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Original article

High protein intake in human/maternal milk fortification for ≤1250 gr infants: intrahospital growth and neurodevelopmental outcome at two years

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Summary. *Background and aim of the study:* Extrauterine growth restriction and failure to thrive remain a major problem in Extremely Low Birth Weight infants. Nutritional support in preterm babies has the objective to improve the achieve rate of growth similar to those of the fetus in utero at the equivalent gestational age. The aim of the study was to evaluate feeding tolerance, intrahospital growth, neurological outcome and anthropometric data until 24 months of corrected age (mca) from different protein intake assumed by preterm babies <1250 g during their stay in NICU. *Methods:* The study evaluates auxological/neurodevelopmental outcomes until 24 months of corrected age (mca) in preterm infants with different protein intake (control group-CG: 3,5g Kg^{-1} perday; intervention group-PSG: 4,8g Kg^{-1} per day). *Results:* PSG group showed a significant higher length growth at 9 mca (p 0,04) and hearing/language score of Griffiths Mental Development Score (GMDS) at 12 (p 0,03) and 18 mca (p<0,05) comparing with CG. PSG-ELBW preterms showed an higher intrahospital head circumference (p 0,02) and length growth rate (p 0,04), greater Performance (p 0,04) and Hearing/Language (p 0,03) scores of GMDS at 3 and 12 mca. PSG-SGA preterms showed significantly higher scores in GMDS scores at 18 and 24 mca except for the locomotor domain. *Conclusions:* Supplemental enteral proteins lead to benefits of reduced postnatal growth restriction and better neurological outcome in preterm infants <1000 g and in those SGA <1250 g. (www.actabiomedica.it)

Key words: preterm infants, growth restriction, extremely low birth weight, protein intake, human milk

Introduction

Inadequate protein and energy intake in very low birth weight (VLBW) infants, and especially in those extremely low birth weight (ELBW), may account for about 45% of the postnatal growth restriction and represents an important negative prognostic factor for the long term outcome (1). Therefore, interventions aimed to the prevention of weight-loss with a satisfactory nutrition are considered very relevant to allow to these neonates to reach their goals (2). In case of premature neonates, the protein intake is associated not only to the appropriate growth of lean body components, mostly the brain, but also the increase of fat mass especially intra-addominal adipose tissue (3), while the degree of under-nutrition and growth inhibition could lead to delay and deficit in the area of cognitive development (4). Despite literature deepened the benefits of human milk and of its enrichment, it is to note that the neonate's entire need for protein intake could be not satisfied by the standard protein fortification (5, 6).

Recently, many studies found that standard protein fortification is associated with better auxological and neurodevelopmental outcome in the first year of infants life (7-9). Globally, to our knowledge, less attention was paid on the outcome on the later development. Only the study of Giannì et al. (2014) (10) assessed small for gestational age (SGA) infants at 24 months of corrected age.

Aim of the study was to fulfill the gap in literature, exploring if the use of protein enriched human/maternal milk is associated to long term advantage in preterm auxological and neurodevelopmental outcomes. Primary outcome was to investigate the impact of infants' nutrition on intrahospital anthropometric growth data. As secondary outcome, we investigated the effects of the supplemented protein regime on the growth and neurodevelopment in the first two years of infants' corrected age. In particular, the influence of protein fortification was assessed in three high-risk conditions: preterm born ≤1250 gr, ELBW and SGA infants.

Subjects and Methods

In the period between January 2010 and March 2011, all preterm infants hospitalized the Neonatal Unit (NICU) of the Bufalini Hospital in Cesena (Italy) were recruited for the study. Inclusion criteria were the following: birth weight under 580-1250 g and gestational <32 wk.

According to the standard rules of the NICU, all preterm neonates were fed with maternal or bank milk of different protein contents from the first day of full enteral feeding with standard fortification (11).

