Short- and long-term effects of individualized enteral protein supplementation in preterm newborns

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SUMMARY: Ergenekon E, Soysal Ş, Hirfanoğlu İ, Baş V, Gücüyener K, Turan Ö, Beken S, Kazancı E, Türkyılmaz C, Önal E, Koç E, Atalay Y. Short- and long-term effects of individualized enteral protein supplementation in preterm newborns. Turk J Pediatr 2013; 55: 365-370.

The aim of this retrospective study was to assess the need for additional enteral protein supplementation in preterm newborns with gestational age (GA) ≤32 weeks after full enteral feeds with either fortified breast milk (FBM) or preterm formula (PF) were reached, and to determine the effects of additional protein on physical and neurological development. After the standard early total parenteral nutrition (TPN) and reaching full enteral nutrition with 150-160 ml/kg/day, preterms were assessed for the requirement of additional protein based on serum blood urea nitrogen (BUN)/prealbumin levels. Additional enteral protein was given for BUN <5 mg/dl and/or prealbumin ≤8 mg/dl with weekly assessments as per Neonatal Intensive Care Unit (NICU) protocol. Growth in the NICU and neurodevelopmental outcome at 18 months' corrected age (CA) were determined.

There were 32 newborns in the non-supplemented group (Group 1) and 33 newborns in the supplemented group (Group 2). All newborns in Group 2 were on FBM. Weight gain and head growth were better and Bayley scores at 18 months' CA were higher in Group 2.

Standard preterm nutrition with FBM may not be sufficient for preterms, and additional enteral protein supplementation may improve the physical growth rate in the NICU and result in better neurodevelopmental outcome at 18 months' CA.

Key words: preterm newborn, enteral protein, growth, neurodevelopmental outcome.

Nutrition of preterm newborns is an ongoing area of research and discussion. Early aggressive nutrition has almost become the standard of care, with early implementation of intravenous (IV) amino acid administration with total parenteral nutrition (TPN) from the very first hours of life in very low birth weight infants[1]. This approach has aimed to prevent the metabolic shock of the preterm after birth and has been reported to be well tolerated, while decreasing the progressive loss of total body protein. The main purpose of aggressive nutrition in preterms is to enable the best possible brain growth in order to obtain a favorable neurodevelopmental outcome in this vulnerable population.

Currently, there is very little doubt amongst neonatologists about when to start the IV amino acid solution and by how much to increase it. However, once the baby is on full enteral feeds receiving either fortified breast milk (FBM) or preterm formula (PF), it is less clear how to proceed, for there are raised concerns about whether this feeding regimen is adequate or too much for the rapidly growing preterm. The content of breast milk (BM) fortified with commercial BM fortifiers for preterm newborns has been assessed, revealing that the protein content of the FBM may actually be inadequate for sufficient growth[2]. The authors of one study have suggested that targeted fortification of BM, i.e., adding supplemental protein by titrating

the amount in BM, may be an alternative to overcome this problem. Arslanoglu et al.[3] looked at adjustable fortification of BM again with enteral protein supplement, but this time aiming at a certain level of blood urea nitrogen (BUN) in the serum. Their results revealed a better protein balance and growth in the study group, and they concluded that despite fortification of BM, the preterms might still be getting less than adequate protein for their needs and that the protein supplementation should thus be individualized[3,4].

On the other hand, nutritional assessment is another point of interest and discussion in the growing preterm. Although BUN levels may show protein balance in healthy individuals, they are still prone to false conclusions due to the rapid changes in the fluid balance in the small preterm. Prealbumin has been considered as another useful tool for assessment of protein accretion in this population[5-7].

The objectives of this retrospective study were 1) to determine the growth parameters during the Neonatal Intensive Care Unit (NICU) stay and neurodevelopmental outcome at 18 months' corrected age (CA) in preterm newborns with gestational age (GA) ≤32 weeks who received additional enteral protein supplement based on BUN and prealbumin levels after reaching full enteral feeds with either FBM or PF, and 2) to compare results with those of infants who did not receive extra enteral protein supplement.

