Need for riboflavin supplementation in small prematures fed with human milk\(^1\)-\(^3\)

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**ABSTRACT**

It is the practice in Finland to feed small premature infants with human milk and without riboflavin supplementation. In this study the riboflavin status was analyzed in 39 such premature infants, 19 with riboflavin supplementation (0.3 mg/day) and 20 without, in their mothers, and in breast-milk samples during a period of 12 wk after delivery. The mean gestational age of the infants was 30.1 wk and their birth weight 1,183 g. Stimulation of erythrocyte glutathione reductase by flavin-adenine-dinucleotide was used as the criterion for riboflavin status in the blood samples. At age 6 wk 47\% of the infants without supplementation had activity coefficient values indicative of riboflavin deficiency. The riboflavin status of the infants receiving supplementation was better (\(p < 0.01\)). The concentration of riboflavin in the human milk samples was dependent on the amount of riboflavin supplementation of the mothers during the period from two to twelve weeks after delivery (\(p < 0.05-0.01\)). These data indicate, in small premature infants the intake of riboflavin may be inadequate without supplementation during the first few weeks after birth. *Am J Clin Nutr* 1986;43:1-6.

**KEY WORDS**

Riboflavin, premature infant, infant nutrition, human milk, lactation

**Introduction**

Riboflavin is a water-soluble vitamin which crosses the placenta by active transport (1). It is phosphorylated to flavin-mononucleotide (FMN) and further to flavin-adenine-dinucleotide (FAD) in the tissues. The flavin coenzymes are involved in many facets of intermediary metabolism including both lipids and amino acids. By splitting FAD to free riboflavin the placenta can maintain a higher concentration of riboflavin in the fetal blood (1). The fetal tissues need FAD, however, and not free riboflavin, but are able to synthesize it from free riboflavin taken up from the fetal blood. Hence, even very premature infants may have a good capacity for synthesizing FAD from riboflavin (1). But, after birth the supply of riboflavin decreases because the milk intake of these infants tends to be low for many days and their ability to absorb riboflavin may be reduced as well. Rapid postnatal growth may also lead to an increased need for the vitamin. For these reasons the very low-birthweight (VLBW) infant may be at risk of riboflavin deficiency during the first weeks of life.

The aim of this study was to investigate the riboflavin status and the need for riboflavin supplementation in VLBW-infants.

**Methods**

A group of 54 VLBW-infants were followed during their initial hospitalization at the Helsinki University Children's Hospital. Fourteen infants who received exchange transfusions were excluded. From the remaining 40 infants (33 boys and 7 girls) 19 were receiving riboflavin supplementation (0.3 mg/day), and 21 were not supplemented. The mothers of the infants were not supplemented with riboflavin during pregnancy. The samples were analyzed for riboflavin concentration and riboflavin status as follows. The concentration of riboflavin in the human milk samples was dependent on the amount of riboflavin supplementation of the mothers during the period from two to twelve weeks after delivery (\(p < 0.05-0.01\)). These data indicate, in small premature infants the intake of riboflavin may be inadequate without supplementation during the first few weeks after birth. *Am J Clin Nutr* 1986;43:1-6.

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fusions were excluded, and the final study population consisted of 39 premature infants whose birth weight were from 620 to 1,520 g (1,183 ± 38 g, mean ± SEM). Their mean gestational age was 30.1 ± 0.3 wk, with a total range from 26 to 35 wk.

All the infants were fed with human milk only. They received fresh pooled mature milk from the Human Milk Bank and/or their own mothers' premature milk. The final daily volume of milk given was 200 ml/kg which was reached from 1 to 4 wk of age.

The infants were randomly supplemented with human milk protein (n = 12), medium-chain triglyceride (MCT) oil (n = 11) or both (n = 9), or given plain human milk (n = 7) (2). Since there was no correlation between protein or fat intake and riboflavin status at any age, no further attention was paid to the detailed fat and protein intake.

Riboflavin supplementation, 0.3 mg/day riboflavin phosphate (Beko®, Orion, Finland), was given to 19 of the infants from the third day of life until the age of 12 wk (Table 1). The infants also received 0.3 mg/day of thiamin and pyridoxin in the same preparation.

Supplementation of vitamins A, E, D, C, B₁₂, and folic acid was within recommendations and has been described earlier (3).

Iron supplementation was started at the age of 2 wk and gradually increased by the age of 6 wk to a maximum 3 to 4 mg/kg/day of iron as ferrous sulfate (3).

