0021-7557/12/88-02/119
Jornal de Pediatria

Comparison between homologous human milk supplements and a commercial supplement for very low birth weight infants

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Abstract

Objectives: To describe the methodology for the preparation of two additives derived from human milk, liquid and powdered, and to compare this composition with the commercial additive FM85®.

Methods: For the preparation of the liquid and powdered supplements, 40 samples of human milk were used. Both supplements have been through three preparation phases: skimming, evaporation and lactose removal. After these phases, the liquid supplement is ready, and the powdered requires a fourth phase – lyophilization. To each sample of the liquid and powdered supplements were added, respectively, 80 mL (group I) and 100 mL (group II) of pooled banked human milk. For comparison, 20 samples of 100 mL of the pool were added to 5 g of the FM85® supplement (Nestlé) (group III). Analyses of carbohydrates, protein, lipids, calcium, phosphorus, sodium, osmolality and caloric content were performed, considering a significant difference $p < 0.05$.

Results: Groups I, II, and III showed, respectively, the following results: protein = 1.81, 2.38 and 1.96 g/dL ($p < 0.001$); carbohydrates = 6.70, 7.25 and 10.06 g/dL ($p = 0.006$); fat = 3.75, 3.75 and 3.73 g/dL ($p = 0.96$); calcium = 36.92, 44.75 and 79.37 mg/dL ($p = 0.001$); phosphorus = 20.02, 23.28 and 56.30 mg/dL ($p = 0.02$); sodium = 14.32, 14.40 and 20.33 mEq/L ($p = 0.143$); osmolality = 391.45, 412.47 and 431.00 mOsmol/kgH_2O ($p = 0.074$); and caloric content = 67.78, 72.27 and 81.65 kcal ($p = 0.001$).

Conclusion: The studied additives differ significantly from the commercial additive FM85® in some of its components, and its composition may or may not meet the quantity of nutrients suggested by the most recent recommendations.

J Pediatr (Rio J). 2012;88(2):119-24: Premature infant, enteral nutrition, human milk, milk bank.

Introduction

The studies on nutrition have contributed to the reduction of morbidity and mortality in the neonatal period. The early introduction of amino acids, the use of appropriate lipid solutions, the concept of minimal enteral nutrition and the adequacy of micronutrients (vitamins, oligoelements and minerals) have improved survival, especially in very low birth weight preterm infants (VLBWI), contributing to reduce length of hospital stay, besides preventing non-communicable chronic diseases in the long term.[1,2]

There is agreement on the use of human milk as a source of nutrients for VLBWI due to its energy content of protein, enzymes, growth factors and, mainly, as a result

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No conflicts of interest declared concerning the publication of this article.

Suggested citation: Thomaz DM, Serafim PO, Palhares DB, Melnikov P, Venhofen L, Vargas MO. Comparison between homologous human milk supplements and a commercial supplement for very low birth weight infants. J Pediatr (Rio J). 2012;88(2):119-24.

Manuscript submitted Aug 6 2011, accepted for publication Nov 16 2011.

http://dx.doi.org/10.2223/JPED.2166

of the immune factors from this source.[3,4] However, some weeks after delivery, the levels of nutrients decrease, and the offer does not meet the nutritional needs, especially in relation to the amount of protein, calcium and phosphorus.[5] Therefore, the addition of fortifiers to human milk from the own mother or from a milk bank becomes necessary.[6-8] Since the quantity of protein in human milk is considered ideal for newborns, the use of additives whose protein is from homologous origin may be the most adequate.[9]

The additives of such origin currently on the market – Prolacta +4, +6, +8, +10® and NeoPro® Prolacta Bioscience®[10] – are costly, what limits their use to neonatal intensive care units of public hospitals.

Studies demonstrate that the modification of human milk by evaporation may be a way to concentrate nutrients and provide nutritional content suitable for VLMW.[11-13] The analysis of these studies led us to the methodology proposed in the present study: modification of banked human milk by fat extraction, lactose removal and lyophilization, aiming to use it as a banked human milk additive that offers nutrients from a homologous origin and in the amount recommended to provide appropriate growth and development in the short and long term.

Methods

For the preparation of the liquid and powdered additives and for the reconstitution of the proposed additives and the commercial additive, human banked milk with a lactation period from 2 months to 1 year from donors of the human milk bank of the School Hospital of the Universidade Federal de Mato Grosso do Sul (UFMS), which authorized the use of the donated milk, after the assent of the Research Ethics Committee of the Hospital's clinical board and of the University, as well as a Free Informed Consent.

