Lactoengineering: A Method for the Estimation of the Human Milk Protein Requirements of Very-Low-Birth-Weight Newborn Infants

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This chapter concerns some of the observed metabolic effects of varying the protein and fat content of human milk. These modifications in the composition of human milk have been achieved without disturbing its immunological properties or lipase activity and without the addition of foreign antigen. These experiments were conducted in order to determine the nutritional requirements of very-low-birth-weight (VLBW) infants.

The hypothesis that the protein requirement is especially high during the initial period after birth in the VLBW infant is based on known fetal accretion rates (1). However, before advocating an optimal food supply, we must take into consideration the infant's metabolic tolerance to the amount given. This is particularly critical in the VLBW infant, who suffers from both limited excretion and metabolic capacity (2). Thus, in our efforts to avoid brain damage by providing sufficient energy and protein during this critical period of development, we are sailing between the Scylla of brain damage from undernutrition and the potential Charybdis of brain damage resulting from overnutrition (Fig. 1). What we need is a method of producing a “human milk formula” (3) without destroying the advantages of using human milk and yet without having to provide large volumes of water (4).
To circumvent this problem, two different methods have been developed. In the first, the mother's own milk, or banked milk, is concentrated to provide an increased concentration of nutrients. The method potentially leads to rough handling of the immune properties of the milk and to high osmolality. The second method, which is the one we have chosen, is that of producing human milk fat and protein fractions to be added to the mother's own fresh milk or banked milk pasteurized with a special plate heat exchanger.

The homeostasis of blood amino acids is a sensitive index of protein and calorie supply in relation to requirement and to metabolic tolerance (5). We have recently reported (6) that the doubling of the human milk protein content was well tolerated by four VLBW infants given 150 to 175 ml/kg per day of their mothers' protein-fortified milk. This was evident from the acid-base balance, the free amino acid levels of whole blood, the serum urea levels, and growth, which followed the intrauterine growth curve. It has also been demonstrated that human milk supplementation prevents hypoproteinemia without causing metabolic imbalance in LBW infants fed expressed breast milk (7).
However, there are serious difficulties in defining normal levels of indices such as the free amino acid concentrations of peripheral blood in a group that should normally have remained in utero. The intrauterine levels are not necessarily relevant to the particular situation of the newborn VLBW infant and perhaps the best criteria we can obtain are the levels of ad libitum breast-fed full-term infants (8). Different groups of VLBW newborn infants have therefore been studied in order to provide a scale of comparison.

MATERIAL AND METHODS

The investigation was approved by the local Ethical Committee, and the parents gave their consent after having received verbal and written information. Twenty-five infants with birth weight below 1,800 g and gestational age below 34 weeks were divided into four groups (Table 1). The experimental period began as soon as the infants could tolerate 150 ml/kg per day intragastrically and ended at 36 weeks of postconceptional age. Heel-prick blood samples were obtained every second day during the first week and then weekly for the analysis of urea, calcium, sodium, potassium, base excess, and bicarbonate. An 8-hr urine collection was performed weekly for the analysis of sodium, potassium, calcium, and osmolality. Tyrosine was determined by a fluorimetric method, as this is a sensitive indicator of protein overload in the LBW newborn (9). Weight, length, and head circumference were plotted weekly on a perinatal growth curve (P. Karlberg, personal communication). All infants were without malformations and had an uncomplicated course during the observation period.

Whole-blood free amino acid levels were determined by a micro-method using capillary blood allowed to drop freely onto a filter paper. The equivalent of 10 μl of blood was eluted from the paper with physiological saline, deproteinized with sulfosalicylic acid, and freeze dried. The samples were stored at −70°C before analysis on an automatic ion-exchange chromatography system (10). The reproducibility of the method was better than ±5% for the amino acids reported here.
**TABLE 1. The four feeding groups**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A (n = 7)</th>
<th>Group B (n = 6)</th>
<th>Group C (n = 6)</th>
<th>Group D (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (g)</td>
<td>Range (g)</td>
<td>Mean (g)</td>
<td>Range (g)</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>1,490</td>
<td>1,240–1,720</td>
<td>1,340</td>
<td>1,060–1,560</td>
</tr>
<tr>
<td>Gestation age (weeks)</td>
<td>30.6</td>
<td>29.0–32.0</td>
<td>30.2</td>
<td>28.0–32.0</td>
</tr>
<tr>
<td>Age when feeding regimen started (days)</td>
<td>11</td>
<td>10–16</td>
<td>15</td>
<td>10–21</td>
</tr>
</tbody>
</table>

*Human milk protein (0.8 g) and human milk cream (1.0 g true fat) added to 100 ml of the mother's fresh milk (n = 5) or banked milk (n = 2) providing 125–140 kcal/kg per day and 3.0–3.4 g protein/kg per day.