Thirty seven mothers were able to lactate; 16 infants were breast-fed exclusively during the whole study period. After delivery 22 of these mothers were without vitamin supplementation and 15 used a multivitamin preparation from which they received 2.5 to 5 mg of riboflavin/day. The supplementation was continued as long as the mother was lactating. Eight of the unsupplemented and 12 of the supplemented mothers had taken multivitamin preparations during pregnancy.

Blood samples were taken at 2, 6, and 12 wk after delivery from both infants and mothers. Stimulation of erythrocyte glutathione reductase (ERG) by flavin adenine dinucleotide (FAD) was used as the criterion for riboflavin status. It was measured as described by Vuillemeier et al (4). The results were expressed as activity coefficients (AC) according to Brubacher's criteria (5) were values above 1.29 indicated a high risk of riboflavin deficiency, values between 1.20 and 1.29 a moderate risk, and values below 1.20 a low risk.

Human milk samples were collected at 1, 2, 6, and 12 wk after delivery; 5 ml of fore and hind milk were collected at each milking during a 24 h period. The samples were frozen until analyzed. The total riboflavin was determined by a fluorimetric method (6). All the samples were analyzed at the Department of Vitamin and Nutrition Research, F Hoffmann-La Roche and Co Ltd, Basle, Switzerland.

Statistical analyses were performed using Biomedical computer programs by the University of California at Los Angeles (7). Tests used were one-way analysis of variance (ANOVA), and simple linear regression and correlation. The values are given as mean ± SEM.

The study was approved by the Ethical Committee, University of Helsinki's Children's Hospital and is in accordance with the Helsinki Declaration.

Results

In the infants without riboflavin supplementation the mean AC-values were 1.26 ± 0.08 at the age of 2 wk, 1.39 ± 0.08 at 6 wk, and 1.12 ± 0.09 at 12 wk. The corresponding values for the infants with supplementation were 1.29 ± 0.07, 1.13 ± 0.05 and 1.11 ± 0.06. The mean values differed at the age of 6 wk (p < 0.01) (Fig 1). There was no difference in riboflavin status between infants who were small-for-gestational age and appropriate-for-gestational age.

The estimated riboflavin intake was from two- to fivefold higher in the infants with riboflavin supplementation than in those without (Table 2). The mean concentration of riboflavin in the banked pooled human milk was 318 µg/l and varied from 180 to 738 µg/l in the individual mothers. There was a negative correlation between the AC-values of the infants fed exclusively by their own mothers' milk and the riboflavin concentration in the milk at ages 2 and 6 wk (r = −0.76, p = 0.046; r = −0.65, p = 0.086, respectively).

Supplementation of the lactating mothers with riboflavin did not result in any significant difference in the riboflavin status of their blood (Fig 2). Two mothers in the unsupplemented group were at high risk of deficiency 2 wk after delivery. During later lactation the mean AC-values tended to increase from 1.08 ± 0.03 at 2 wk to 1.16 ± 0.05 at 12 wk (p = 0.013). By contrast, the values for the mothers in the supplemented group during the same period were similar (1.04 ± 0.01 and 1.05 ± 0.04, respectively). There was no correlation between the AC-values of the mothers and of the infants who were fed exclusively with their own mothers' milk and were unsupplemented.

The concentration of riboflavin in the milk samples taken 1 wk after delivery was the same

<p>| TABLE 1 |</p>
<table>
<thead>
<tr>
<th>Description of the infants</th>
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<tbody>
<tr>
<td>With riboflavin supplementation</td>
</tr>
<tr>
<td>Infants (n)</td>
</tr>
<tr>
<td>Birth weight (g)</td>
</tr>
<tr>
<td>Gestational age (wk)</td>
</tr>
<tr>
<td>Small-for-gestational age infants (n)</td>
</tr>
<tr>
<td>Male/female</td>
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<tr>
<td>Phototherapy (n)</td>
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</table>
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FIG 1. Activity coefficients of EGR in infants with (○) and without (□) riboflavin supplementation at ages 2, 6, and 12 wk. The limits of riboflavin deficiency (5) are also shown.

for both the unsupplemented and the supplemented groups of mothers (Fig 3). The mean concentration of riboflavin increased until the second week of lactation, then remained at the same level through the twelfth week. However, between the second and twelfth week of lactation the range of mean values was from 83–140 μg/l in the two groups (p < 0.05–0.01). The riboflavin concentrations in the milk and the AC-values in the blood had a negative correlation at the second (r = −0.39, p = 0.015) and sixth week of lactation (r = −0.41, p = 0.027). The riboflavin concentration in the milk did not correlate with the gestational age of the infant but correlated negatively with the birth weight (r = −0.42, p = 0.034).

