

New human milk fortifiers for the preterm infant

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Abstract

Given its unique nutritional and functional advantages, human milk (HM) should be considered as the first choice for the nutrition of all infants, including preterm newborns. Since its protein, mineral and energy contents are not suitable to meet the high needs of very-low-birth-weight (VLBW) infants, HM should be fortified for these components. Fortification of HM is an important nutritional intervention in order to provide appropriate nutritional intake and appropriate growth. The standard fortification strategy has yielded inadequate protein intakes, resulting in slower growth as compared to preterm formulas. Improvement of outcomes depends on new fortification strategies, considering the large variability of HM composition. Individualized fortification, either targeted or adjustable, has been shown to be effective and practical in attaining adequate protein intakes and growth.

Most commercially available multi-nutrient fortifiers and protein concentrates are derived from bovine milk (BM), which has a protein composition very different from that of HM. The use of BM proteins has been recently questioned for possible association with intestinal inflammation in VLBW infants. Recently, one HM-based fortifier was shown to be associated with lower necrotizing enterocolitis rates and lower mortality in extremely premature infants, compared to BM-based products. Other milk sources are currently under evaluation: a randomized, controlled, single-blind clinical trial, coordinated by the Neonatal Unit of the University of Turin in collaboration with the Italian National Research Council of Turin and the University of Cagliari, is being carried out to evaluate the adequacy of fortifiers derived from donkey milk for the nutrition of preterm infants.

Keywords

Human milk, targeted fortification, adjustable fortification, bovine milk, quality, preterm.

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The importance of an adequate nutrition for the preterm infant

Early postnatal growth failure due to inadequate nutrient intakes is still very common in very-low-birth-weight (VLBW) infants [1-4]. Early postnatal period corresponds to a critical window for brain development, during which undernutrition can have permanent effects on the development of the central nervous system [5-6]. Clinical trials with preterm infants indicate that nutritional quality and growth in the early postnatal period have an important impact on the long-term neurological and cognitive performance [7-11]. Thus, provision of optimal nutrition in Neonatal Intensive Care Units, particularly for VLBW infants, has become a priority. The goal of nutritional management is to ensure that intakes meet needs; however, this is not so simple with the preterm infants. During early life of the preterm infants, deficit of nutrients, particularly proteins, occurs, due to delays in establishing and maintaining adequate nutritional intakes [1-4]. This deficit is difficult to recover during the unstable phase. At this point, feeding clinically stable preterm infant becomes crucial to replace the accumulated deficit, and to prevent morbidities related to postnatal growth failure.

Human milk (HM) has an essential role in the nutrition of preterm infants due to its bioactive and immunomodulatory components [12-14]. Recent evidence shows that preterm infants fed HM have lower rates of infection and necrotizing enterocolitis (NEC) [15-19] and improved neurodevelopmental outcomes [9, 20-24] compared to infants fed preterm formula. The Vermont Oxford Network declared that, among efforts to reduce the nosocomial bacteremia for VLBW infants, early feeding with HM should have the highest priority [25]. The American Academy of Pediatrics strongly

recommends HM use for preterm infants, because of its unique advantages with respect to host protection and improved developmental outcomes [26].

Fortification of human milk

Given its unique advantages, HM should be considered as the first choice for the nutrition of preterm infants. But, since the protein, mineral and energy content of HM is not suitable to meet the high needs of VLBW infants, it should be fortified [27-29]. The awareness of the need of HM fortification is largely based on studies comparing “intrauterine nutrient intake and intrauterine growth” with the composition of HM and postnatal growth [1, 27]. Therefore, the nutritional goal is to support growth that “approximates the *in utero* growth of a normal foetus”. Minimum and maximum protein and energy intakes for VLBW infants were recommended by a consensus group [30] and an Expert Panel of the Life Sciences Research Office of the American Society for Nutritional Sciences [31].

