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## Individualized Protein Fortification of Human Milk for Preterm Infants: Comparison of Ultrafiltrated Human Milk Protein and a Bovine Whey Fortifier [Original Articles]

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## ABSTRACT

**Background:** To improve the nutritional management of preterm infants, a new individualized human milk fortification system based on presupplementation milk protein analyses was evaluated.

**Methods:** In an open, prospective, randomized multicenter study, 32 healthy preterm infants (birth weights, 920-1750 g) were enrolled at a mean of 21 days of age (range, 9-36 days) when tolerating exclusive enteral feedings of 150 ml/kg per day. All infants were fed human milk and were randomly allocated to fortification with a bovine whey protein fortifier ( $n = 16$ ) or ultrafiltrated human milk protein ( $n = 16$ ). All human milk was analyzed for protein content before fortification with the goal of a daily protein intake of 3.5 g/kg. During the study period (mean, 24 days) daily aliquots of the fortified milk were obtained for subsequent analyses of the protein content.

**Results:** Both fortifiers were well tolerated, and growth gain in weight, length, and head circumference, as well as final preprandial concentrations of serum urea, transthyretin, transferrin, and albumin were similar in both groups. The ultimate estimated protein intake was equivalent in both groups (mean  $3.1 \pm 0.1$  g/kg per day). Serum amino acid profiles were similar in both feeding groups, except for threonine (significantly higher in the bovine fortifier group) and proline and ornithine (significantly higher in the human milk protein group).

**Conclusions:** Protein analyses of the milk before individual fortification provides a new tool for an individualized feeding system of the preterm infant. The bovine whey protein fortifier attained biochemical and growth results similar to those found in infants fed human milk protein exclusively with the corresponding protein intakes.

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Human milk (HM) is preferred in feeding all infants, including preterm and sick newborn infants (1). There is general agreement that preterm infants have a higher protein requirement than is usually achieved by feeding exclusively HM as the nutrient source (2), particularly if banked HM is used (3). Therefore, to increase the protein intake of these infants, specially designed preterm formulas have been developed, and alternatively, when HM is used, supplementation with an HM fortifier is advocated (4-6). Both methods have been shown to improve the nutritional management of preterm infants with increased growth rates and improved metabolic responses. Also, recent studies indicate the superiority of HM over formula for feeding of preterm infants, considering both short-term and long-term outcome data (7,8).

Fortification exclusively with HM protein should theoretically be the gold standard, at least if it is assumed that the amino acid profile of HM protein is superior to any bovine protein. However, because of the large variations in the nutrient composition of HM from mother to mother and during the course of lactation (9-13), it is difficult to obtain accurate nutrient levels using current blind fortification schemes.

To assess an individualized feeding system based on HM protein analyses before fortification, we evaluated growth and protein metabolism in preterm infants fed HM supplemented with ultrafiltrated HM protein (HMP) or a bovine whey fortifier (BF). Our hypothesis was that preterm infants fed similar protein intakes irrespective of protein quality in the fortifier would not differ significantly in short-term growth and indices of protein metabolism.

## **MATERIALS AND METHODS**

### **Study Design and Subjects**

The trial was conducted at the two neonatal units of the University of Lund, Sweden, located in Lund and Malmö and at the neonatal unit of the Department of Perinatal Pathology, Provincial Maternity Hospital, Milan, Italy, from August 1994 through October 1995. The study design was an open, randomized (sealed envelopes), prospective trial involving two groups of HM-fed preterm infants assessing fortification with BF or HMP. The study outcome was markers of protein utilization and growth performance, and the sample size was primarily chosen to show significant differences in protein responses. The sample size was chosen to detect a difference of 10% to 15% in any individual amino acid concentration between the groups with a  $p = 0.05$  and a power of 80%.

Infants were eligible to randomization if their birth weight was between 900 and 1750 g, if they tolerated exclusive enteral feeding of 150 ml/kg per day (no intravenous fluids), and if they showed no signs of systemic disorders or major malformation. Informed consent was obtained from all parents. Thirty-two infants completed the trial, comprising 16 infants in each group. The desired minimal study period was 3 weeks but was not fulfilled by all infants.

