Human milk protein supplementation for the prevention of hypoproteinemia without metabolic imbalance in breast milk-fed, very low-birth-weight infants

In a group of 18 infants with birth weights of 1,500 gm or less, either preterm transitional or mature human milk was given during the time of initial hospitalization. Half of the infants were given protein supplement isolated from mature human milk which increased the protein content of the ingested milk by 0.8 gm/dl. The protein intake of these infants was increased by 0.6 to 1.6 gm/kg/day between two and 12 weeks after birth. The infants in the unsupplemented group developed hypoproteinemia at 8 to 12 weeks of age whereas those who received protein supplementation did not. We conclude that the hypoproteinemia resulted from nutritional lack of protein and did not represent a physiologic phenomenon of preterm development. There was no difference in the growth of the two groups. There was no evidence of any imbalance in amino acid metabolism even though there were significant correlations between individual protein intakes and plasma concentrations of tyrosine and phenylalanine. Protein intake of more than 3 gm/kg/day resulted in a mean serum urea nitrogen concentration of more than 15 mg/dl at 2 weeks of age, indicating that excessive protein intake should be avoided soon after birth.

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The survival rate of even the smallest preterm infants has increased dramatically during recent years. The risks of both early malnutrition as a contributing factor to later disease and protein excess, with resulting imbalance of amino acid metabolism and metabolic acidosis, have been emphasized. Some investigators have stressed the need to develop adapted milk formulas; others believe that human milk is the superior basic food which should be supplemented according to the special needs of very low-birth-weight infants. We designed the present study after being convinced that some VLBW infants who receive human milk from milk banks have low serum concentrations of protein during the second month of life. In Finland, the basic nutrition for all preterm infants consists of human milk. We decided, therefore, to isolate human milk protein from large volumes of human milk and to add this concentrate in a random fashion to human milk. The primary questions were whether this manipulation prevents hypoproteinemia and whether it results in any of the risks that are reported when milk formulas are supplemented with nonhuman protein.

SUBJECTS

The series consisted of 23 infants with birth weights of less than 1,500 gm; they were selected for follow-up if,
Table I. Protein intake (gm/kg/day) of infants in milk protein-supplemented (P+) and unsupplemented (P−) groups*

<table>
<thead>
<tr>
<th></th>
<th>2 wk</th>
<th>6 wk</th>
<th>12 wk</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>P+</td>
<td>P−</td>
<td>P+</td>
</tr>
<tr>
<td>2.6 ± 0.43</td>
<td>2.0 ± 0.13</td>
<td>3.6 ± 0.09</td>
<td>2.0 ± 0.08</td>
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<tr>
<td>0.7–4.5</td>
<td>1.4–2.6</td>
<td>3.4–4.1</td>
<td>1.8–2.5</td>
</tr>
<tr>
<td>0.7–4.5</td>
<td>1.4–2.6</td>
<td>3.4–4.1</td>
<td>1.8–2.5</td>
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</tbody>
</table>

*Values are based on individual measurements as indicated in text; mean ± SE and range are shown.

Table II. Plasma amino acid concentrations in protein-supplemented and control infants two weeks after birth*

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Valine</th>
<th>Tyrosine</th>
<th>Phenylalanine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>121 ± 15</td>
<td>67 ± 11</td>
<td>42 ± 2</td>
</tr>
<tr>
<td>Protein-supplemented</td>
<td>10</td>
<td>165 ± 22</td>
<td>113 ± 25</td>
<td>49 ± 4</td>
</tr>
</tbody>
</table>

*Values are shown as µmol/l (means ± SE).

during the first two days of life, they had developed no serious illness and had no major malformations. However, the data of five infants were excluded from the study because they subsequently developed major disease, died, or were lost to follow-up. Thus, this series consisted of 18 infants whose mean birth weight was 1,190 ± 59 gm (SE), with a total range from 620 to 1,500 gm. Gestational age, determined in each case by the method of Dubowitz et al., averaged 30.3 ± 0.5 weeks and ranged from 27 to 36 weeks. Four infants were small for gestational age.

FEEDING REGIMENS

Milk feeding. All infants were given human milk exclusively. Pooled banked human milk was given from the Breast Milk Bank unless the infant's own mother produced it. Infants were receiving their own mother's milk in 11 cases at 2 weeks and in six cases at 6 weeks of age. Banked human milk was given at those times to five and nine infants, respectively. The rest of the subjects received a mixture of human milk from both sources.

The banked milk was obtained from a pool of several milk donors who met the following two additional criteria: the milk contained few or no bacteria in prior samplings and was brought to the hospital within 24 hours after lactation. At the hospital it was either used within the subsequent 24 hours or frozen for a maximum period of one month, then thawed, and used within the next 24 hours. These milk specimens were not heat treated.