The HM nutrient content provides insufficient quantities of protein, sodium, phosphate and calcium, magnesium, copper, zinc and many vitamins (B2, B6, C, D, E, K, folic acid) to meet the estimated needs of the preterm infant [31]. Large fluid volumes should be required to provide sufficient calories to maintain adequate growth. The composition of HM varies within a single feed, among feeds, and throughout the course of lactation. By the end of the first month of lactation, the protein content of preterm milk is inadequate to meet the needs of most preterm infants [32-34], so that serum albumin and blood urea nitrogen concentrations may decline in premature infants as a result of inadequate dietary protein intake [35, 36]. Preterm infants fed unsupplemented HM can develop poor radiological bone mineralization, rickets, and fractures at 4-5 months of age, due to inadequate dietary intakes of calcium and phosphate and consequent osteopenia [37]. Other metabolic complications associated with the long-term use of unsupplemented HM in preterm infants include hyponatremia at 4 to 5 weeks [38], hypoproteinemia at 8 to 12 weeks [39], and zinc deficiency at 2 to 6 months [40].

Now, food-industry produced multicomponent fortifiers are available to supplement HM. Most of the available fortifiers contain varying amounts of protein, carbohydrate, calcium, phosphate, other minerals (zinc, manganese, magnesium, and copper), vitamins, and electrolytes.

Strategies for human milk fortification

The main factor responsible for limited success with HM fortification is that standard fortification strategy is based on routine assumptions about the composition of HM: the common practice is to add a fix amount of fortifier, assuming that HM has an average protein content and the infant has an average protein requirement. But the protein concentration of preterm milk is variable and decreases with the duration of lactation [33, 34]. Also, the protein concentration of banked donor milk, which is most often provided by mothers of term infants, is likely to be lower [41, 42]. Hence, most HM fed to preterm infants during the fortification period is likely to have an inadequately low protein concentration [4, 36]. Actual protein intakes are consistently and significantly lower than we assume when we fortify in standard fashion.

Novel fortification strategies should deal with the problem of ongoing protein undernutrition. Individualized fortification approach appears to be promising for the optimization of fortification methods [43-45]. Individualized fortification is now believed to be the best solution to the problem of variability of the composition of HM and donor HM. Currently, there are two proposed methods for individualization: the first, targeted fortification, depends on milk analyses; the second, adjustable fortification, depends on the metabolic response of each infant.

In the targeted approach, proposed by Polberger et al. [44], the concept is to analyze the HM and to fortify it in such a way that each infant always receives the amount of nutrient needed. The milk is thus analysed periodically and a target nutrient intake (in this case, proteins) is chosen according to the predefined requirements of preterm infants. The amount of fortifier is added considering the protein content of the milk to reach the targeted intake. The two main shortfalls of targeted fortification are that a milk analyser has a high cost, the procedures are labour intensive, and may not be practical for the routine use of each nursery, and that the quantities of protein and fat are relatively small, and need to be measured with extreme accuracy.

In the approach of adjustable fortification, protein intake is adjusted on the basis of the infant's metabolic response, evaluated through periodic determinations of blood urea nitrogen (BUN) [43, 45, 46]. Adjustable fortification method was shown

to be effective in providing the preterm infants with adequate protein intakes and appropriate growth approximating intrauterine intakes and growth [46]. The adjustable fortification does not make any assumptions regarding an infant's protein requirements; it directly monitors the metabolic response taking into consideration the actual protein status in each infant. Moreover, it does not need frequent milk analyses and expensive equipment, and it is not labour intensive: in other words, it is practical for routine use in the nurseries. BUN determination is considered an excellent index for adequacy of protein intake in clinically stable preterm infants [45, 46].

Composition of human milk fortifiers

The optimal qualitative composition of fortifiers is also a critical issue. Most commercially available multi-nutrient fortifiers are derived from bovine milk (BM), which has a different protein composition with respect to HM. The composition of currently available fortifiers based on BM is shown in **Tab. 1**. Commercial products have a protein fraction composition showing different casein to whey protein ratio, as well as different degree of protein hydrolysis. Since whey proteins are known to have a higher biological value, due to the higher concentration in essential aminoacids, these differences may have a relevant impact on the nutritional value of fortifiers. For this reason, some fortifiers contain only whey proteins as nitrogen source. Moreover, the degree of protein hydrolysis may affect its tolerability: aminoacid formulations should improve tolerance issues with the sensitive premature gastrointestinal tract, but extensive hydrolysis also prevents any functional effect of the protein fraction. Very recently, acidified liquid formulations for HM fortifiers were tested in order to ensure liquid product sterilization, but these tests were abandoned because of observed increase in clinical complications such as acidosis, due to preterm infant's inability to buffer this acid load [47].