### **Human Milk Analyses**

After having surveyed available methods of determining protein and energy concentrations of HM (14), we have in cooperation with the dairy industry established a routine infrared (IR)

method (15) calibrated against HM, to analyze total protein, fat, and carbohydrates (lactose) in HM (13). Energy is calculated using the factors 4.4 kcal/g protein, 3.95 kcal/g carbohydrates, and 9.25 kcal/g fat (3). This method has been found to be the most reliable and least expensive method (in our setting, less than \$15 per sample) for determining the macronutrient content of HM (13). Because of the variation in macronutrient content between pumpings, we have found it important that the sample be taken from a thoroughly mixed 24-hour collection of the infant's own mother's milk (OMM).

We have in Lund, Sweden, during the past years analyzed 437 HM samples from 241 mothers for macronutrient composition and have found a large variation, particularly in the protein and lipid contents (Table 1). This is in agreement with previous reports (9-13). Therefore, to diminish the effects of this variation in macronutrient content in HM between mothers and during the course of lactation, the study was performed using an individualized protein feeding regimen based on presupplementation macronutrient analyses of the milk.

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TABLE 1. Human milk protein content in a group of Swedish mothers

## Feeding Regimens

After informed parental consent was obtained, eligible infants were randomized to one of two feeding regimens: HM fortified with the bovine whey fortifier (BF group) produced by Wyeth Nutritionals International (Philadelphia, PA, U.S.A.), or HM fortified with HMP (HMP group), as previously used (3). Infants were fed exclusively HM supplemented with either BF or HMP. The total enteral volume intake during the study period was maintained between 150 and 170 ml/kg per day. Preferentially, OMM was used for feeding, or, when not available or inadequate in volume, banked HM was used. All milk was expressed using an electric pump, and the milk was stored at  $-20^{\circ}\text{C}$  before use. Contrary to OMM, all banked HM was heat treated before use (Holder pasteurization). No formula was used during the study period.

Before randomization, a representative sample from a 24-hour collection of OMM was analyzed to determine protein content. All banked HM used during the study period was also analyzed for protein content. During the study time, 24-hour collections of OMM were analyzed for protein content once a week for subsequent individualized fortification. After randomization, BF or HMP was added to HM in incremental amounts and gradually increased over a 3- to 7-day period until full strength was achieved. Full strength was defined as the amount of fortifier added to HM to provide a targeted total protein intake of 3.5 g/kg per day. The reason for the goal of daily protein intake of 3.5 g/kg was our previous finding that an intake of 3 to 3.5 g/kg per day yields optimal growth in very low birth weight infants (3). This is in agreement with current recommendations (16,17). The amount of daily fortification was individually adjusted based on infant weight, volume intake, and tolerance of the supplement, as well as the protein content of the milk, to maintain this protein intake throughout the study period. For each infant in the study, the HM was supplemented in the morning with BF or HMP for the following 24 hours. The infants were maintained in the study until they began breast-feeding or when a weight of approximately 2200 g was achieved.

From the fortified milk, daily samples (5-10 ml) were removed and stored at  $-20^{\circ}\text{C}$ . They were subsequently thawed and pooled to representative weekly samples (same amounts obtained each day) and analyzed for protein content using the Kjeldahl procedure (total nitrogen) with Tecator Kjeltac equipment at the Department of Nutrition, Chemical Center, Lund. Protein content was calculated from nitrogen (in grams)  $\times 6.25$ . This procedure provided an accurate estimation of the protein intake throughout the study period for each participating infant.

## Human Milk Fortifiers

Both forms of the supplement (BF or HMP) were in powder form. Human milk protein was prepared in a semiindustrialized scale using a previously described method (18). Pasteurized HM was defatted and subjected to ultrafiltration, leaving a protein concentrate that was freeze dried and packed in sterile aluminum pouches. The powder consisted of 68% protein (mean). Wyeth Nutritionals International has developed a new powdered bovine 100% whey protein fortifier. The composition is presented in [Table 2](#).

Nutrients in 4 g 100% whey powder	Amount
Energy (kcal)	15
Protein (100% whey), g	4
Fat (g)	0.05
Carbohydrate (g)	5
Lactose (g)	0.3
Minerals	
Calcium (mg)	80
Phosphorus (mg)	40
Calcium phosphate (mmol)	2.1
Magnesium (mg)	7
Iron (mg)	100
Zinc (mg)	11.2
Manganese (mg)	4
Copper (mg)	24.1
Iodine (mg)	24.1
Selenium (mg)	1.6
Fluorine (mg)	2.1
Chloride (mg)	1.4
Vitamins	
A (IU)	700
B <sub>1</sub> (mg)	1.4
B <sub>2</sub> (IU)	200
B <sub>6</sub> (IU)	1.5
K <sub>1</sub> (mg)	10
B <sub>12</sub> (microgram)	100
B <sub>12</sub> (microgram)	100
B <sub>12</sub> (microgram)	100
B <sub>12</sub> (microgram)	100
B <sub>12</sub> (microgram)	100
Ascorbic acid (mg)	900
Other acid (mg)	10
Phosphoric acid (mg)	100
Other acid (mg)	1.2
C (mg)	10