Milk from mothers of preterm infants was usually collected at home and brought to the hospital within 24 hours. Samples of this milk usually contained some bacterial contamination so it was pasteurized prior to use.

The protein concentration remained stable in the pools of banked milk and averaged 0.9 gm/dl. The protein concentrations of milk obtained from mothers of subjects were higher soon after birth; mean values were 1.6 gm/dl at one week and 1.3 gm/dl at two weeks. However, this phenomenon had only a minor influence on the protein intake of the infants soon after birth because of the relatively small volumes of milk ingested. But it did result in large individual variation in the protein intake at 2 weeks of age (Table I).

Protein supplementation. Infants were grouped into four categories of gestational age; less than 30 weeks, 31 to 33 weeks, 34 to 36 weeks, and more than 37 weeks. In each category, subsequent infants belonged to the supplemented (test) and to the unsupplemented (control) group. The five infants lost to follow-up were replaced by the subjects born next in the respective gestational age categories.

The test group consisted of ten infants whose birth weights averaged 1,180 ± 98 gm (range 620 to 1,480 gm) and gestational age 30.0 ± 0.7 weeks (range 27 to 36 weeks).

The control group consisted of eight infants of average birth weight 1,200 ± 58 gm (range 1,010 to 1,500 gm). Their mean gestational age was 30.6 ± 0.9 weeks (range 28 to 36 weeks).

Two infants in each groups were small for gestational age.

Influence of supplementation on protein intake. Individual protein intake was estimated by the volume of milk ingested during the second, sixth, and twelfth weeks of life and by the concentration of milk protein at two, six, and 12 weeks of lactation or in the banked milk, respectively (Table I).

Vitamin and mineral supplementation. Vitamin supplementation, started on the third day of life, included vitamins A, B₁₂, C, D, E, and folic acid. The individual doses of vitamins C, E, and folic acid were variable. In addition, infants were randomly given supplementary vitamins B₆, B₁₂, and B₉. Iron supplementation was started in each infant at or after 2 weeks of age. By 2 months the dosage had reached the maximum (3 to 4 mg/kg/day divided into three oral doses of iron as the ferrous salt).
Calcium was also given to the control infants (10 mg/kg/day) to compensate for the additional intake of calcium through the protein concentrate (see below).

**METHODS**

**Milk sampling.** Individual milk samples for the laboratory studies were collected during a 24-hour period, 5 ml prior to and at the end of each milking.

**Preparation of protein concentrate from human milk.** In this study we used 750 L of pooled, banked, human milk collected at the Helsinki Breast Milk Bank and stored at -18°C for less than three months prior to use. The protein concentration was prepared at the Research Unit of Kuivamaito Oy, Lapinlahti, Finland, in three batches, each containing 250 L of frozen milk which was thawed up to +5°C in a water bath. The fat was then removed in a milk separator. The protein was concentrated by ultrafiltration at 30 to 35°C, a procedure which resulted in removal of lactose and electrolytes. The concentrate was divided into aliquots, frozen, and stored at -18°C until use. The maximum period of storage was always less than three months. Prior to use the concentrate was thawed, pasteurized at 62°C for 30 minutes, and mixed either with the pooled banked human milk or with the individual milks from the infants' mothers.

The concentration of protein in the three lots varied from 6 to 8 gm of protein/dl. Thus the exact volume of these supplements depended on the batch of concentrate used. The fat, lactose, sodium, potassium, and calcium...
Table III. Increments in measurements of growth in supplemented and control groups (means ± SE)

<table>
<thead>
<tr>
<th>Group</th>
<th>Weeks of age</th>
<th>Weight (gm/kg*/day)</th>
<th>Height (cm/m*/day)</th>
<th>Head circulation (cm/m*/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0-12</td>
<td>18.3 ± 1.2</td>
<td>0.31 ± 0.02</td>
<td>0.41 ± 0.03</td>
</tr>
<tr>
<td>Control</td>
<td>6-12</td>
<td>27.2 ± 3.2</td>
<td>0.33 ± 0.05</td>
<td>0.47 ± 0.04</td>
</tr>
<tr>
<td>Supplemented</td>
<td>0-12</td>
<td>17.6 ± 1.2</td>
<td>0.37 ± 0.02</td>
<td>0.39 ± 0.02</td>
</tr>
<tr>
<td>Supplemented</td>
<td>6-12</td>
<td>25.3 ± 1.1</td>
<td>0.39 ± 0.02</td>
<td>0.44 ± 0.05</td>
</tr>
</tbody>
</table>

*At birth.

Protein determinations. The milk protein concentrations were determined by a Biuret method after precipitation of the protein. Nonprotein nitrogen in milk should not interfere with these measurements. The protein in the concentrates was determined by a Kjeldahl method based on the total nitrogen. We assume that most of the nonprotein nitrogen was removed from the concentrates by ultrafiltration prior to these assays. Serum protein was quantitated by the Biuret method. Plasma amino acids were determined as described earlier.