A similar degree of variability could be seen for the carbohydrate and lipid fractions. First generation fortifiers did not contain lipids, while newly available fortifiers include plant oils as a source of energy, in addition to maltodextrins, which are the most used sugar source. Lipid fraction may contain medium chain triglycerides (MCT), which are known to be more readily absorbable than long chain fatty acids. Carbohydrate fraction

Table 1. Composition of human milk fortifiers.

Name (Brand)	Multicomponents										Protein concentrate					
	SMA BMF (Nestlé)	FM85 (Nestlé)	Entamil HMF (Mead Johnson)	Similac HMF (Abbott Nutrition)	Eoprotein (Mullupa)	Aptamil FMS (Mullupa)	Nutriprem (Cow & Gate)	Entamil HMF Acidified Liquid (Mead Johnson)	Similac HMF Acidified Liquid (Abbott Nutrition)	Similac HMF Concentrated Liquid (Abbott Nutrition)	Prolact + H2MF (Proclacta)	Milte FR (Milte)	Beneprotein (Nestlé)	ULK Protein (Dietetic Metabolic Food)	Prolitar (Danone)	Aptamil/ Mellin PS (Danone)
Formulation	Powder	Powder	Powder	Powder	Powder	Powder	Powder	Liquid	Liquid	Liquid	Liquid	Powder	Powder	Powder	Powder	Powder
Protein g (Nx6.25)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
<i>Casein:whey</i>	0:100	0:100	60:40	60:40	50:50	40:60	50:50	0:100	100:0	50:50	40:60 (human milk)	0:100	0:100	0:100	80:20	50:50
<i>Hydrolysis</i>	No	Yes	Yes (whey)	No	Yes	Yes	Yes	Yes	Yes	No	No	No	No	No	No	Yes
Carbohydrate g	2.4	3.3	0.4	1.8	3.5	2.8	2.9	0.5	0.5	1.5	1.5	1.1	0.0	0.1	0.0	0.0
<i>Lactose</i>	-	0.3	0.4	-	-	-	-	0.5	-	-	1.5	-	-	0.1	-	-
<i>Maltodextrins</i>	2.4	3	-	1.8	3.5	2.2	2.9	-	0.5	1.5	n/a	1.1	-	-	-	-
<i>GOS/FOS</i>	-	-	-	-	-	-	-	-	-	-	n/a	0.4	-	-	-	-
Lipids g	0.2	0.0	0.9	0.4	0.0	0.0	0.0	1.0	1.0	0.4	1.5	0.0	0.0	0.5	0.0	0.0
<i>Source</i>	Plant	-	MCT/soy	MCT	-	-	-	MCT/soy/sunflower	MCT/soy/coconut	MCT	human milk	-	-	bovine milk	-	-
Kcal	15	17	13	14	18	14	13	14	14	14	23	8	4	4	4	4
Calcium mg	90.0	75.0	81.8	117.0	63.3	86.0	78.0	52.2	52.7	60.0	85.8	46.4	3.3	5.4	15.3	6.4
Phosphorus mg	46.0	45.0	40.9	67.0	66.7	57.5	57.0	28.3	28.6	34.0	45.0	25.8	-	2.7	7.9	6.3
Sodium mg	18.0	26.0	14.5	15.0	33.3	31.8	12.5	12.2	12.3	10.0	30.8	7.2	2.5	1.6	0	9.3
Chloride mg	17.0	23.0	11.8	38.0	25.0	22.7	21.7	12.6	12.7	26.0	24.2	n/a	-	0.5	1.4	0.8
Potassium mg	28.0	66.0	26.4	63.0	4.0	20.9	20.0	20.3	20.5	42.0	41.7	n/a	5.0	3.2	1.0	15.0
Magnesium mg	3.0	4.0	0.9	7.0	3.5	8.5	8.6	0.8	0.8	4.2	3.9	0.5	-	0.6	0.3	0.6
Vitamin C mg	40	18	11	25	25	18	17	7	7	15	<0.2	n/a	n/a	n/a	n/a	n/a
Added active ingredients	-	-	-	-	-	-	-	DHA, ARA	DHA	DHA, ARA	-	Lactoferrin	-	-	-	-
Osmolarity mOsm/L	394 ^b	314 ^a /385 ^b	377 ^a /401 ^b	385 ^b	371 ^a /394 ^b	343 ^a /436 ^b	400 ^b	326 ^a	339 ^a	385 ^b	335 ^b	n/a	n/a	n/a	n/a	n/a