At age 2 wk there was no difference in riboflavin status in infants who had received phototherapy for jaundice soon after birth. Furthermore, there was no correlation between the duration of the phototherapy after birth and the riboflavin status after the age of 2 wk.

Clinical signs of riboflavin deficiency such as angular stomatitis, cheilosis, or glossitis etc,

TABLE 2
Estimated riboflavin intake of the infants (ranges in parentheses) according to age and mean weight (g)

<table>
<thead>
<tr>
<th>Age</th>
<th>Weight</th>
<th>With riboflavin supplement (μg/day)</th>
<th>Without riboflavin supplement (μg/day)</th>
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<tr>
<td></td>
<td></td>
<td>2 wk: 1,162 ± 40</td>
<td>6 wk: 1,721 ± 73</td>
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<td></td>
<td></td>
<td>368 ± 7 (315–442)</td>
<td>442 ± 13 (362–590)</td>
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<tr>
<td></td>
<td></td>
<td>76 ± 7 (13–133)</td>
<td>125 ± 8 (52–260)</td>
</tr>
</tbody>
</table>
Discussion

FAD-saturation of erythrocyte glutathione reductase has been shown to be a sensitive measure of riboflavin nutritional status in man (8) and is one way of studying the riboflavin status of VLBW-infants. In this study the saturation values indicated that one-third to one-half of the infants were at risk of riboflavin deficiency during the first weeks of life.

Earlier studies did not show any evidence of term infants being riboflavin deficient at birth (9, 10, 11, 12). However, Hovi et al found that in term infants fed with human milk AC-values increased during the first week of life and then decreased gradually during the next few weeks to normal (11). Lucas et al found in their study of premature infants fed with human milk elevated AC-values during the first 2 wk of life (13). In the present study of VLBW-infants, AC-values were maximal at the age of 6 wk. Thus, although the high AC-values seen during the first weeks of life in term and premature infants may be a physiological phenomenon, there is strong evidence that they are a sign of riboflavin deficiency.

Another way of studying the riboflavin status in VLBW-infants is to investigate the influence of the riboflavin concentration in breast milk on the AC-values in the infants. In the present study a negative correlation was found indicating that even small premature infants are able to utilize the riboflavin available in human milk.

After supplementing half the infants with 0.3 mg of riboflavin, 20 to 30% were still at high risk of deficiency up to the age of 6 wk. Their total riboflavin intake ranged from 315 to 590 μg/day during the second and sixth week of life which was in accordance with the recommended 400 μg/day intake of riboflavin for premature infants (14).
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None of the infants in the unsupplemented group, however, attained this intake by the age of 12 wk. Although the use of riboflavin supplementation improved the AC-values of the infants, as expected, it nevertheless did not guarantee a level equivalent to an adequate riboflavin status in healthy adults.

The mean AC-values we found in the mothers after delivery were at the same level as in the former studies (9, 10, 15). Vir et al considered that about one-fifth of unsupplemented Caucasian women were riboflavin-deficient during the third trimester of pregnancy (16). In the present study, 2 wk after delivery 2 of the 20 mothers in the unsupplemented group were at high risk of riboflavin deficiency. However, during the proceeding lactation the riboflavin status of this group tended to get worse. Thus, supplementation of lactating mothers with riboflavin may prove useful if lactation continues for over 12 wk.

The mean concentration of riboflavin in the premature milk of our unsupplemented mothers was slightly higher than the values reported earlier in both premature and term milks (17, 18). This could be the result of a better riboflavin status among the mothers participating in the present study. Riboflavin supplementation increased the concentration of the vitamin in breast milk, as reported earlier (19). The concentration of riboflavin was about 100 μg/l higher in the milk of mothers supplemented with 2.5 to 5 mg of riboflavin. However, even the premature milk of these mothers could not provide the VLBW-infants with a riboflavin intake sufficient to maintain their riboflavin status at normal adult levels. After all, there is no evidence that such an adult level is necessary or even desirable for the infants.

The present study found that a large number of VLBW-infants fed exclusively with human milk had elevated AC-values indicative of marginal or poor riboflavin nutrition and that human milk with a higher concentration of riboflavin or a daily riboflavin supplementation reduced these values. Thus, it is clear that riboflavin supplementation during the first months of life improves the riboflavin status of VLBW-infants. The supplementary amount should be higher than 0.3 mg/day, probably 0.4–0.5 mg/day. However, Spicket et al (20) and Ennever et al (21) have shown in studies in vitro that photodynamic activation of riboflavin may cause alteration in intracellular DNA. In the view of this, the introduction of riboflavin supplementation should probably be postponed until after phototherapy.

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References