

ORIGINAL ARTICLE

Adjustable fortification of human milk fed to preterm infants: does it make a difference?

S Arslanoglu¹, GE Moro¹ and EE Ziegler²¹Center for Infant Nutrition, Department of Neonatology, Macedonio Melloni Hospital, Milan, Italy and ²Fomon Infant Nutrition Unit, Department of Pediatrics, University of Iowa, Iowa City, USA

Background: Inadequate nutrition leading to growth failure is common among premature infants. Although fortified breast milk (breast milk plus commercially prepared fortifier) is the preferred feeding, nutrient intakes achieved with fortified breast milk fall short of meeting nutrient needs. This is mainly due to inadequate protein content of fortifiers and variability in composition of expressed breast milk.

Objective: A new adjustable fortification regimen has been designed to ensure that protein needs of premature infants are met at all times. The new regimen encompasses increasing the amount of fortifier and adding extra protein to breast milk guided by periodic determinations of blood urea nitrogen (BUN). The study tested the hypothesis that infants fed according to the new regimen have higher protein intakes and improved weight gain compared to infants fed according to standard fortification regimen.

Methods: In a prospective, controlled trial, preterm infants with birth weights of 600–1750 g and gestational ages between 26 and 34 weeks were fed their own mother's milk or banked donor milk or both. Infants were randomly assigned before 21 days of age to either the new adjustable fortification regimen or the standard regimen. The study period began when feeding volume reached 150 ml/kg/day and ended when infants reached a weight of 2000 g. Standard fortification (STD) consisted in the use of the recommended amount of fortifier. Adjustable fortification (ADJ) consisted in the use, in addition to standard fortification, of extra fortifier and supplemental protein guided by twice-weekly BUN determinations. The primary outcome was weight gain, with serum biochemical indicators and nutrient intakes as secondary outcomes.

Results: Thirty-two infants completed the study as planned (16 ADJ, 16 STD). Infants receiving the ADJ regimen had mean protein intakes of 2.9, 3.2 and 3.4 g/kg/day, respectively, in weeks 1, 2 and 3, whereas infants receiving the STD regimen had intakes of 2.9, 2.9, 2.8 g/kg/day, respectively. Infants on the ADJ regimen showed significantly greater gain in weight (17.5 ± 3.0 vs 14.4 ± 3.0 g/kg/day, $P < 0.01$) and greater gain in

head circumference (1.4 ± 0.3 vs 1.0 ± 0.3 ; $P < 0.05$) than infants on the STD regimen. Weight and head circumference gain were significantly ($P < 0.05$) correlated with protein intake. No significant correlations were found between growth parameters and intake of fat and energy. There were no significant differences between groups in BUN and other serum chemical values. In the ADJ group, BUN concentrations increased significantly ($P < 0.001$) over time but were not significantly higher than in the STD group.

Conclusion: Premature infants managed with the new adjustable fortification regimen had significantly higher weight and head circumference gains than infants managed with standard fortification. Higher protein intake appears to have been primarily responsible for the improved growth with the adjustable regimen. The new fortification method could be a solution to the problem of protein undernutrition among premature infants fed human milk.

Journal of Perinatology (2006) **26**, 614–621. doi:10.1038/sj.jp.7211571; published online 3 August 2006

Keywords: human milk fortification; breast milk fortification; individualized fortification; VLBW infant; neonatal nutrition; protein intake

Inadequate nutrition during vulnerable periods of development has been shown to be associated with impaired brain development in animal models^{1–8} and with impaired neurocognitive development in human preterm infants.^{9,10} Breast milk is the preferred feeding for very low birth weight (VLBW) infants,^{11–24} but it alone cannot meet the high nutrient needs of VLBW infants without nutrient fortification.^{25–26} Current fortification methods produce significantly improved growth in comparison with unfortified maternal milk.²⁷ However, current methods of breast milk fortification still fall short of ensuring an adequate nutrient supply at all times.²⁵ Although VLBW infants frequently show postnatal growth failure,^{28–32} infants fed breast milk fortified according to current standard methods consistently show slower growth than infants receiving equicaloric amounts formulas.^{28,33–35} Assessment of nutrient intakes^{28,36} suggests that protein is the limiting nutrient when maternal milk is fortified according to standard methods,

although energy intake is often low, too, and can become limiting if protein intake is at a satisfactory level.

There are mainly two reasons for inadequate protein intakes, namely the protein content of fortifiers and the variable protein content of maternal milk. Commercial fortifiers are designed to raise the protein content of breast milk to a level that meets the protein needs of the VLBW infant. But commercial fortifiers raise the protein level from the assumed 2.1–2.4 g/100 kcal only to about 3.25 g/100 kcal. This level falls short of meeting the protein needs of the VLBW infant, which are around 3.6 g/100 kcal.³⁷ The other reason is that maternal milk has the assumed protein content of 2.1–2.4 g/100 kcal only at about day 14 of lactation.³⁸ Milk produced earlier is higher in protein content and milk produced later is lower in protein content. As most milk fed to the VLBW infant is either maternal milk produced after day 14 of lactation, or is donor milk provided by mothers of term infants (protein content <1.5 g/100 kcal), the protein content of fortified breast milk fed to VLBW infants is almost always less than 3.25 g/100 kcal. It is therefore not too surprising that VLBW infants fed fortified breast milk show poor growth. The matter is further complicated by the enormous variability of the protein and fat content of expressed breast milk.^{39–41}