TABLE 2. Composition of the bovine whey protein fortifier

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## Clinical Routines

Enteral feedings were begun in all infants on their first day of life. Throughout the study period the feedings were delivered as a bolus through a nasogastric tube every 3 hours, except in a few infants who were fed by bottle at the end of the study. Gastric residual volumes were monitored as part of the clinical routine. The infants were nursed in closed incubators until subsequently placed in open cribs. Throughout the study, all infants with birth weights less than 1500 g were supplemented with extra vitamins and minerals. From the third day of life, a multivitamin preparation (Protovit, Roche, Switzerland) was administered including a total of 1150 IU of vitamin D (calciferol). From the fifth day of life extra calcium (30 mg/kg per day) and phosphorus (20 mg/kg per day) were also administered. This schedule was continued during the study except for the BF group: If the BF supplied exceeded 2 g (one package) per 100 ml, extra calcium and phosphorus was discontinued, and only one-half of the standard dose of the multivitamin preparation was given. Furthermore, from 4 weeks of age, 2 to 3 mg/kg per day of elemental iron (Ferromyn S, Hässle, Mölndal, Sweden) was administered to all infants. Infants with birth weights of 1501 to 1750 g were only given the multivitamin preparation from the third day of life and elemental iron from 4 weeks of age.

## Study Population

Thirty-two infants completed the study protocol. Of these, 19 were from Milan (9 BF, 10 HMP) and the remaining 13 from the University of Lund (7 BF, 6 HMP). Another six infants were originally enrolled but were withdrawn during the study because of deterioration of clinical condition, including suspicion of infections ( $n = 4$ ; 2 BF, 2 HMP) or parental desire to withdraw

the infant from the study ( $n = 2$ ; 1 BF, 1 HMP). Characteristics of the study population are shown in Table 3. Birth weights differed from 940 to 1750 g in the HMP and 920 to 1750 g in the BF group. Age at the beginning of the study varied from 9 to 36 days in both groups. Seven of the infants in the HMP and 11 in the BF group were boys. All infants completing the study were discharged from the hospitals in good clinical condition. One infant was later found by definition to be small for gestational age; all other infants were appropriate for gestational age. The study period was 20 to 40 days, except for four infants with a shorter observation time (2 HMP and 2 BF; 12, 13, 17, and 18 days). No infants were supported by mechanical ventilation or continuous positive airway pressure treatment during the study period.

	HMP (n = 16)	BF (n = 16)
Characteristics		
Birth weight (g)	1446 ± 264	1447 ± 274
Birth gestational age (weeks)	36.8 ± 2.8	36.7 ± 2.2
Age at study start (days)	20.7 ± 7.0	20.3 ± 6.9
Study period (days)	22.1 ± 7.7	22.9 ± 6.7
Breast protein intake (g/kg per day)		
Mean ± SD	3.13 ± 0.14	3.06 ± 0.15
Growth indices		
Weight gain (g/kg)	26.8 ± 3.4	27.1 ± 4.2
Weight gain (g/kg per day)	14.7 ± 3.2	15.6 ± 2.6
Length growth (cm/week)	1.82 ± 0.23	1.97 ± 0.38
Head circumference (cm/week)	1.82 ± 0.23	1.86 ± 0.21

TABLE 3. Characteristics of the study groups, protein intake, and indices of growth

HMP, human milk protein; BF, bovine formula; NS, nonsignificant. Data are mean ± standard deviation. P is nonsignificant.

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## Anthropometric Measurements <sup>†</sup>

The infants were weighed to the nearest 5 g each morning before the next meal as a routine procedure performed by the attending nurse. Measurements of crown-heel length and largest occipitofrontal head circumference were performed weekly by one of two investigating nurses in each unit. A sliding board with a built-in reader in millimeters was used to measure length, and an individual plastic-coated tape measure was used for recording the head circumference.