A total of 61 preterm neonates' were included in the study (birth weight ranging between 580 and 1250 g and gestational age 23-32 wk). They were randomly assigned to two group according to different feeding condition. The first one was named Protein Supplemented Group (PSG): it was composed by 34 neonates that received supplemented protein intake, corresponding to Fortification level 2 and 3 obtained by graded amounts of protein (Protifar, Nutricia). For PSG, we considered eligible only the infant that completed all the period of feeding with protein intake. In case of interruptions, infants were excluded from the study. Before entering in the study, an informed consent from the parents of these babies was obtained.

PSG was compared to Control Group (CG), that included 27 preterm babies that received Fortification level 1, which is the routine target in the unit (6): it was achieved by increasing the amount of BMF (Aptamil) until 5 g/100 ml.

Globally, for all preterm neonates, milk was provided by the infants' own mother (60%) and by hospital's pasteurized donor milk bank (40%). The quantity of proteins in human milk was adjusted according to the neonate's metabolic capacity derived by blood urea values (12), that were being monitored twice per week: if the urea value was less than 40 mg/ml, fortification was yielded by one level, while no change in fortification was made when the value was 40 to 45 mg/dl without metabolic acidosis (pH >7,30 and BE <-4). In case of the CG, the max fortification was obtained by increasing BMF to 5% and was kept fixed around urea values of 45-50 mg/dl, while in the PSG it was reached with BMF 5% plus 1% of Protifar depending on the tolerance of the neonate (12). Assuming human milk protein content of 0,8-1, 1 gr/dl and the prescribed diet volume intake of 160 ml Kg^{-1} per day the max protein intake should have been about 3,5 g Kg^{-1} per day in the CG whereas the PSG in the level 3 would achieve a protein intake of 4,8 g Kg^{-1} per day. The energy intake group was 135 $Kcal^{-1}$ per day and 141 $Kcal^{-1}$ per day in the CG and PSG respectively.

The end of the study was set at the time of discharge/transfer, or when the baby was able to ingest >50% of his prescribed quantity directly from the breast of his mother.

In order to consider the level of severity of prematurity, analyses were run also in other two specific conditions: 19 PSG and 13 CG infants were ELBW (birth weight 580-980 g), while 8 PSG and 6 CG infants were small for gestational age (SGA; birth weight <10^{th} percentile).

According to primary outcome, during hospitalization, growth, biochemical indices of nutritional status, enteral intake, feeding tolerance (13), clinical histories and morbidity were assessed serially. Length was measured to the nearest cm using a length board (14).

After discharge, infants' growth and neurodevelopment continued to be monitored.

At 40 GA weeks MRI was performed in 25/34 neonates and 24/27 neonates in the PSG and CG respectively.

All preterm infants were also included in the clinical follow up (8) at 3, 6, 9, 12, 15, 18 and 24 months of corrected age. During each assessment, firstly a neonatologist evaluated infants' growth according to weight, length and head circumference. Secondly, Milani Comparetti and Amiel Tison neurological assessment corrected for gestational age were used to assess neurological outcome. Neurologic outcome was defined by the presence or the lack of any of the following neurological impairment (NI): Cerebral Palsy, index score <80 at GMDS, deaf/hearing loss requiring amplification in both ears or bilateral blind. Finally, a psychologist, blind to infants' nutrition group, evaluated the level of infant development according to Griffiths Mental Development Scales (GMDS) (15), that were a well-recognized measure for infants mental and psychomotor development and are widely used in the clinical follow-up of the preterm infants (7, 8, 10, 16). A global score (General Quotient-GQ) represents the mean score of the 5 subscale scores (Locomotor, Personal-Social, Hearing and Language, Eye-Hand Coordination, Performance).

Anova and χ^2 test were used for analysis, significance was accepted when $p<0,05$. All analyses were run in the whole sample, and in the subsamples of ELBW and SGA infants.