If postnatal growth failure with its risk of impaired neurocognitive development is to be avoided in infants fed fortified maternal milk, it is necessary to find ways of providing sufficient amounts of protein so that the needs of the infant for protein are met at all times. Increasing the amount of protein added to maternal milk carries the risk of providing excessively high protein intakes in cases where the protein content of maternal milk is higher than assumed. Polberger *et al.*⁴² devised a method whereby the amount of fortifier is adjusted in accordance with weekly determinations of milk protein content to achieve target protein intakes at all times. This individualized approach, besides being very labor-intensive, depends on the availability of milk analyses. We have devised an individualized approach that does not depend on milk analysis and in which the adjustment of protein intake is based on the metabolic response of the infant.⁴³ The method uses blood urea as metabolic parameter. In the presence of normal renal function, blood urea reflects protein intake, with very low levels indicating low (inadequate) protein intake and very high levels indicating possibly excessive protein intake. Thus, the monitoring of blood urea could help detecting infants with inadequate protein intakes while at the same time safeguarding against excessive protein intakes, a theoretical possibility with any aggressive fortification scheme that does not involve determination of milk protein content. The feasibility of the method was demonstrated by Moro *et al.*⁴³ in a study that showed that the individualized method did lead to higher protein intakes than standard fortification and resulted in somewhat improved growth. The present study was designed to further explore this individualized approach in VLBW infants. Contrary to the earlier

study, the present study used graded additions of complete fortifier and of protein in order to achieve protein intakes that better meet protein needs than the protein intakes achieved with standard fortification.

Methods

Study design

The study was a prospective, randomized, controlled trial in which infants received either the new adjustable (ADJ) fortification regimen or the standard (STD) regimen. The study hypothesis was that infants fed according to the new ADJ regimen would have higher protein intakes and improved weight gain compared to infants fed according to the STD regimen. The study design is illustrated in Figure 1. Infants were enrolled and randomized to one of the feeding groups – STD regimen or ADJ regimen – if and when they reached a feeding volume of 90 ml/kg/day. The actual study began when the feeding volume reached 150 ml/kg/day with full-strength standard fortification. The study ended whenever infants reached a weight of 2000 g. Infants received the regimen to which they were assigned (STD or ADJ) throughout the study. Predetermined random assignments to feeding groups were kept in sequentially numbered sealed opaque envelopes. Randomization used stratification by birth weight (≤ 1250 , 1251–1500 and 1501–1750 g). It was not possible to blind investigators to study group assignment, but caregivers responsible for infants' care and feeding were not involved in the investigation. Subjects were considered to have completed the study as planned if they completed at least 14 days in the study.

Subjects

Infants with birth weight between 600 and 1750 g and gestational age between 24 and 34 weeks were eligible if they were free of

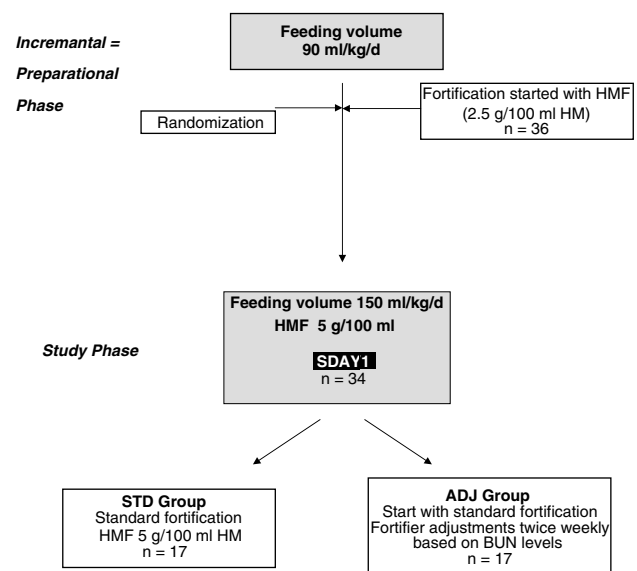


Figure 1 Feeding schedule at the incremental phase and study phase.

major congenital abnormalities, chromosomal aberrations, systemic disease, sepsis, necrotizing enterocolitis or intraventricular hemorrhage, reached a feeding volume of 90 ml/kg/day before DOL (day of life) 21 and were not ventilator-dependent on DOL 21. Gestational age was determined on the basis of menstrual history and antenatal ultrasound or by physical examination when discrepancies were present. All infants were Caucasian. Multiple births were not eligible.

The Institutional Review Board of Macedonio Melloni Hospital reviewed and approved the study protocol and informed written consent was obtained from one or both parents. Thirty-six infants whose parents gave consent were enrolled between April 2002 and July 2003.