## Blood Analyses <sup>†</sup>

No blood was obtained if a blood transfusion had been administered within 3 days of the time of blood sampling, to ensure minimal impact on the study results. Serum was obtained from the infants at the beginning and the end of the study by peripheral venipuncture. All blood samples were taken within 1 hour before the next meal (preprandial sampling). Every attempt was made to coordinate blood sampling with the blood testing that is routine in neonatal care. One blood sample was lost at the end of the study in the BF group. Hematocrit and concentrations of hemoglobin and electrolytes were all analyzed at the usual chemical laboratory at each participating hospital. Serum proteins (albumin, transthyretin = prealbumin, transferrin, C-reactive protein [CRP]) and serum urea were analyzed at the Department of Clinical Chemistry at the University Hospital in Lund using routine methods.

Amino acid analyses were performed on the supernatant obtained after precipitation of one part of serum with five parts of a 3% solution of sulfosalicylic acid. After centrifugation for 10 minutes, the supernatant was assayed for amino acid concentrations using an automatic amino acid analyzer (Model 119 CL; Beckman Instruments; Palo Alto, CA, U.S.A.). All amino acid analyses were performed at the Department of Clinical Chemistry of the University Hospital in Malmö.

## Ethics <sup>†</sup>

The study protocol was reviewed and approved by the Research Ethics Committees at the University of Lund and the Provincial Maternity Hospital in Milan.

## Statistical Analysis <sup>†</sup>

Weight gain and increments of length and head circumference were calculated as the difference between values at the beginning and the end of the study period, divided by the number of days of the interval. Expression of weight gain per kilogram body weight was accomplished using the mean weight for the interval studied. Significance of differences between the two feeding groups was analyzed by the Mann-Whitney test.

## RESULTS

### Feedings

Both feeding regimens were well tolerated by the infants. One infant received no enteral feeding for 2 days because of surgery for an inguinal hernia but remained in the study. All other infants continued with enteral feeding throughout the study period. The mean daily volume intake during the trial for the HMP and BF group was  $160 \pm 4$  ml/kg and  $161 \pm 7$  ml/kg (mean  $\pm$  SD), respectively.

Most infants received all or almost all of the milk (>70%) as OMM (BF:  $n = 8$ , HMP:  $n = 12$ ). Seven infants in the BF group and four in the HMP group were fed banked HM exclusively during the study.

### Protein Intake

The actual protein intake could be calculated, because a daily sample of the fortified milk was obtained and pooled to weekly samples subsequently analyzed for nitrogen content, as described. Mean weight gain and volume intakes were calculated, leaving the mean volume intake expressed as ml/kg per day during the weekly milk collection period. By multiplying this value by the determined protein level (nitrogen  $\times$  6.25), a mean protein intake was found. By calculating the weighted means during the study period and excluding the first week of increasing protein intakes, a mean protein intake for the study time was established. As seen in Table 2, mean protein intake was similar in both groups,  $3.13 \pm 0.14$  g/kg per day and  $3.05 \pm 0.15$  g/kg per day for the HMP and BF groups, respectively, thus constituting approximately 10% less than the targeted 3.5 g/kg per day.

### Growth

Weight gain and increments of length and head circumference were very similar in both groups (Table 3).

### Blood Chemistry

No significant differences were found between the groups for hemoglobin, hematocrit, and serum electrolytes (Table 4). Also, the serum concentrations of the different markers of protein metabolism (urea, transthyretin, transferrin, and albumin) showed no significant differences between the groups, at the beginning or the end of the study period (Table 4). Serum concentrations of CRP were also analyzed for all infants concomitantly. All CRP values were normal (<10 mg/l). The reason for including serum CRP was to use an acute-phase reactant to exclude an inflammatory reaction, which may affect the concentrations of other serum proteins.

	Start of study		End of study	
	HMP	BF	HMP	BF
Urea (mg/dl)	4.4 $\pm$ 0.5	4.4 $\pm$ 0.5	4.3 $\pm$ 0.5	4.4 $\pm$ 0.5
Transferrin (mg/dl)	125 $\pm$ 10	125 $\pm$ 10	125 $\pm$ 10	125 $\pm$ 10
Transthyretin (mg/dl)	35 $\pm$ 2	35 $\pm$ 2	35 $\pm$ 2	35 $\pm$ 2
Albumin (g/dl)	3.5 $\pm$ 0.1	3.5 $\pm$ 0.1	3.5 $\pm$ 0.1	3.5 $\pm$ 0.1
Hemoglobin (g/dl)	10.5 $\pm$ 0.5	10.5 $\pm$ 0.5	10.5 $\pm$ 0.5	10.5 $\pm$ 0.5
Hematocrit (%)	32 $\pm$ 1	32 $\pm$ 1	32 $\pm$ 1	32 $\pm$ 1
Serum electrolytes	212 $\pm$ 5	212 $\pm$ 5	212 $\pm$ 5	212 $\pm$ 5
Protein (g/dl)	6.5 $\pm$ 0.5	6.5 $\pm$ 0.5	6.5 $\pm$ 0.5	6.5 $\pm$ 0.5