N/a: not reported; ^aestimated; ^bliterature.

is enriched by galactoligosaccharides (GOS) and fructoligosaccharides (FOS) in some formulations, due to their prebiotic function. Carbohydrates and polysaccharides are primarily responsible for the increase in osmolarity of HM. A relevant increase of osmolarity secondary to the addition of a fortifier might lead to abdominal distension, reduced gastric emptying, and frequent stool, that may cause the interruption of fortification [48].

The recent Consensus Statement on Human Milk in Feeding Premature Infants [49] drew attention on routinely used BM-based fortifiers, whose bovine proteins might be associated with intestinal inflammation in extremely-low-birth-weight (ELBW) infants [50]. Investigation on exclusive HM diets (human milk-based fortifier and donor HM, if own mother's milk is unavailable) have been carried out in recent years. Currently, one HM-based fortifier, whose composition is reported in **Tab. 1**, is available on the market. Clinical trials reported that exclusive HM diet is associated with less NEC, less NEC requiring surgery, and lower mortality in ELBW infants than in those who receive own mother's milk supplemented with BM-based products [51, 52]. Hair et al. [53] recently reported decreased retinopathy of prematurity, bronchopulmonary dysplasia, patent ductus arteriosus, and ventilator days in infants with a exclusive HM diet. Ghandehari et al. [54] also observed a significant reduction of the need of total parenteral nutrition for extremely premature infants fed with an exclusive HM diet, when compared to a standard diet of HM supplemented with BM fortifier and preterm formula. Further studies are needed to establish the real potential of HM-based fortifier, since the early results are promising. The Panel of the recent Consensus Statement on Human Milk in Feeding Premature Infants concluded: "Human milk-based fortifiers are available, probably are of better quality than cow's milk based fortifiers, but are very expensive at the moment" [49]. Volumes of donor HM necessary to produce such fortifiers are extremely high, and the amounts collected by HM banks at the moment are not enough to satisfy the needs of all VLBW infants, thus posing an ethical issue.

New perspectives: donkey milk-based fortifiers

Recently, donkey and human milk diet integration was shown to be associated with a decrease of inflammatory status and with the improvement of lipid and glucose metabolism in a

murine model, when compared to a diet integration with BM [55]. The functional similarity of human and donkey milk is probably due to their closeness in quantitative and qualitative protein, glucidic and lipid fractions composition, that differ to that of BM [56, 57].

Based on these considerations, the Neonatal Unit of the University of Turin is currently coordinating a randomized, controlled, single-blind clinical trial to evaluate the use of a multi-component fortifier and a protein concentrate derived from donkey milk for the nutrition of VLBW or gestational age < 32 weeks infants. This trial is performed in collaboration with the Italian National Research Council of Turin and the University of Cagliari. Aim of the trial is to assess whether adjustable fortification with a multi-component supplement and a protein supplement, both derived from donkey milk, affects differently feeding tolerance and clinical, metabolic, neurological and auxological outcomes at short- and long-term, with respect to BM-based fortifiers.

Conclusion

Current standard fortification methods have yielded inadequate protein intakes resulting in slower growth compared to preterm formulas. Improvement of the outcomes depends on new fortification strategies and on the optimization of fortifiers composition. Recently, new ingredients are being evaluated in order to improve nutrients bioavailability, feeding tolerance and clinical outcomes.

Declaration of interest

EB, GEM and LC have competing interests since they are inventors of a food composition based on donkey milk (Patent WO2015056166 [A1]-2015-04-23). All other Authors declare that they have no conflicts of interest. No funding and material support was acknowledged for the present manuscript.

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