Feedings

Most infants (83.3%) received parenteral nutrition starting soon after birth using central venous catheters. Feedings were initiated during the first 3 days of life in the majority (80.6%) of the babies. Infants were fed their own mother's milk or donor milk from the hospital's Human Milk Bank. Milk was initially unfortified. Own mother's milk was some times fed fresh, but mostly had been frozen for some time. Donor milk had been stored frozen. Formula was not fed at any time. Fortification with human milk fortifier (HMF) (FM 85, Nestlé, Italy) was initiated at the time of enrollment, that, when milk intake volume reached 90 ml/kg/day (Figure 1). HMF was initially used at half strength (2.5 g/100 ml of milk) for 2–4 days before it was increased to full strength (5 g/100 ml of milk). Feeding volume was increased gradually as tolerated by the infant. The study period began (SDAY 1) the day when the feeding volume reached 150 ml/kg at full-strength fortification. Beginning on SDAY 1, infants assigned to the adjustable (ADJ) regimen were managed according to the rules for that regimen, whereas infants assigned to the standard regimen (STD) were managed according to nursery routine (Figure 1). During the study, feeding volumes were maintained at 150–160 ml/kg/day. The daily milk supply of each infant was prepared each morning by the addition of the appropriate amount of HMF plus, in the case of infants in the ADJ group, supplemental protein (see below). Fortified human milk was kept refrigerated until fed. The volume of milk fed at each feeding was recorded. All infants received daily 750 IU of vitamin A, 750 IU of vitamin D, and 50 mg/kg of vitamin C starting from the 15th day of life. Zinc in the form of zinc sulfate was added at 0.24 mg/100 ml to fortified milk. From 30 days of age, all infants were provided with 2 mg/kg of iron in the form of ferrous sulfate.

Standard fortification

Infants in the standard fortification group (STD) received human milk fortified with HMF in the standard amount (5 g/100 ml of HM) throughout the study. As indicated in Table 1, the HMF provided (per 100 ml of breast milk) 0.8 g of protein in the form of

hydrolyzed bovine whey proteins and 18 calories (from protein and maltodextrins).

Adjustable fortification

Infants started out with standard fortification but then adjustments of fortification were made based on twice-weekly (Monday and Thursday) determinations of blood urea nitrogen (BUN). Adjustments were made in the amount of HMF and of additional protein added. If the BUN was between 9 and 14 mg/dl (3.2–5.0 mmol/l), no adjustment was made. Every time the BUN was <9 mg/dl (<3.2 mmol/l), fortification was increased by one level. If the BUN was >14 mg/dl (>5.0 mmol/l), a decrease in fortification by one level was made. Table 2 shows the amounts of HMF and additional protein used at the different fortification levels. For level 1 the amount of HMF was increased, whereas for levels 2 and 3 in addition protein was added in the form of a bovine whey protein concentrate (Pro-Mix, Corpak Medsystems, Wheeling, IL, USA). The protein powder was weighed to the nearest 0.1 g using a MonoBloc B2002-S Scale (Mettler Toledo, Switzerland).

Outcome measures

The primary outcome was weight gain (g/kg/day, g/day) determined from SDAY1 to the time infants reached a weight of

Table 1 Nutrient composition of human milk fortifier (HMF) and of supplemental protein (quantities added to 100 ml of milk) according to manufacturers

	HMF FM 85	Supplemental protein (Pro-mix)
Amount of fortifier	5 g	0.4 g
Energy (kcal)	18	1.6
Protein (g)	0.8	0.3
Carbohydrate (g)	3.6	0.05
Fat (g)	0.01	0.016
Calcium (mg)	52	1.6
Phosphorus (mg)	36	1.2
Sodium (mg)	27	0.8
Chloride (mg)	18.5	0.6
Potassium (mg)	11.5	2.3
Magnesium (mg)	2	—

Table 2 Amount of HMF and protein at the various fortification levels

Fortification level	Amount added (g/100 ml milk)
3	HMF 6.25+prot 0.8
2	HMF 6.25+prot 0.4
1	HMF 6.25
0	HMF 5
–1	HMF 3.75
–2	HMF 2.5

2000 g. Additional growth measures included length and head circumference. Anthropometric measurements were performed by experienced nurses. Body weight was determined twice weekly using electronic scales (± 10 g). Length was measured weekly by two measurers to the nearest 0.1 cm using a measuring board with fixed headboard and movable footboard. Head circumference was measured weekly to the nearest 0.1 cm using a nonstretchable tape measure. Weight gain in g/day was calculated as the difference between the initial and final weight, divided by the number of days elapsed, and in g/kg/day by dividing gain in g/day by the average weight during the observation period.

Secondary outcome measures were BUN and serum concentrations of creatinine, albumin, calcium (Ca), phosphorus (P) and alkaline phosphatase (ALP). Blood samples for BUN were drawn twice weekly by heel stick and for creatinine and albumin weekly and for Ca, P and ALP every 2 weeks by venipuncture. BUN was analyzed by a urease method using Stat Profile Critical Care Analyzer Xpress (Nova Biomedical, Waltham, MA, USA). Serum albumin was determined by a colorimetric method using a Roche/Hitachi Modular Analyzer (Roche Diagnostics, Mannheim, Germany). Calcium, phosphorus and alkaline phosphatase were determined by colorimetric assays using a Roche/Hitachi 912 Analyzer (Roche Diagnostics, Mannheim, Germany).