TABLE 4. Blood and serum biochemical values

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The serum concentrations of 21 amino acids are presented in Table 5. No differences were found between the groups at the beginning of the study. However, at the end of the study, serum levels of threonine were significantly higher in the BF group, whereas the levels of proline and ornithine were significantly higher in the HMP group. Because of loss of one blood sample, only 15 samples were available for the final biochemical analyses in the BF group.

	First analysis		End of study		P value
	BF	HMP	BF	HMP	
Alanine	117 ± 7.2	120 ± 10.0	125 ± 10.0	120 ± 7.0	NS
Arginine	28 ± 3.0	32 ± 4.7	31 ± 3.0	28 ± 2.0	NS
Asparagine	276 ± 30.0	281 ± 17.0	287 ± 10.0	259 ± 30.0	0.009
Aspartic acid	270 ± 17.0	265 ± 13.0	240 ± 9.0	262 ± 16.0	NS
Cysteine	50 ± 4.0	48 ± 10.0	70 ± 10.0	48 ± 11.0	NS
Glutamine	117 ± 4.0	120 ± 10.0	120 ± 10.0	120 ± 11.0	NS
Glutamic acid	331 ± 23.0	325 ± 13.0	340 ± 10.0	340 ± 17.0	NS
Glycine	294 ± 11.0	295 ± 10.0	275 ± 7.0	276 ± 10.0	0.0005
Isoleucine	250 ± 10.0	270 ± 10.0	260 ± 10.0	260 ± 10.0	NS
Leucine	17 ± 1.0	17 ± 1.0	17 ± 1.0	17 ± 1.0	NS
Phenylalanine	12 ± 1.0	12 ± 1.0	12 ± 1.0	12 ± 1.0	NS
Proline	10 ± 1.0	10 ± 1.0	10 ± 1.0	10 ± 1.0	NS
Threonine	15 ± 1.0	15 ± 1.0	15 ± 1.0	15 ± 1.0	NS
Tyrosine	100 ± 10.0	100 ± 10.0	100 ± 10.0	100 ± 10.0	NS
Valine	10 ± 1.0	10 ± 1.0	10 ± 1.0	10 ± 1.0	NS
Ornithine	10 ± 1.0	10 ± 1.0	10 ± 1.0	10 ± 1.0	NS
Other	10 ± 1.0	10 ± 1.0	10 ± 1.0	10 ± 1.0	NS

TABLE 5. Serum amino acid concentrations (µmol/l)

## DISCUSSION

To our knowledge, the present study is the first to present data on the actual protein intake in preterm infants using an individualized HM fortification system based on presupplementation milk protein analyses. Available handling instructions by the manufacturers of various HM fortifiers in general do not take into consideration the preexisting macronutrient content of the milk to be fortified. There is a substantial risk of causing protein overnutrition, particularly when enriching preterm OMM to full strength (5,11). Also, especially when fortifying mature banked HM, there is a risk of causing protein undernutrition if an insufficient amount of fortifier is added to the milk (3).

The importance of this problem can be illustrated as follows: If the infant's OMM, as presented in Table 1, had been fortified according to the manufacturer's recommendations with, for example, 1.0 g protein/100 ml of HM (full strength), the protein intake in infants fed HM with the mean protein content of 15.9 g/l would have been 4.1 g/kg and without fortification 2.5 g/kg per day (with the assumption of a volume intake of 160 ml/kg per day, as in this study). To obtain an estimated protein intake of 3.5 g/kg per day with this HM, fortification with 0.6 g protein/100 ml HM would have been advisable. For infants fed HM with a protein content of -1 SD, (i.e., 12.2 g/l), the corresponding protein intake would have become 3.6 g/kg (fortified) or 1.9 g/kg (unfortified) per day. In contrast, if fed HM with a protein content of +1 SD (19.6 g/l), the intake would have been 4.7 g/kg or 3.1 g/kg per day, respectively. In the extreme, an infant fed HM with only 6.2 g of protein per liter would have been given a protein intake of 2.6 g/kg per day even if maximally fortified, whereas an infant fed a high-protein HM of 27.3 g/l would receive a protein intake of 6.0 g/kg (fortified) or 4.4 g/kg (unfortified) per day.