The study used 40 samples of 200 mL for the preparation of modified human milk, which was performed in three stages: fat removal; evaporation, for concentration of nutrients and lactose removal; and lyophilization.

The fat was removed from each sample through a small cream separator, model 18 GR, Casa das Desnatadeiras®, which by centrifugal force separates the fat from the rest; this process, however, retains about 120 mL of the milk sample in the rotor. After fat removal, 80 mL samples were subjected to the Marconi MA 120® rotary evaporator, reducing the volume to 20 mL with concentration of nutrients. This concentrate was stored in conical tubes at -20 ºC for 24 hours.

The samples were then centrifuged at 4,000 rpm for 20 minutes at 4 ºC in a Sigma 3K30® refrigerated centrifuge, forming the lactose precipitate in the samples, which were heated in water bath at 37 ºC for the supernatant removal, discarding the precipitate and giving rise to the liquid form of the additive, a lactose removal technique already proposed in a study developed by Santos.[12]

The same process is repeated for the formation of the powdered additive, but the volume of each sample to be evaporated is 70 mL. The content, after evaporation and removal of lactose, was frozen at -20 ºC and lyophilized with an Edwards® equipment, originating the additive in the powdered form.

The additive in the liquid form of each sample was added to a human milk pool, obtaining the volume of 100 mL (group I – evaporated). The powdered form additive of each sample was added to 100 mL of the same human milk pool (group II – lyophilized), being then pasteurized, a necessary condition for the use in preterm newborn feeding.

To compare with the commercial additive, 20 samples of the same pool of milk used in groups I and II, supplemented with FM85® (Nestlé) in the proportion of 5 g% (group III).

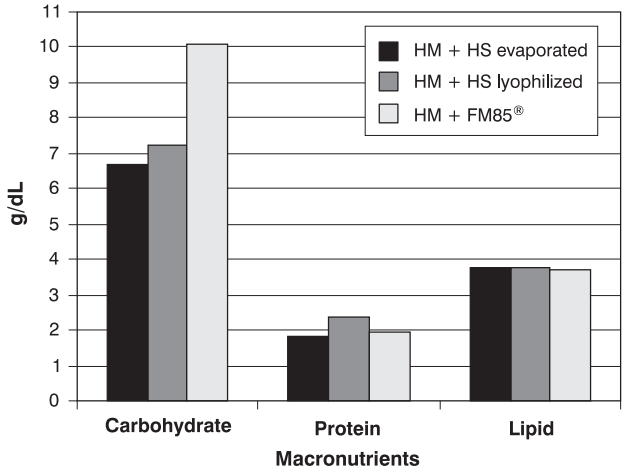
All the samples, after reconstitution, went through the milk bank evaluation concerning dilution, and stabilization, and were subjected to pasteurization and microbiological analysis. Only after approval on this evaluation, a quantitative assessment of the following elements was performed: carbohydrates (technique of reducing glucids formation); protein (protein nitrogen quantification by the microKjedahl method); calcium (ashing method using colorimetry with chloranil acid); phosporus (photocolorimetry); sodium (flame photometry); osmolality (freezing point osmometry); fat (Gerber method); and caloric content (Atwater formula); techniques described and recommended by the Adolfo Lutz Institute.[14]

The comparison between the measured variables was performed with the ANOVA test with Tukey post-test for the determination of mean and mean standard error, considering significant difference $p < 0.05$. The study used Microsoft Excel 2003 for data spreadsheet and the 2.0 version of the Sigma Start Software.[15]