Feeding volume and feeding tolerance (abdominal distension, gastric residuals, emesis and withheld feedings) were recorded daily.

Nutrient intakes. Aliquots of fortified milk were collected twice weekly from each infant's daily supply of milk and combined to form weekly pools. Samples were stored frozen at -20°C until analyzed. Milk fat was determined by a modified Folch procedure⁴⁴ and total nitrogen was determined by a micro-Kjeldahl method as described previously.⁴⁵ Protein (g/l) was calculated as nitrogen $\times 6.38$, assuming that nonprotein nitrogen contributed 295 mg/l and that 27% of nonprotein nitrogen was bioavailable, that is equivalent to protein nitrogen.⁴⁶ Milk energy was calculated assuming a lactose concentration of 70 g/l and using the factors 4.0 kcal/g for protein and lactose and 9.0 kcal/kg for fat. Intakes of protein and energy were calculated from recorded feeding volumes and protein concentration and energy density.

Sample size

To be clinically relevant, growth with the adjustable fortification regimen would have to be improved to such a degree that hospital stay would be shortened by 3 days. This would require a difference in weight gain of 3.5 g/day. Based on the data of Moro *et al.*⁴³ to detect such a difference at $\alpha = 0.05$ with 80% power, 15 infants per group were needed.

Statistical analyses

Analysis of variance procedures were used to compare continuous variables between feeding groups and to assess for effects of time

within feeding groups. When equality of variances were not present, Kruskal Wallis and Mann–Whitney *U* nonparametric tests were used. Repeated dependent variables (biochemical measurements, milk composition and nutrient intake values, fortification levels) were evaluated by repeated measures ANOVA. When a significant feeding by time interaction was detected, means were compared at each time point. Bivariate correlations were determined by Pearson's coefficient of correlation. Statistical significance was set at the 5% level of probability. All statistical analyses were performed using the SPSS 10.0 program for Windows. Unless indicated otherwise, the data are expressed as mean \pm s.d. values.

Results

Subjects

A total of 36 infants were enrolled but two left the study early because of feeding intolerance in terms of abdominal distension and emesis, leaving 34 who were randomized to either the ADJ group ($n = 17$) or the STD group ($n = 17$). All infants completed the protocol, but two (one in ADJ, one in STD) reached a weight of 2000 g after < 14 day in the trial, so their data were excluded from analysis. Characteristics of the remaining 32 subjects are presented in Table 3. The groups were similar with respect to gestational age, weight, head circumference and length at birth, Apgar scores, age, and weight at SDAY1. The clinical course before study entry was similar in both groups. Most of the infants were initiated on minimal enteral feedings on the second or third day of life (mean age 2.8 ± 2.3 day STD group, 2.4 ± 1.3 day ADJ group). Full feedings were reached at a median age of 11 day (range: 24 day) in STD and 9.5 day (range: 26 day) in ADJ. Before study entry and during the study, all infants were fed human milk, of which about 60% was provided by the infants' own mothers and 40 % was pasteurized donor milk from the Human Milk Bank.

Milk fortification and nutrient intakes

Infants received fortified breast milk for an average of 4.9 day (STD group) and 4.8 day (ADJ group) before they reached SDAY 1 and

Table 3 Baseline characteristics of study subjects

	STD ($n = 16$)	ADJ ($n = 16$)
Gestational age at birth (wk)	31.3 ± 2.1	31.8 ± 1.7
Birthweight (g)	1407 ± 258	1386 ± 283
Length at birth (cm)	39.6 ± 2.1	39.6 ± 2.6
Head circumference at birth (cm)	27.7 ± 2.2	27.3 ± 2.1
AGA/SGA	12/4	11/5
Sex (female/male)	9/7	12/4
Apgar score (5 min, median)	8.5	9
Postnatal age at SDAY1 (d)	18.9 ± 7.5	18.7 ± 6.4
Weight at SDAY1 (g)	1526 ± 181	1501 ± 252

Values are mean \pm s.d.

Differences between study groups were not statistically significant.

remained in the study for a similar duration (mean 20.8 ± 8.0 day STD group and 20.9 ± 9.0 day ADJ group). Mean fortification levels in the ADJ group increased significantly ($P < 0.001$) over time, with mean levels being $+0.9$, $+1.7$ and $+2.3$, respectively, in successive study weeks. All 16 babies in the ADJ group reached at least level $+1$, with 14 reaching level $+2$ and 8 reaching level $+3$. Only one baby needed level -1 for 3 days.

