released into fetal circulation predominantly as free riboflavin after coenzyme hydrolysis. Hydrolysis of this type was shown to be possible earlier (11). A smaller part of vitamin B_2 seems to be transferred in its coenzyme forms via placenta

(see above). The concentration of vitamin B_2 in placental tissue is a matter of controversy. Dancis *et al.* (13) described a nearly equal distribution of free riboflavin and its coenzyme forms in placental tissue (riboflavin *versus* FMN *versus* FAD ~50%:10%:40%). However, Lust *et al.* (11) reported FAD to be the most abundant metabolite in placental tissue. They determined concentrations which amounted to 205 μg of FAD/100 g, wet weight and 9 μg of FMN + riboflavin/100 g, wet weight. Their results are in agreement with our values if coenzyme forms, which we assessed as total flavocoenzymes, are predominantly composed of FAD. The placental concentration of coenzyme forms in the full-term group was somewhat higher than that observed by Lust *et al.*

Other factors that might have an influence on fetal vitamin uptake were also considered. A significant dependence of fetal vitamin concentrations on maternal vitamin status was observed for full-term infants. This is in agreement with previous results (5, 10). No significant dependence on maternal status was observed for preterm infants. We assume that this is due to the low number of infants in this sample group (n = 10 for regression analysis). This should be kept in mind during evaluation of all data obtained for this group (preterm infants, in total n = 13). For both infant groups, there was no significant dependence of fetal plasma concentration on placental vitamin concentration. Also, there was no dependence of placental concentration on maternal status. This maybe due to the fact that we analyzed total placenta, not taking into account a possible segmentation of the placental vitamin B_2 pool. FAD- and FMN-dependent enzymes are not equally distributed within cells. Some are located predominantly in the supernatant fraction, others are found in diverse organelles (33–35). An unequal distribution of accompanying flavocoenzymes in cells can therefore not be dismissed, even though only ≤10% of FAD is covalently protein-bound (34). We speculate that the unbound vitamin B_2 in the cytosol of placental cells correlates with the vitamin concentration in the maternal and fetal blood plasma. Further studies should focus on the subcellular level to examine this hypothesis. We also speculate that fetal supply of free riboflavin is influenced by the activity of flavocoenzyme hydrolyzing enzymes in the placenta. These enzymes include nucleotide pyrophosphatase which cleaves FAD and phosphatases which degrade FMN (34).

Fetal vitamin uptake was strongly dependent on the umbilical plasma flow. It is difficult to determine umbilical plasma flow because of its strong dependence on the blood vessels' diameter. This diameter influences the calculation of blood flow to a large degree. The related problems have been discussed in detail elsewhere (14). We state that our results of umbilical blood flow are in the upper limits of the normal range. Künzel *et al.* (14) reported a blood flow of 300 mL/min at term. Other investigators have reported a blood flow of 125 ± 7.5 mL/min/kg body weight (17) and 117 ± 7.5 to 130 ± 8.2 mL/min/kg body weight (18) in the umbilical vein. These values are close to our data.

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