RESULTS

The serum levels of protein were higher in the protein-supplemented group than in the control group at eight, ten, and 12 weeks after birth (P < 0.01, P < 0.01, and P < 0.05) (Fig. 1). Thus, the supplementation prevented the development of the hypoproteinemia that occurred at about 2 months of age in the human milk-fed infants. However, the mean plasma concentration of valine was not different (Table II).

We could demonstrate no difference in any of the measurements of growth between the two groups of infants (Table III).

Potential harmful effects were estimated by determining the plasma concentrations of tyrosine and phenylalanine at 2 weeks of age, which should be the most sensitive age for such detection. However, there was no difference between the mean concentrations (Table II). We also compared the average daily intake of protein by each infant during the second week of life with the respective concentration of amino acid. This analysis resulted in a significant correlation between the protein intake and plasma tyrosine concentration (Fig. 2). A similar correlation was also found between the protein intake and plasma phenylalanine values (r = 0.65, P < 0.01). The highest individual plasma concentration of phenylalanine was 80 μmol/L.

The mean concentration of nitrogen urea was about twice as high in the protein-supplemented group as in the control subjects; this difference persisted throughout the study (Fig. 3). Two of the values in either feeding group exceeded 20 mg/dl at any age. At 2 weeks of age the individual levels of nitrogen urea and the amount of protein intake correlated (r = 0.72, P < 0.01). According to this regression line, protein intakes of 2.8 gm/kg/day and 3.5 gm/kg/day resulted in serum urea nitrogen concentrations of about 15 mg/dl and about 20 mg/dl, respectively.

There were no differences in the mean values measuring acid-base balance in the two groups at 2, 4, 6, and 8 weeks of age. However, at 2 weeks of age the pH was 7.32 in the test and 7.37 in the control group (P < 0.05).

DISCUSSION

Our data indicate that human milk-fed VLBW infants develop hypoproteinemia which is prevented by human milk protein supplementation without any harmful effects or metabolic imbalance.

The protein concentration of pooled mature human milk averages about 0.9 mg/dl. Many of the earlier estimations indicated somewhat higher contents; this was related to the unusually large proportion of nonprotein nitrogen in human milk and the nitrogen-based protein analyses. The protein content of human milk is highest soon after delivery and decreases during the first month of lactation to the level of that in mature milk. Atkinson et al showed that the nitrogen concentration in milk of mothers who have given birth to preterm infants is about 20% higher than that in specimens from mothers delivered of full-term infants, although most of the individual values overlapped. Recently, however, Gross et al reported considerably higher protein concentrations in morning samples obtained during the first month of lactation after both preterm and term pregnancy. On the other hand, the preterm milk contained, on the average, about 30% more nitrogen than the term milk. These data, together with our findings, indicate that the protein intake in preterm infants may vary considerably in different hospitals where human milk...
is used. In addition, individual variations in milk protein content further enhance the individual differences in actual intake.

We have documented the development of hypoproteinemia in VLBW infants who receive their own mothers' milk or pooled mature human milk during the second month of life. These data also suggest that the hypoproteinemia is related to a lack of protein intake rather than to a physiologic phenomenon of preterm development, although no effect on growth of the infants was found. This point of view is supported by the observation that the hypoproteinemia was abolished by the addition of human milk protein. However, our data allow only speculation on the optimal human milk protein intake in these conditions.

The plasma concentration of valine has been used in estimations of protein deficiency and excess, but even those results are difficult to interpret because of the linear correlation between protein intake and plasma valine values. In contrast, our observations on the concentration of urea nitrogen indicate that a safe maximal limit for protein intake might be about 3 gm/kg/day at 2 weeks of age.

High plasma concentrations of tyrosine and phenylalanine have been found in formula-fed preterm infants, especially at 2 weeks of age and are regarded as evidence of metabolic imbalance that should be avoided. In our series the average values were less than 10% of those that might be considered dangerous, although the individual levels did directly correlate with the protein intake. Thus, we conclude that supplementation of human milk with human milk protein to double the concentration in mature milk was not associated with evidence of imbalance of amino acid metabolism. The disadvantages of a higher serum protein level were not studied. These might be the consequences for the oncotic pressure of the plasma, for renal function, for water clearance, and for the proportion of total body water to body solids.

Our findings suggest that intake of more protein is necessary in VLBW infants, especially during the second month of life, in order to prevent the hypoproteinemia found in infants fed human milk. The higher protein intake can be achieved while the infant is receiving his own mother's milk during the first month of life, although this practice hardly augments the intake of protein during the second month of life, at which time the need seems to be critical. The protein supply can be guaranteed by the use of human milk as the basic nutrition without obvious risks. This practice may have other beneficial effects on the infant. For practical reasons, however, the effect of similar supplementation of human milk by nonhuman protein should be studied.

REFERENCES