Volume of intake was maintained around the target volume of 150 ml/kg/day in both groups. As indicated in Table 4, average fat and energy content of fortified milk were similar in both groups and did not change during the course of the study. On the other hand, protein concentration in the ADJ group increased

Table 4 Milk composition and nutrient intakes

	STD	ADJ
<i>Energy density (kcal/100 ml)</i>		
1st week	85.0 ± 5.4	85.0 ± 8.7
2nd week	83.9 ± 7.9	83.7 ± 6.9
3rd week	80.5 ± 4.9	85.2 ± 3.9
<i>Protein concentration (g/100 ml)</i>		
1st week	1.9 ± 0.3	$1.9 \pm 0.2^{\#}$
2nd week	$2.0 \pm 0.2^{**}$	$2.2 \pm 0.3^{**\#}$
3rd week	$1.9 \pm 0.2^{**}$	$2.3 \pm 0.3^{**\#}$
<i>Fat concentration (g/100 ml)</i>		
1st week	3.8 ± 0.5	3.5 ± 0.9
2nd week	3.7 ± 0.8	3.3 ± 0.8
3rd week	3.4 ± 0.6	3.6 ± 0.9
<i>Volume of intake (ml/kg/day)</i>		
1st week	148.4 ± 6.1	149.7 ± 3.9
2nd week	151.1 ± 2.8	150.0 ± 3.3
3rd week	150.1 ± 2.8	150.2 ± 4.6
<i>Energy intake (kcal/kg/day)</i>		
1st week	125.9 ± 7.9	127.2 ± 12.1
2nd week	126.6 ± 11.8	125.6 ± 11.6
3rd week	120.5 ± 8.3	128.0 ± 8.3
<i>Protein intake (g/kg/day)</i>		
1st week	2.9 ± 0.4	$2.9 \pm 0.3^{\#}$
2nd week	$2.9 \pm 0.3^{*}$	$3.2 \pm 0.4^{*\#}$
3rd week	$2.8 \pm 0.2^{**}$	$3.4 \pm 0.5^{**\#}$
<i>Fat intake (g/kg/day)</i>		
1st week	5.7 ± 0.7	5.3 ± 1.3
2nd week	5.6 ± 1.3	4.9 ± 1.2
3rd week	5.0 ± 1.0	5.4 ± 1.5

Values are mean \pm s.d.

***Significant differences between groups; $P = 0.05$, $P < 0.05$, respectively.

*,**Significant changes over time; $P = 0.01$, $P < 0.01$, respectively.

significantly over time and was significantly higher during weeks 2 and 3 than in the STD group. Consequently, protein intake increased significantly over time in the ADJ group and was significantly greater in the ADJ group than in the STD group, in which protein intake remained constant. Figure 2 shows mean protein intakes for the study groups throughout the study. For reference, intrauterine protein requirements determined by the factorial method¹⁸ are also indicated.

Growth

Infants in the ADJ group gained significantly more weight and had greater increases in head circumference than infants in the STD group (Table 5). Although linear growth was somewhat faster in the ADJ group, the difference did not reach a statistical significance. Protein intake was significantly correlated with weight gain (g/kg/day) ($r = 0.392$, $P = 0.027$) and head circumference gain ($r = 0.389$, $P = 0.029$). No correlations were found between energy and fat intakes and growth variables.

Serum chemical data

Serum albumin and creatinine levels did not differ between the groups and did not change significantly during the study (Table 6). On the other hand, in the ADJ group mean BUN, as

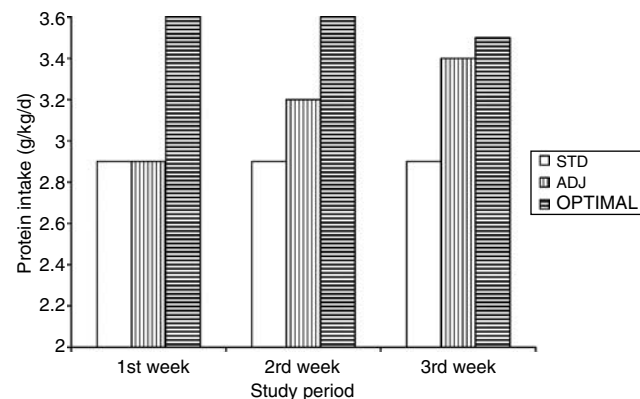


Figure 2 Average protein intakes during the study compared with intrauterine protein intakes. *Optimal intakes reflect intrauterine protein intakes of the fetus with a body weight corresponding to the mean body weights of the study infants for each week.

Table 5 Weight, length and head circumference gains during the study period

Outcome variable	STD	ADJ	P-value
Weight gain (g/day)	24.8 ± 4.8	30.1 ± 5.8	<0.01
(g/kg/day)	14.4 ± 2.7	17.5 ± 3.2	<0.01
Length gain (mm/day)	1.1 ± 0.4	1.3 ± 0.5	>0.05
Head circumference gain (mm/dy)	1.0 ± 0.3	1.4 ± 0.3	<0.05

Values are mean \pm s.d.

Table 6 Serum chemical values

	STD	ADJ
<i>BUN (mg/dl)</i>		
1st week	8.3±5.2	7.7±2.7 [#]
2nd week	8.9±4.3	9.4±3.6 [#]
3rd week	9.3±4.7	11.9±2.6 [#]
<i>Creatinine (mg/dl)</i>		
1st week	0.54±0.1	0.50±0.1
2nd week	0.49±0.2	0.47±0.1
3rd week	0.49±0.1	0.48±0.1
<i>Albumin (g/dl)</i>		
1st week	3.0±0.9	3.0±0.3
2nd week	3.1±0.2	3.0±0.4
3rd week	3.1±0.2	3.2±0.6

Values are mean±s.d.