Material and Methods

The study was performed on the data of newborns admitted to Gazi University NICU from 2006-2010. Preterm newborns with GA ≤32 weeks who were discharged from the NICU were considered eligible for the study. According to our unit's nutrition protocol, each infant is started on IV amino acid solution 1-1.5 g/kg/day in the first 24 hours of life, IV lipid solution is added the second day, and both are increased by 1 g/kg/day up to 3-4 g/kg/day IV amino acid and 3 g/kg/day IV lipid dose. Enteral feeds are usually started as soon as the baby is stable as per our unit's protocol. The mother's own milk is the choice of nutrition if available; otherwise, PF is given, as donor milk banking is not yet established in our country.

According to our enteral nutrition protocol, one week after the newborn reaches full enteral feeds (150-160 ml/kg/day) with fully FBM including 5.5 g/100 ml Eoprotin® (Aptamil-Milupa breast-milk fortifier), which supposedly provides 87 kcal/100 ml and 2.2 g protein/100 ml of BM (assuming 1g of protein in 100 ml of BM), or PF (Prematil®/Milupa), serum BUN and prealbumin levels are measured. Then, additional enteral protein Protifar® (Nutricia), which provides 2.2 g of protein/1 scoop, is added to the diet if BUN is <5 mg/dl and/or prealbumin is ≤8 mg/dl. The supplement is started as 1 g/kg/day additional enteral protein. The same values are checked within 7-10 days to determine if the supplementation resulted in an increase in BUN or prealbumin levels, and another increase in protein supplement is made if no response is obtained. BUN and prealbumin levels were checked in the non-supplemented group every 7-10 days, and they were given additional protein if the levels

Table I. Demographic Data and Physical Growth Data of the Study Group during NICU Stay

	GA (wk)	BW (g)	Weight Gain (g/kg/day)	Increase in length (cm/week)	Increase in HC (cm/week)
Group 1: No Additional Enteral Protein					
N=32 (12 F, 20 M)	30.5 (28-32.5)	1417.5 (1262-2350)	11.5 (9.4-23)	0.7 (0-1.3)	0.6 (0.25-1.25)
Group 2 : Additional Enteral Protein					
N=33 (18 F, 15 M)	30 (23-32)	1190 (613-1813)	17 (11-31)	0.9 (0.5-1.5)	0.75 (0.5-1.25)
P	0.3	0.04	0.0001	0.085	0.007

GA: Gestational age. BW: Birth weight. HC: Head circumference. F: Female. M: Male.
Data are given as median (range).

were below the previously mentioned cut-off level. The non-supplemented group received 3.5 g/kg/day of protein with 160 ml/kg/day PF or FBM, whereas the supplemented group received 4-5 g/kg/day of protein depending on the biochemical measurements. The highest enteral protein intake did not exceed 5 g/kg/day together with feeds and supplement. The supplementation was decreased gradually once the BUN reached 9≥ mg/dl and prealbumin reached 8≥ mg/dl, and was discontinued once the baby reached 40 weeks' CA, if the BUN and prealbumin were within the target levels.

Supplemental protein was discontinued if feeding intolerance, including increased gastric residuals, abdominal distention or constipation after protein supplementation, were recorded.

According to the study design, the weight gain in the NICU was assessed weekly as g/kg/day based on the previous week's weight. Increase in length and in head circumference as cm/week and growth percentiles determined by Fenton curves at admission and at discharge were evaluated[8]. Newborns with pathological head ultrasound, including intraventricular hemorrhage, periventricular leukomalacia or ventricular dilatation, or history of feeding intolerance necessitating the discontinuation of feeds, BM fortifier or protein supplement were excluded from the statistical analysis.