Human milk cream added to the mother's fresh milk (n = 3), to both mother's and banked milk, or to banked milk only (n = 1) providing 120–135 kcal/kg per day and 1.8–2.0 g protein/kg per day.

Human milk protein added to mother's fresh milk (n = 3) or to banked milk (n = 3) providing 110–125 kcal/kg per day and 3.0–3.4 g protein/kg per day. Sodium chloride added giving a total amount of 20 mEq/liter.

Mother's fresh milk only (n = 5) or both mother's milk and banked milk (n = 1) providing 105–120 kcal/kg per day and 1.8–2.0 g protein/kg per day.
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The donors to the milk bank receive instructions for the hygienic collection of their milk and boil all contaminated parts of the electrical breast pump. Mothers are supplied with disinfected glass bottles into which the expressed breast milk is poured, cooled under running water, and then refrigerated. If the mother develops sore nipples, milk congestion, or infection, she is requested to call the milk bank. The donated milk is delivered to the local grocery store, where it is collected by the daily dairy products transportation. To be accepted for use, the bacterial concentration of the unpasteurized milk has to be below 100,000/ml. An acceptable milk is then pasteurized at 72°C for 15 sec (Alfa-Laval, type P 20-RB, modified plate heat exchanger).

Processing of the milk is shown in Fig. 2. The cream is separated in a simple Alfa-Laval separator type 100. The lactose and salts are separated by means of ultrafiltration through polysulfone membranes with a total area of 0.6 m² (the membrane from Kalle AG and the module from GKSS, West Germany). The milk is freeze dried in separate polypropylene cans (Hostalen PP, Hoechst) for 5 hr at −20°C and for 48 hr at −5°C (Hetosicc, CD 206, Hetolab Equipment AS, Denmark). Each can is cooled and stored under nitrogen. Bacterial controls are performed five times during the process.

The composition of the dry human milk powder is protein, 59.7%, fat, 7.7%, lactose, 10.9%, ash, 1.8%, Na, 0.12%, K, 0.24%, Ca, 0.49%, and Mg, 0.036%. In Group C, the supernatant of the "creamatocrit" (11) was used for sodium analysis, and sodium chloride was added to reach a final concentration of 20 mEq/liter, giving an osmolality of 320 mOsm/kg.

RESULTS

The growth rates of the infants in the different groups were similar. The group being given supplements of sodium chloride had similar serum sodium levels as the other groups (141 mM, range 131–148, n = 6; as against 139, range 134–145, n = 17), but higher concentrations of sodium in the urine (14 mM, range 4–25; as
FIG. 2. The processing of human milk protein powder.
compared with 4, range 1–12). Serum urea was unaffected by the type of feeding, as were the other routine biochemical parameters (Table 2). The urinary C-peptide concentrations in the 6- to 8-hr urine were occasionally higher in the protein/calorie-imbalanced groups.

The whole-blood free amino acid levels for the "critical" amino acids indicating under- or overfeeding (5) are given in Fig. 3. The bars indicate the groups A, B, C, and D (from left to right). For comparison, the free amino acid levels of peripheral blood plasma of ad libitum breast-fed normal infants between 1 and 5 months of age (8) are indicated by a broken horizontal line. They correspond very closely with those of LBW infants given 170 ml/kg per day of pooled human milk determined by Rassin et al. (12,13).

It is evident from Fig. 3 that the blood amino acids show grossly elevated levels in the group supplemented with protein only (group C), whereas the addition of fat "normalized" the spectrum. The proline levels were elevated in group C, and low levels were seen in group B as compared to the unmodified human milk or the doubly supplemented group. In comparison to the plasma free amino acids of full-term breast-fed infants, the blood levels of the VLBW infants were generally lower except for tyrosine and glycine in the protein-only-supplemented group, where the levels were doubled. The plasma free amino acid levels of venous cord blood in term (14) and in premature deliveries (15) are generally higher (alanine and valine four times higher) than the blood levels of VLBW infants, again with the exception of glycine and tyrosine levels of the protein-only-substituted group, where the levels were twice as high.

DISCUSSION AND CONCLUSIONS

The absence of differences in growth rate between the infants who received protein-supplemented human milk and infants fed on preterm milk only has been demonstrated by others (7). Preterm milk has been reported to contain higher protein concentrations during the first weeks post-delivery (16); however, most of this increase is probably caused by secretory IgA (sIgA), which is not absorbed. In this study, the protein content was therefore assumed
TABLE 2. Results of the chemical analyses in the four groups after 2 weeks on the different feeding regimens (group D at 1 month of age)*