[#]Change over time significant at $P<0.0001$.

expected, increased significantly over time, but was not significantly ($P=0.57$) higher than in the STD group. Although some BUN values were <4 mg/dl, indicative of inadequate protein intake, no value was >20 mg/dl, our upper limit of normality. Serum calcium levels did not differ between the groups during the study and did not change during the study, whereas phosphorus levels and alkaline phosphatase activity increased significantly over time in both groups but did not differ between study groups.

Feeding tolerance and clinical course

There were no statistically significant differences between feeding groups with respect to the number of days with at least 1 episode of emesis (1.9 ± 3.0 in STD, 0.9 ± 1.1 in the ADJ groups); and percentage of the withheld feedings (2.1 ± 1.7 in STD, 2.3 ± 1.2 in ADJ groups). One infant in STD group had abdominal distension before SDAY1 and two infants in the ADJ group had abdominal distension during the study while being on fortification level +2 and +3, respectively. In these cases abdominal distension resolved when, per nursery routine, fortification was withheld for 1 day. Thereafter fortification was resumed without further abdominal distension. No study infant had necrotizing enterocolitis or systemic infection.

Discussion

Confirming our earlier findings,⁴³ the present study demonstrates the general feasibility and safety of our BUN-based individualized method of breast milk fortification. The present study furthermore shows the method to be effective. To our knowledge, this is the first study to demonstrate that protein supplementation can be

increased safely beyond the amount provided by standard fortifiers and that increased protein intake leads to improved growth of VLBW infants. Thus, it appears that our method of BUN-based adjustable fortification offers for the first time a practical and safe method of raising the protein intake of VLBW infants to a satisfactory level.

Securing an adequate protein intake for VLBW infants fed maternal milk presents a challenging problem because of the variable and, in practice, always unknown protein content of maternal milk. The challenge is somewhat different with donor milk with its consistently low protein content, but even there the problem of protein fortification has not been solved. The evidence is overwhelming that with current fortification methods VLBW infants fed breast milk, both maternal and donor, receive inadequate protein intakes. As a result not only inadequate protein intakes but also excessively high protein intakes need to be avoided, it is thought that any method for increasing the protein intake of VLBW infants would need to be individualized.

Among the individualized approaches, a method based on periodic analysis of maternal milk has been shown to lead to improved protein intake.⁴² Although this method holds promise, it depends on the availability of milk analysis and is therefore currently not widely applicable.

Our individualized ('adjustable') approach makes use of the metabolic response of the infant to guide the addition of protein above and beyond that which is provided by the commercial fortifier. It is a truly individualized method in that it makes no assumptions regarding an infant's protein requirements. Its advantage over other individualized approaches⁴² include that it does not depend on milk analysis and that it safeguards against unduly high protein intakes. Although blood urea (BUN) is influenced by renal function and by hydration state, we used BUN as metabolic indicator because, all other influences being equal, it is proportional to protein intake^{47–50} and responds rapidly to changes in protein intake. BUN determinations are routinely performed by clinical laboratories and are thus readily available. In the present study, periodic determination of BUN proved satisfactory in that it led to improved protein intake while avoiding excessively high protein intakes. It is possible that indicators of protein nutritional status, such as retinol-binding protein or transthyretin, could be used in place of BUN, although it does not appear that any of these indicators are useful in detecting excessively high protein intakes.

The adjustable fortification method improved both weight gain and head growth compared to standard fortification. It has been clearly demonstrated⁵¹ that early postnatal head growth is a strong predictor of neurodevelopmental outcome. In VLBW infants, perinatal growth failure, as evidenced by a subnormal head circumference at 8 months of age, has been found to be associated with poor cognitive function, academic achievement, and behavior at 8 years of age.⁵² Thus, promotion of growth and particularly of

the head circumference, possibly will have a long-term positive impact that needs to be further investigated.

One theoretical disadvantage of our BUN-based adjustable fortification method is that it does not permit adjustment of energy intake. However, energy intake was not correlated with weight gain in the present study. This lack of effect of energy intake on growth confirms the notion that protein intake is usually the limiting nutrient with regard to growth of VLBW infants.²⁸ Protein intake, on the other hand, was found to be strongly related to growth. This serves to reinforce the paramount importance of protein among the nutritional determinants of growth of VLBW infants. It seems entirely appropriate to concentrate efforts on improving the protein intake of VLBW infants.

Acknowledgments

We thank L Grella, responsible nurse of Human Milk Bank, for her precious help in collecting milk samples. We also thank L Guida, RN and all NICU staff for their assistance in collecting blood samples and for their contributions to the study.