After discharge, the newborns were followed in the neonatal follow-up clinic as part of the routine protocol, and neurodevelopmental assessment was done at regular intervals. Bayley III test scores at 18 months' CA were recorded and statistically analyzed for correlation with growth parameters, oxygen treatment duration during their NICU stay, and maternal education.

The study was approved by the local ethics committee, and informed consent was obtained from parents.

The Statistical Package for the Social Sciences (SPSS) 15.0 statistical package was used for statistical analysis. Nonparametric tests were used for comparison of the groups who were supplemented with enteral protein and those who were not. Spearman correlation was used to correlate neurodevelopmental test scores with growth parameters and duration of oxygen during NICU stay. Multiple linear regression with backward analysis was used to analyze the effects of GA, birth weight (BW), and physical growth in the NICU expressed by growth in head circumference in both groups. Results were expressed as median (range) unless stated otherwise, and $p<0.05$ was accepted as significant.

Results

During the period 2006-2010, 75 preterms who were discharged from the NICU were eligible for the study. However, 10 patients were excluded for abnormal cranial ultrasound findings or gastrointestinal problems, leaving 65 patients for statistical analysis: 32 who did not receive enteral protein supplement (Group 1), and 33 who did receive additional enteral protein based on BUN and/or prealbumin levels (Group 2). The demographic data including BW and GA, gender, weight gain, and increase in length and head circumference during NICU stay of both groups are shown in Table I. The infants requiring additional enteral protein (Group 2) had significantly smaller GA and BW compared to the non-supplemented group; however, they had larger weight gain and larger increase in head circumference during the NICU

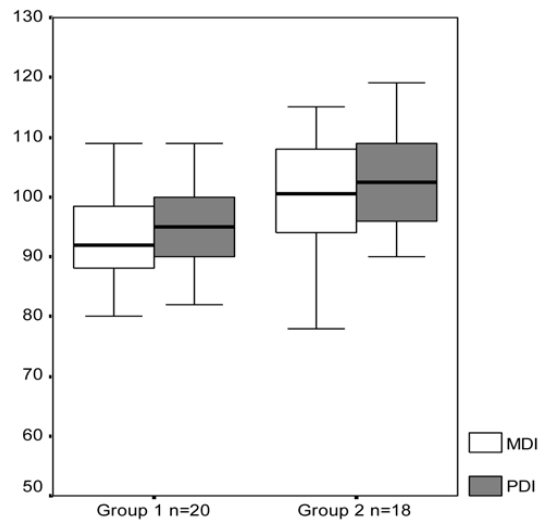

Fig.1. Bayley mental and psychomotor test scores (MDI: Mental developmental index, PDI: Psychomotor developmental index).
∗ : MDI was significantly higher in the enteral protein-supplemented group compared to the non-supplemented group, p=0.03.
δ : PDI was significantly higher in the enteral protein-supplemented group compared to the non-supplemented group, p=0.033.

stay. Two patients in Group 1 (6%) were small for gestational age (SGA) determined as being below the 10th centile for GA according to Fenton curves; however, this number increased to 13 (40%) during discharge. There was a 6.5-fold increase in the number of SGA infants at the time of discharge in Group 1. Seven patients (21%) in Group 2 were SGA at the beginning, and the number increased by 3.5-fold, reaching 23 (70%) growth- retarded patients. Serum prealbumin levels were significantly higher in Group 2 at discharge compared to the initial values at the beginning of enteral protein supplementation, whereas BUN levels were not significantly different. Initial prealbumin was 6.1 mg/dl (1.8-14.8) and final prealbumin 8.22 mg/dl (4-14.5) (p=0.013). Initial BUN was 4 mg/dl (4-28) and final BUN 5 mg/dl (2-15) (p=0.8). All the infants in Group 2 received FBM, whereas Group 1 was more heterogeneous: 6 babies received FBM, 12 babies received PF only, and 14 babies received both FBM and PF.