Results

Intrahospital outcome

The PSG had a higher CRIB score (2,6±2,1 versus 1,7±1,1 p 0,02), lower neonatal weight g (939±196 versus 1012±163 p 0,08) and smaller GA week (27,8±2,3 versus 28,4±0,81 p 0,11) than the CG and this, with the moderate protein regime intake of the CG, could have blunted the statistical significance of the analysis. Although the neonates in the CG were primarily more mature and their milk intake was slightly larger, their growth rate was less than the others': levels of Urea were significantly higher in PSG versus the CG (mg/dl 32,9±7,4 versus 26,2±14) p 0,02 and lower no significant Ph values (7,32±0,03 versus 7,33±0,03). The milk volume given to the CG was slightly more than the PSG (162,7±6,5 versus 160,4±4,0) p 0,08. The tolerance to the hyperproteic diet was quite acceptable, metabolic acidosis and ipercreatinine were not detected more than previous time.

Despite during the hospitalization, PSG infants did not show any significant advantage when compared to CG in speed regarding the growth of weight (g/Kg/day: 19,0±2,2 versus 18,8 ± 2,1),of head circumference (cm/wk: 0,88±0,17 versus 0,88±0,35) and length (cm/wk: 1,04±0,2 versus 0,88±0,35), when only ELBW neonates were considered, the protein supplemented neonates yielded an advantage in length (p 0,04) and head circumference (p 0,02) growth, whereas the difference in weight gain was smaller (p 0,05) (Table 1).

After-discharge assessment

MRI showed clearly pathologic images in one neonate in the CG who demonstrated NI later.

The evaluation of the anthropometric data until 24 months of corrected age (m.c.a.) showed that the PSG had a better catch-up growth than the CG: the weight, length and head circumference mean z-score values were higher for the protein supplemented regime although the difference became significant only for the height at 9 m.c.a. (-0,85 versus -1,27 p 0,04) (Table 2).

Table 1. Anthropometric data growth in the ELBW of both groups in NICU

	PSG (n=19)	CG (n=13)	P value
Weight gain mean, g/Kg/per day (SD)	19.5 (2.4)	18.1 (1.0)	**.05**
Length growth, cm/wk (SD)	1.07 (0.25)	0.91 (0.2)	**.04**
Head circumference, Growth cm/wk (SD)	0.92 (0.1)	0.82 (0.1)	**.02**
Milk volume intake, ml/Kg/per day	160.2 (4.3)	163.1 (5.6)	**.07**

PSG: protein supplemented group; CG: control group

Table 2. Mean z-score values calculated with World Health Organization curves, at 3, 6, 9, 12, 15, 18 and 24 months of corrected age (m.c.a.) in the two groups of premature babies

Follow-up	Weight		Head circumference		Length	
	PSG (n =34)	CG (n = 27)	PSG (n =34)	CG (n = 27)	PSG (n =34)	CG (n= 27)
3 mca	-0.95 (± 0.4)	-1.6 (± 0.6)	0.14 (± 0.47)	0.17 (± 0.64)	-1.07 (± 0.33)	-1.58 (± 0.87)
6 mca	-0.63 (± 1.2)	-0.83(± 0.9)	+0.23 (± 0.49)	-0.13 (± 0.62)	-0.08 (± 0.46)	-1.4 (± 0.54)
9 mca	-0.51 (± 1.2)	-0.68 (± 0.86)	+0.19 (± 0.47)	+0.19 (± 0.62)	**-0.85 (± 0.55)**	**-1.27 (± 0.55)**
12 mca	-0.48 (± 1.12)	-0.56 (± 0.92)	-0.33 (± 1.23)	0.34 (± 0.58)	-0.46 (± 1.04)	-0.62 (± 1.07)
15 mca	-0.57 (± 1.03)	-0.75 (± 1.05)	0.17 (± 1.25)	0.49 (± 0.68)	-0.89 (± 0.82)	-1.42 (± 0.81)
18 mca	-0.20 (± 1.28)	- 0.17 (± 1.10)	0.21 (± 1.43)	0.74 (± 0.70)	-0.34 (± 0.84)	-0.81 (± 1.02)
24 mca	-0.79 (± 1.02)	-0.69 (± 0.86)	0.18 (± 1.22)	0.78 (± 0.77)	-0.85 (± 1.29)	-0.86 (± 1.29)