References

- 1 Dobbing J. Early nutrition and later achievement. *Proc Nutr Soc* 1990; **49**: 103–118.
- 2 Grantham-McGregor SM, Ani CC. Undernutrition and mental development. In: Fernstrom JD, Uauy R, Arroyo P (eds). *Nutrition and Brain*. Vevey: Nestec Ltd, and Karger: Basel, 2001, pp 1–14.
- 3 Uauy R, Mena P, Peirano P. Mechanisms for nutrient effects on brain development and cognition. In: Fernstrom JD, Uauy R, Arroyo P (eds). *Nutrition and Brain*. Vevey: Nestec Ltd, and Karger, Basel, 2001, pp 41–72.
- 4 Diaz-Cintra S, Cintra L, Ortega T, Kemper T, Morgane PJ. Effects of protein deprivation on pyramidal cells of the visual cortex in rats of three age groups. *J Comp Neurol* 1990; **292**: 117–126.
- 5 Faundez V, Cordero ME, Rosso P, Alvarez C. Calibers and microtubules of nerve fibers: differential effect of undernutrition in developing and adult rats. *Brain Res* 1990; **509**: 198–204.
- 6 Escobar C, Salas M. Neonatal undernutrition and amygdaloid nuclear complex development: an experimental study in the rat. *Exp Neurol* 1993; **122**: 311–318.
- 7 Escobar C, Salas M. Dendritic branching of claustral neurons in neonatally undernourished rats. *Biol Neonate* 1995; **68**: 47–54.
- 8 Montanha-Rojas EA, Ferreira AA, Tenorio F, Barradas PC. Myelin basic protein accumulation is impaired in a model of protein deficiency during development. *Nutr Neurosci* 2005; **8**: 49–56.
- 9 Lucas A, Morley R, Cole TJ. Early diet in preterm babies and developmental status at 18 months. *Lancet* 1990; **335**: 1477–1478.
- 10 Lucas A, Morely R, Cole TJ. Randomised trial of early diet in preterm babies and later intelligence quotient. *BMJ* 1998; **317**: 1481–1487.
- 11 Lucas A, Cole TJ. Breast milk and neonatal necrotising enterocolitis. *Lancet* 1990; **336**: 1519–1523.
- 12 Anderson JW, Johnstone BM, Remley DT. Breast-feeding and cognitive development: a meta-analysis. *Am J Clin Nutr* 1999; **70**: 525–535.
- 13 Bier JA, Oliver T, Ferguson AE, Vohr BR. Human milk improves cognitive and motor development of premature infants during infancy. *J Hum Lact* 2002; **18**: 361–367.
- 14 Blaymore Bier J, Oliver T, Ferguson A, Vohr BR. Human milk reduces outpatient upper respiratory symptoms in premature infants during their first year of life. *J Perinatol* 2002; **22**: 354–359.
- 15 Feldman R, Eidelman AI. Direct and indirect effects of breast-milk on the neurobehavioral and cognitive development of premature infants. *Dev Psychobiol* 2003; **43**: 109–119.
- 16 Hylander MA, Strobino DM, Dhanireddy R. Human milk feedings and infection among VLBW infants. *Pediatrics* 1998; **102**(3): E18.
- 17 Hylander MA, Strobino DM, Pezzullo JC, Dhanireddy R. Association of human milk feedings with a reduction in retinopathy of prematurity among very low birth weight infants. *J Perinatol* 2001; **21**: 356–362.
- 18 Lucas A, Morley R, Cole TJ, Gore SM. A randomised multicentre study of human milk versus formula and later development in preterm infants. *Arch Dis Child* 1994; **70**: F141–F146.
- 19 Lönnerdal B. Nutritional and physiologic significance of human milk proteins. *Am J Clin Nutr* 2003; **77**(suppl): 1537S–1543S.
- 20 McGuire W, Anthony MY. Donor human milk versus formula for preventing necrotising enterocolitis in preterm infants: systematic review. *Arch Dis Child Fetal Neonatal Ed* 2003; **88**: F11–F14.
- 21 Rønnestad A, Abrahamsen TG, Medbø S, Reigsatd H, Lossius K, Kaarensen PI *et al*. Late-onset septicemia in a Norwegian national cohort of extremely premature infants receiving very early full human milk feeding. *Pediatrics* 2005; **115**: e269–e276.
- 22 Schanler RJ. The use of human milk for premature infants. *Pediatr Clin North Am* 2001; **48**: 207–219.
- 23 Kilbride HW, Wirtschafter DD, Powers RJ, Sheehan MB. Implementation of evidence-based potentially better practices to decrease nosocomial infections. *Pediatrics* 2003; **111**(4 pt 2): e519–e533.
- 24 American Academy of Pediatrics. Policy statement. Section on breastfeeding. *Pediatrics* 2005; **115**: 496–506.
- 25 Ziegler EE. Breast-milk fortification. *Acta Paediatr* 2001; **90**: 720–723.
- 26 Hay Jr WW, Lucas A, Heird WC, Ziegler EE, Levin E, Grave GD *et al*. Workshop summary: nutrition of the extremely low birth weight infant. *Pediatrics* 1999; **104**: 1360–1368.
- 27 Kuschel CA, Harding JE. Multicomponent fortified human milk for promoting growth in preterm infants. *Cochrane Rev* 2004; **1**: Cochrane Library.
- 28 Carlson SJ, Ziegler EE. Nutrient intakes and growth of very low birth weight infants. *J Perinatol* 1998; **18**: 252–258.
- 29 Ehrenkranz RA, Younes N, Lemons JA, Fanaroff AA, Donovan EF, Wright LL. Longitudinal growth of hospitalised very low birth weight infants. *Pediatrics* 1999; **104**: 280–289.
- 30 Lemons JA, Bauer GR, Oh W. 2001 Very-low-birth-weight outcomes of the NICHD Neonatal Research Network, January 1995 through December 1996. *Pediatrics* 2001; **107**(1): E1.
- 31 Dusick AM, Pointdexter BB, Ehrenkranz RA, Lemons JA. Growth failure in the preterm infant: can we catch up? *Semin Perinatol* 2003; **27**: 302–310.
- 32 Embleton NE, Pang N, Cooke RJ. Postnatal malnutrition and growth retardation: an inevitable consequence of current recommendations in preterm infants? *Pediatrics* 2003; **107**: 270–273.
- 33 Schanler RJ, Shulman RJ, Lau C. Feeding strategies for premature infants: beneficial outcomes of feeding fortified human milk versus preterm formula. *Pediatrics* 1999; **103**: 1150–1157.