Therefore, to improve the nutritional management of preterm HM-fed infants, we have developed an individualized feeding system based on protein analyses of HM before fortification (13). By a few simple calculations the protein concentration of HM was adjusted with the goal of a daily protein intake of 3.5 g/kg. This was performed daily based on current volume intake, weight of the infant and analyses of the protein content of the milk. However, the ultimate protein intake turned out to be 3.1 g/kg per day (mean), compared with the expected 3.5. This can mainly be explained by the gradual decrease in the protein content of the OMM that occurs in most women during the course of lactation. Because there is a delay from the time the sample is obtained to the time the result of the protein analysis is available and the milk is finally fortified and fed to the infant, the final amount of protein supplied is lower than originally calculated. This phenomenon was accounted for in the study design with a goal of protein intake of 3.5 g/kg per day, whereas the optimal intake is considered to be 3.0 to 3.5 g/kg per day (16,17).

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In fact, during the study period, the protein content of OMM in some mothers decreased to 50% to 60% of the original level, whereas the protein content of a few milks remained unchanged. A protein intake of 3.0 g/kg per day is probably sufficient for adequate nutrition of these infants, which is also supported by our growth and biochemical data.

We are well aware that a Kjeldahl-calibrated nitrogen method of estimating protein content of HM has its limitations, because some of the nitrogen included is of nonprotein origin, and not all of the protein can be considered to be nutritionally available. However, the nitrogen analysis is well established as the predominant protein assay in clinical studies, and in our opinion this feeding system is far better than the alternative (i.e., when not taking into account the large variation in milks that are fortified for feeding vulnerable preterm infants).

The amino acid concentrations found in the present study were in the same range as in previous studies (19-22). The only significant differences between the two feeding groups were found in the levels of threonine, proline, and ornithine; no differences were seen in growth or biochemical data including serum urea and transthyretin, albumin, and transferrin. All infants tolerated the feedings, and growth rate corresponded to the estimated intrauterine growth rate of the third trimester indicating clinical usefulness of the new BF.

As previously discussed (19), the closest comparison to the levels of plasma essential amino acids in preterm infants fed exclusively with human milk protein at an intake of 3.6 g/kg per day was seen in healthy breast-fed term infants, whereas the levels of amino acids were generally higher in fetuses and cord blood. This suggests that breast-fed term infants are a more appropriate model when evaluating concentrations of amino acids in growing preterm infants.

The higher serum threonine concentration in the BF group at the end of the study is attributable to the use of 100% whey protein, which is in agreement with another report evaluating a 100% whey protein fortifier (22). However, in infants fed whey-predominant formulas the threonine levels were generally higher than in our BF group (21,23,24). The content of proline is much higher in casein than in whey protein. This is probably the reason the serum proline levels were significantly higher in the HMP group, because BF consists of 100% whey protein compared with HMP (approximately 60%). The higher serum ornithine levels found in the HMP group is more difficult to explain; however, the levels found in both groups were all in the normal range as presented in the literature.

Except for these three amino acids, the levels of the serum amino acids were similar although not identical in both groups, with no signs of protein undernutrition. Also, the sum of the final mean concentrations of nine essential amino acids (val, leu, ile, thr, met, lys, his, phe, tyr) in the two groups was almost identical (HMP  $962 \pm 50$   $\mu\text{mol/l}$ , BF  $955 \pm 70$   $\mu\text{mol/l}$  [mean  $\pm$  SD]).

Final concentrations of serum urea as well as the three serum proteins were numerically but not significantly lower in the BF group, corresponding to a slightly lower protein intake in this group. This suggests similar or even better protein utilization in the BF group than in the HMP group and suggests adequate metabolic tolerance of the new BF. The levels of serum urea corresponded well with data from previous studies (8,22,25) as well as for transthyretin (22,26-29) and albumin (26-28).

In summary, an individually adjustable fortification regimen based on HM macronutrient analyses may become a valuable tool in the nutritional management of preterm infants. We also found that the whey protein BF fortifier was well tolerated and produced biochemical and growth data similar to HMP in preterm infants fed equivalent amounts of protein.

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