All of the infants in both groups lost percentile in weight during their NICU stay. The changes in percentiles in the weight, length and head circumference were similar between groups. Time to reach full enteral feeds, the duration of hospital stay, oxygen treatment, postnatal infection rates, and maternal education levels were similar between groups, whereas patent ductus arteriosus (PDA) was more frequent in Group 2.

Long-term neurodevelopmental follow-up at 18 months' CA could be performed in 20 and 18 infants in Group 1 and Group 2, respectively (overall 55%). Infants in Group 2 had better weight gain and increase in head circumference compared to Group 1 (p=0.001 and p=0.009, respectively). Bayley mental and psychomotor test scores are shown in Figure 1. Both mental and psychomotor developmental scores were significantly higher in Group 2 compared to Group 1 at 18 months' CA: mental developmental index (MDI): 92 (64-109) in Group 1 and 100 (63-115) in Group 2 (p=0.03), psychomotor developmental index (PDI): 95 (82-115) in Group 1 and 102 (56-119) in Group 2 (p=0.033). MDI and PDI scores were correlated with BW and GA in Group 1 (correlation coefficients 0.56 and 0.53, and p values 0.009 and 0.01, respectively), but not in Group 2. Despite significant difference in physical growth between the two groups during the NICU stay, we were not able to show a statistically significant correlation between physical growth in the NICU and neurodevelopmental test scores. Similar results were obtained by multiple linear regression analysis where 18 months' CA Bayley scores were influenced by GA and BW in Group 1 (B=2.9, p=0.041 and B=4.1, p=0.022, for psychomotor and mental test scores, respectively), whereas there was no effect of either GA, BW or physical growth in the NICU in Group 2, as shown by regression analysis. Oxygen treatment duration and maternal education were not correlated with the long-term neurodevelopmental scores at 18 months' CA.

Discussion

Survival of extremely low birth weight infants has increased in the last 2-3 decades owing to better ventilation strategies, antenatal steroids, better neonatal intensive care facilities, and very importantly, better nutrition practises. More protein intake during the first 28 days of life has been shown to result in better weight gain in very low birth weight infants[9]. Optimal protein intake during this period is estimated as 3.5-4 g/kg/day10. Once the baby reaches full feeds, there are two alternatives for enteral feeding: one is FBM, where fortification is made with commercially available BM fortifiers enriching the content of BM and making it almost equivalent to PF, and the other is PF, of which the contents are believed to meet the requirements of the rapidly growing preterm. If a preterm baby is fed with fully FBM or with PF with 2.2 g of protein/100 ml with 160 ml/kg/day volume, the protein intake is assumed to be 3.5 g/kg/day and the energy intake is assumed to be 130 kcal/kg/day, both of which are considered to be close to adequate for the baby[11]. However, it is also known that the protein content of BM is variable, making the daily protein intake calculation less accurate. In our unit, we judge protein balance based on both serum BUN and prealbumin levels. Prealbumin is known to have a rapid turnover, making it a valuable tool to assess the response to protein intake. The fact that BUN may be affected by the hydration status of the infant also makes prealbumin more

reliable for that purpose. The patients analyzed in this study were not differentiated based on formula or BM intake while deciding about the additional enteral protein supplementation. The only criteria were BUN/prealbumin levels and the stability of the baby with regards to gastrointestinal tract findings, although the groups ended up such that additional enteral protein was given to babies on FBM only. Additional enteral protein supplement was well tolerated by all infants and resulted in better head growth and weight gain. Serum prealbumin levels increased significantly after protein supplement, although no significant increase in BUN levels in the supplemented group was observed, making prealbumin measurements more meaningful while assessing positive protein balance. Total protein intake in our study group was always <6 g/kg/day, which is considered to be excessive according to earlier references[12]. Despite close nutritional follow-up and additional protein supplementation, there was still a high rate of percentile loss at the time of discharge, which is consistent with the literature[13]. The percentage of growth-retarded infants increased considerably in both groups, although the increase was less prominent in the protein- supplemented group (6.5-fold versus 3.5-fold). This is an important finding showing that even with the higher protein supplementation, some infants are still behind the intrauterine growth rates during their NICU stay. This finding itself should be taken seriously and lead to reconsideration of our feeding practises.