- 34 Pieltain C, deCurtis M, Gerard P, Rigo J. Weight gain composition in preterm infants with dual energy x-ray absorptiometry. *Pediatr Res* 2001; **49**: 120–124.
- 35 O'Connor DL, Jacobs J, Hall R, Adamkin D, Auestad N, Castillo M *et al*. Growing and development of premature infants fed predominantly human milk, predominantly premature infant formula, or a combination of human milk and premature formula. *JPGN* 2003; **37**: 437–446.
- 36 Olsen IE, Richardson DK, Schmid CH, Ausman LM, Dwyer JT. Intersite differences in weight growth velocity of extremely premature infants. *Pediatrics* 2002; **110**: 1125–1132.
- 37 Ziegler EE, Thureen PJ, Carlson SJ. Aggressive nutrition of the very low birthweight infant. *Clin Perinatol* 2002; **29**: 225–244.
- 38 Lemons JA, Moye L, Hall D, Simmons M. Differences in the composition of preterm and term human milk during early lactation. *Pediatr Res* 1982; **16**: 113–117.
- 39 Gross SJ, Geller J, Tomarelli RM. Composition of breast milk from mothers of preterm infants. *Pediatrics* 1981; **68**: 490–493.
- 40 Michaelsen KF, Skafte L, Badsberg JH, Jørgensen M. Variation in macronutrients in human bank milk: Influencing factors and implications for human milk banking. *J Pediatr Gastroenterol Nutr* 1990; **11**: 229–239.
- 41 Weber A, Loui A, Jochum F, Bühner C, Obladen M. Breast milk from mothers of very low birth weight infants: variability in fat and protein content. *Acta Paediatr* 2001; **90**: 772–775.
- 42 Polberger S, Raiha NCR, Juvonen P, Moro GE, Minoli I, Warm A. Individualized protein fortification of human milk for preterm infants: comparison of ultrafiltrated human milk protein and a bovine whey fortifier. *JPGN* 1999; **29**: 332–338.
- 43 Moro GE, Minoli I, Ostrom M, Jacobs JR, Picone TA, Riih   NCR *et al*. Fortification of human milk: evaluation of a novel fortification scheme and of a new fortifier. *J Pediatr Gastroenterol* 1995; **20**: 162–172.
- 44 Polberger S, L  nnerdal B. Simple and rapid macronutrient analysis of human milk for individualized fortification: basis for improved nutritional management of very-low-birthweight infants? *J Pediatr Gastroenterol Nutr* 1993; **17**: 283–290.
- 45 Fomon SJ, Thomas LN, Filler Jr LJ, Anderson TA, Bergmann KE. Requirements for protein and essential amino acids in early infancy. *Acta Paediatr Scand* 1973; **62**: 33–45.
- 46 Fomon SJ. Requirements and recommended dietary intakes of protein during infancy. *Pediatr Res* 1991; **30**: 391–395.
- 47 Eggum BO. Blood urea measurement as a technique for assessing protein quality. *Br J Nutr* 1979; **24**: 983–988.
- 48 Polberger S, Axelsson IE, Raiha NCR. Urinary and serum urea as indicators of protein metabolism in very low birth weight infants fed varying human milk protein intakes. *Acta Paediatr Scand* 1990; **79**: 737–742.
- 49 Fomon SJ. *Nutrition of Normal Infants*. Mosby-Year-Book: St Louis, 1993 pp 129–131.
- 50 Davies DP, Saunders R. Blood urea. Normal values in early infancy related to feeding practices. *Arch Dis Child* 1973; **48**: 563–565.
- 51 Gross SJ, Oehler JM, Eckerman CO. Head growth and developmental outcome in very low-birth-weight infants. *Pediatrics* 1983; **71**: 70–75.
- 52 Hack M, Breslau N, Weissman B, Aram D, Klein N, Borawski E. Effect of very low birth weight and subnormal head size on cognitive abilities at school age. *N Engl J Med* 1991; **325**: 231–237.