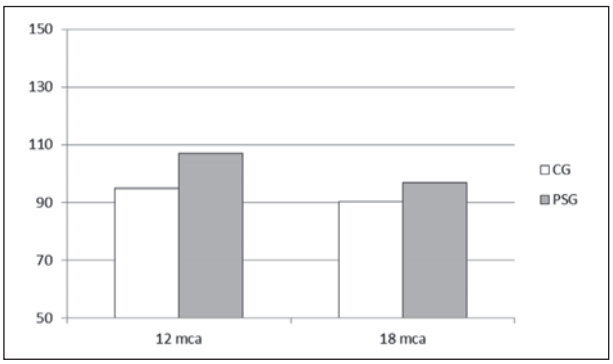

Figure 1. GMDS at 12 and 18 mca PSG expressed higher significantly score in the subscale hearing/language respect to CG (106,06 versus 98,97 p 0,03 ; 90,41 versus 96,71 p<0,05)

GMDS showed difference between groups at 12 (106,06 versus 98,97 p 0,03) and 18 m.c.a. (90,41 versus 96,71 p <0,05), where PSG expressed a significantly higher Hearing and Language score than CG (Figure 1). However, the average scores showed by PSG was persistently higher in all the other domains than those of CG.

For the ELBW neonates fed with a protein supplemented regime, the evaluation made with covariate CRIB, demonstrated a significant advantage in the Performance (p 0,04) at 3 m.c.a. and in the Hearing and Language quotients at 12 m.c.a. (p 0,03) (Table 3).

When SGA infants were considered, we found that PSG infants had a significantly higher score in all the items except Locomotor when compared to those of CG at 18 and 24 m.c.a. (Table 4).

Table 3. GMDS at 3 and 12 months' corrected age in ELBW with covariate CRIB; significant different between groups is showed in the performance items (95,5 versus 109,8 p 0,04) and Hearing and Language items (107,04 versus 94,83 p 0,03)

		PSG (n=19)	CG (n=13)	F	P value
3 mca	Locomotor	114,33±13.99	102,82±23.87	2.725	.111
	Personal-Social	106,06±15.32	104,45±20.04	.299	.589
	Hearing and Language	105.33±15.97	106,18±14.98	.337	.566
	Eye-Hand Coordination	108,00±19.65	108.36±19.44	.222	.641
	Performance	107,89±14.84	97,63±17.56	4.447	**.042**
	General Quotient	107,89±13.75	104,09±17.16	1.295	.265
12 mca	Locomotor	96.00±17.55	86.56±15.80	1.82	.192
	Personal-Social	94.07±10.54	91.00±20.66	.688	.417
	Hearing and Language	107.04±11.43	94.83±8.66	4.858	**.038**
	Eye-Hand Coordination	98.07±15.38	90.56±20.22	1.682	.209
	Performance	99.79±12.66	99.00±14.80	.379	.545
	General Quotient	98.93±12.29	92.22±14.62	2.219	.152

Table 4. GMDS at 18 and 24 months' corrected age performed in SGA preterm of both group: Protein supplemented group (PSG) had higher significantly scores in all items but locomotor respect to Control group (CG)

		PSG (n=6)	CG (n=8)	F	P value
18 mca	Locomotor	85.33±30.21	111.13±16.82	4.182	.063
	Personal-Social	87.00±21.38	112.50±7.80	9.865	**.009**
	Hearing and Language	86.33±17.96	106.88±14.97	5.456	**.038**
	Eye-Hand Coordination	91.67±23.22	114.25±11.54	5.786	**.033**
	Performance	89.33±27.53	114.13±14.74	4.762	**.050**
	General Quotient	86.50±22.98	111.25±10.51	7.384	**.019**
24 mca	Locomotor	110.60±37.47	131.63±4.66	2.593	.136
	Personal-Social	94.60±26.24	114.75±4.95	4.699	**.050**
	Hearing and Language	80.00±21.91	107.00±10.04	9.396	**.011**
	Eye-Hand Coordination	93.80±26.21	117.63±5.76	6.446	**.028**
	Performance	94.40±26.41	115.63±3.89	5.267	**.042**
	General Quotient	92.00±24.46	115.75±3.81	7.650	**.018**

Discussion

Aim of the study was evaluate if the use of protein enriched human/maternal milk was associated to long term advantage in preterm auxological and neurodevelopmental outcomes.