Optimal physical growth is only one aim of adequate nutrition in the preterm. Better neurodevelopmental outcome may be a more important purpose of good nutrition in this group of patients. There is a large body of evidence in the literature showing that better nutrition results in better neurological outcome in the preterm. Casey et al.[14] showed that preterm infants with postnatal growth problems have lower cognitive scores and academic achievement at 8 years of age. Lucas et al.[15] showed this in male preterm infants at 7-8 years of age. He also commented on the positive impact of BM in favorable neurodevelopmental outcome in his study group[16]. In a large group of patients, Ehrenkranz et al.[17] showed that growth velocity in the NICU has a significant effect on 18-22 months' CA neurodevelopmental outcome in extremely low birth weight infants in the NICU. Particularly, better head growth has been found to correlate with better neurodevelopmental outcome at 5.4 years of age[18]. However, using head circumference as an index of brain growth has raised some suspicion considering the increased extracerebral space in preterm infants[19]. More recently, Tan et al.[20,21] looked at the effects of early nutrition on head growth and magnetic resonance imaging (MRI) findings at 40 weeks' CA and neurodevelopmental test scores at 3 and 9 months' CA. Their results suggest that early nutrition in the NICU with enriched energy and protein results in better head growth and better MRI findings; however, the neurodevelopmental test scores were not significantly different between groups at 9 months' CA. The study group consisted of infants with abnormal imaging findings including periventricular leukomalacia, porencephalic cysts, and ventricular dilatation. Our study design excluded patients with pathological head ultrasounds from the statistical analysis; maternal education was similar between groups, and none of the patients had necrotizing enterocolitis. Oxygen treatment duration and chronic lung disease frequency were similar between groups, altogether excluding very important confounding variables with a potential effect on neurodevelopmental prognosis. The additional enteral protein supplementation group had better neurodevelopmental follow-up results at 18 months' CA compared to the non-supplemented group, showing that higher protein intake results in better brain development. In fact, the favorable effects of a high energy-high protein diet on better head growth and axonal diameters in the corticospinal tract has been shown in both term and preterm infants with brain injury[22]. However, in our group, when looking at the correlation between the physical growth rate and neurodevelopmental outcome, no significant correlation was observed. It is possible that this is due to the small number of patients. The finding that neurodevelopmental outcome was correlated with BW and GA in the non-supplemented group but not in the supplemented group is interesting. It might be due to the favorable effect of additional protein and BM somewhat balancing the untoward effects of low BW and GA on neurological outcome. However, regardless of the answers,

it is clear that preterm newborns who receive additional enteral protein supplementation added to FBM have better physical growth in the NICU and better neurodevelopmental outcome at 18 months' CA.

With our results we may conclude that:

Preterm newborns do need additional enteral protein supplementation for better growth once they reach full enteral feeds with FBM. Whether preterm babies who are fed with PF also need additional protein supplementation requires further research.

Prealbumin measurements may be better than BUN analysis for following protein balance.

Despite good nutrition according to actual recommendations, a considerable percent of preterm babies still lose percentile during the NICU stay.

Additional enteral protein supplementation results in better neurodevelopmental test scores at 18 months' CA.

Better neurodevelopmental test scores in the additional enteral protein-supplemented group might be due to effects of protein combined with BM, other than better physical growth, but this requires further investigation. This effect may even surpass the adverse effects of low BW and GA on neurodevelopmental prognosis.

Whether we should give additional enteral protein supplementation regardless of the BUN/prealbumin values and how the amount of supplementation should be decided are questions to be answered.

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