<table>
<thead>
<tr>
<th></th>
<th>Group A (n = 7)</th>
<th>Group B (n = 6)</th>
<th>Group C (n = 6)</th>
<th>Group D (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum urea</td>
<td>4.3</td>
<td>2.5</td>
<td>4.1</td>
<td>4.1</td>
</tr>
<tr>
<td>Mean</td>
<td>1.7–7.6</td>
<td>1.7–3.2</td>
<td>2.9–5.6</td>
<td>1.6–8.3</td>
</tr>
<tr>
<td>Serum calcium</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.4</td>
</tr>
<tr>
<td>Mean</td>
<td>2.3–2.8</td>
<td>2.4–2.9</td>
<td>2.1–2.8</td>
<td>2.2–2.6</td>
</tr>
<tr>
<td>Blood base excess</td>
<td>+0.3</td>
<td>-2.2</td>
<td>-0.8</td>
<td>-1.3</td>
</tr>
<tr>
<td>Mean</td>
<td>-4.0 to +3.1</td>
<td>-3.8 to -1.0</td>
<td>-4.2 to +1.8</td>
<td>-5.0 to +3.3</td>
</tr>
<tr>
<td>Blood bicarbonate</td>
<td>23.5</td>
<td>23.4</td>
<td>23.2</td>
<td>24.4</td>
</tr>
<tr>
<td>Mean</td>
<td>21.0–26.7</td>
<td>21.2–27.9</td>
<td>20.7–24.3</td>
<td>22.0–26.7</td>
</tr>
<tr>
<td>Urinary calcium</td>
<td>2.0</td>
<td>3.5</td>
<td>5.3</td>
<td>2.0</td>
</tr>
<tr>
<td>Mean</td>
<td>1.2–3.0</td>
<td>1.0–6.4</td>
<td>1.5–16.8</td>
<td>0.6–3.9</td>
</tr>
<tr>
<td>Urinary mOsm/kg</td>
<td>100</td>
<td>113</td>
<td>162</td>
<td>87</td>
</tr>
<tr>
<td>Mean</td>
<td>70–165</td>
<td>90–155</td>
<td>80–315</td>
<td>55–160</td>
</tr>
<tr>
<td>Urinary C-peptide (nm)</td>
<td>1.7</td>
<td>2.9</td>
<td>2.8</td>
<td>1.2</td>
</tr>
<tr>
<td>Mean</td>
<td>1.0–2.6</td>
<td>1.1–6.1</td>
<td>1.1–9.5</td>
<td>0.3–2.5</td>
</tr>
</tbody>
</table>

*Urine collection is over 6–8 hr. Data expressed in millimoles unless otherwise indicated.
FIG. 3. Capillary whole blood free "critical" amino acid levels of 25 VLBW newborn infants (mean ± SE) in, from left to right, the four feeding groups A, B, C, and D. In group A the mother's milk was supplemented with human milk protein and fat, in group B with fat only, in group C with protein only, and in group D mother's own milk only was given. The differences between group C and the other groups are highly significant. The differences between the proline levels of groups A and B and the tyrosine levels of groups A and D are statistically significant (p<0.02).

to be 1.2 g per 100 ml (based on N content), the addition providing a total of around 2 g per 100 ml of human formula. The total amount of sIgA (17) was 0.023 g/g of protein in the human milk protein isolate used. Moreover, the sIgA activity against a pool of eight E. coli O antigens, as measured by the ELISA method (18), was 57% of that of a reference milk pool. This may be potentially the most significant effect of raising the protein content of human milk for VLBW infants in this way, yielding a "hyperimmune breast milk."

The amino acid levels of plasma are lower than those of whole blood (19). Therefore, higher levels of free amino acids of blood...
plasma (full-term infants and cord blood) than those of whole blood (VLBW infants) cannot be explained by the fact that different amino acid pools were studied. “Normal” glycine levels (as compared to full-term breast-fed infants) and low alanine and branched-chain amino acid levels (valine, leucine, and isoleucine) of the doubly supplemented group, as well as the unfortified human-milk-fed group, at 150 to 175 ml/kg per day indicate (5) that the protein content of 3.0 to 3.4 g human milk protein/kg per day at a caloric intake of 125 to 140 kcal/kg per day is well tolerated. The imbalanced addition of protein only or fat only to the mother’s milk produces an imbalance in the homeostasis of free amino acids in peripheral blood plasma. Since the brain uptake of free amino acids is correlated with the arterial levels (20), this could be disadvantageous to the supply of nutrients to the brain during this period of rapid brain growth.

Further investigations to reveal the optimal supply of protein will require the combination of the present methods with those of balance studies. A metabolic bed for incubators has been constructed, and such studies are in progress. An additional reason for adding human milk fat to the mother’s own fresh milk is the presence of bile-salt-stimulated lipase activity in unprocessed human milk (21), which may contribute to the superior utilization of human milk lipids (22).

The human milk protein requirement will depend to some extent on the caloric intake achieved. The water and caloric requirements will depend on the external environment of the VLBW infants. The future development of firm recommendations on optimal protein intake will depend to a large extent on the more precise determination of optimal water and calorie requirements of the VLBW newborn infants during and after intensive neonatal care.

REFERENCES