Our results were consistent with previous literature (17) showing that, in the intrahospital period, the protein supplemented regime gives advantages on the growth were acquired for high risk neonates, as showed by data of ELBW premature babies. At lower neonatal weight and gestational age, the efficiency of protein deposition and gain progressively increases (13), with a significantly negative relationship between the amount of protein intake and the development of fat mass, mostly central adiposity (18). In the present study, we observed the main rate growth gain on head circumference and length than on weight, correlating with lean body mass increases. Once birth weight has been regained, the closely monitoring the rate of in-hospital growth became extremely relevant. The daily target growth velocity of the weight and head circumference should be ≥18 g/Kg per day and ≥0,8 cm/per week respectively for all ELBW babies. In case that those rates falter, the increase of dietary protein/energy ratio is needed. This strategy has been implemented as standard practice for ELBW preterm admitted to NICU since the end of this study. During intrahospital period we constantly pursue an early autonomy in breast feeding whose advantages, we believe, exceed any enrichment practices and ensure that the baby can assume the milk quantity which he/she needs. In this study, 83,7% of infants were discharged home with partly maternal milk, and 64% with exclusively maternal milk. The time upon which the infant is left to feed entirely to maternal breast represent again a critical point. Despite this usually happens at 35-36 wk gestation and it is followed by an expected falter growth rate, it is unclear if this time is too big of a penalty for the baby's brain growth. It is probable that nutrients and growth factors strongly regulate the brain development until 42 wk (19). The results of the present studies are relevant because they seem to support that the brain plasticity at 35-36 wk outweighs its vulnerability to minimal nutrient repletion. On the other hand, the significantly negative relationship between the amount of protein intake and the development of fat mass persists during the first month of corrected age. This suggests that a high protein intake provided in the early postdischarge period promotes lean mass gain (18).

The highest neurodevelopment score was seen in the PSG at 12 and 18 m.c.a., although only in the subscale Hearing and Language. This suggests that the advantages of the regime of nutrition made during the NICU stay may affect the cognitive function in a long lasting manner. The ELBW infants of PSG

exhibited a significantly higher score at 3 m.c.a. when compared to those in the CG, as well as in the Hearing and Language quotients at 12 m.c.a. Because the previous literature underline how preterm infants were at high-risk for delay in fine movement and language skills (20), these results are relevant.

SGA infants appear to be at risk of developing abnormal body composition and long–term morbidity, including metabolic syndrome, especially when experiencing rapid postnatal weight gain (21). When we compared the SGA preterm of both groups on GMDS, we found a higher score, at 18 and 24 m.c.a., in all domains except for the locomotor one in those previous fed with hyperproteic regime. This would be a further evidence that the protein deposition with an enhanced protein nutrition is more efficient at lower weight and improves brain growth and its functions.

Since end of the study the same increased protein intake was given to all the ELBW and SGA prematures <1250 g admitted to the NICU (7).

We believe that the intrahospital nutritional regime with high protein intake may present an early window of opportunity to positively influence the growth potential of these infants, while other nutritional post-discharge management showed a scarce evidence of improvement in the later parameters (14). As previously stated, our gold standard to improve neurodevelopmental outcome and decrease the incidence of long-term morbidity, including metabolic syndrome (22), is to obtain breast feeding for all preterm infants as soon as possible, and we believe an optimal nutritional regime is the adequate basis for achieving this target (23). Although promising, our results could be confirmed by further studies. For example, it could be relevant explore the possible effect also on total amino acids composition and vitamins. So, investigation on wider samples could help to a deeper knowledge on the benefits of protein supplement intake on more feature of infant development.

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