Results

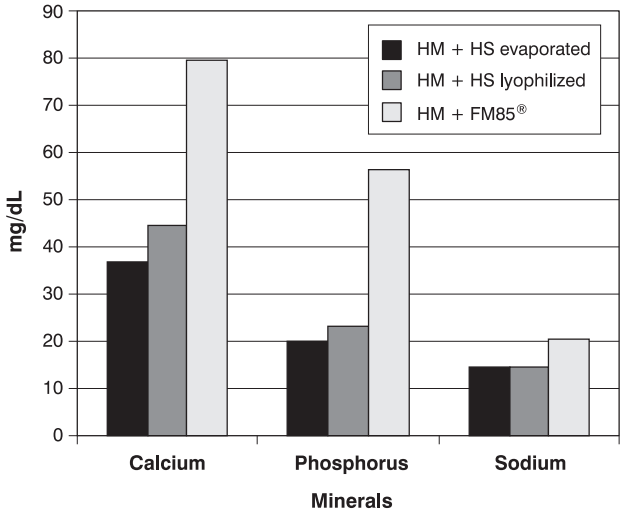
The results of the nutritional content of human milk supplemented with liquid (evaporated), powdered (lyophilized) and FM85® supplements were, respectively: protein = 1.81±0.01, 2.38±0.03 and 1.96±0.01 g/dL ($p < 0.001$); carbohydrate = 6.70±0.20, 7.25±0.25 and 10.06±0.05g/dL ($p = 0.006$); lipids = 3.75±0.16, 3.75±0.16 and 3.73±0.07 g/dL ($p = 0.96$) (Figure 1); calcium = 36.92±1.09, 44.75±1.21 and 79.37±0.34 mg/dL ($p = 0.001$); phosphorus = 20.02±0.95, 23.28±0.95 and 56.30±0.18 mg/dL ($p = 0.02$); sodium = 14.32±0.69, 14.4±0.061 and 20.33±0.033 mEq/L ($p = 0.143$) (Figure 2); osmolality = 391.45±7.22, 412.00±5.99 and 431.00±0.50 mOsmol/kgH_2O ($p = 0.074$) (Figure 3); and

caloric content = 67.78±2.01, 72.27±2.56 and 81.65±0.87 (p = 0.001) (Figure 4).



HM = human milk; SH = homologous supplement.

Figure 1 - Comparison of macronutrients: carbohydrates, proteins and lipids of the samples of 100 mL of human milk with evaporated homologous supplement (liquid), lyophilized homologous supplement (powdered) and FM85®. The columns represent the median values, and the bars, the median standard error. In the comparison between the groups (ANOVA test with Tukey post-test), there was a significant difference regarding the quantity of carbohydrates (p = 0.006)* and protein (p < 0.001)*

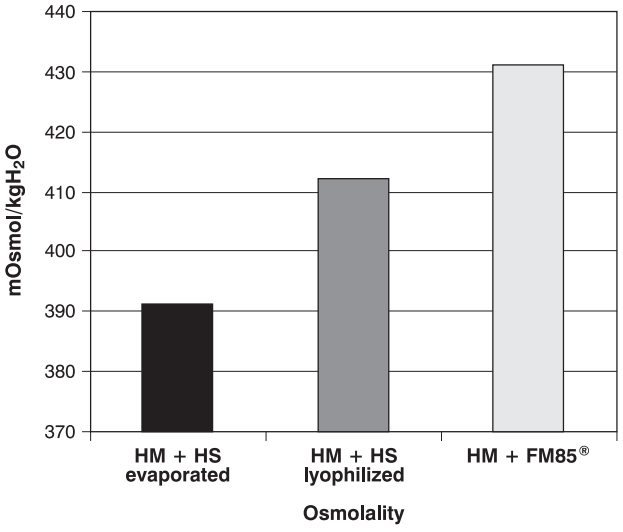


HM = human milk; SH = homologous supplement.

Figure 2 - Comparison of the following minerals: calcium, phosphorus, sodium and potassium of the 100 mL samples of human milk added to evaporated homologous supplement (liquid), lyophilized homologous supplement (powdered) and FM85®. The columns represent the median values, and the bars, the mean standard error. In the comparison between the groups (ANOVA test with Tukey post-test), there was a significant difference in relation to the quantity of calcium (p = 0.001)* and phosphorus (p = 0.02)*

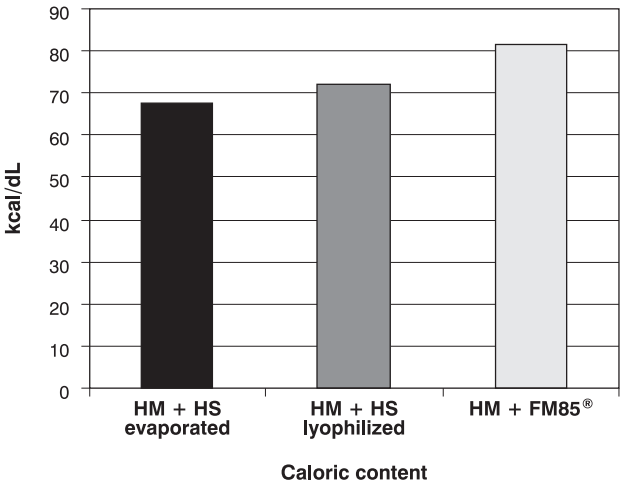
Discussion

The adequacy of the amount of nutrients supplied by the banked human milk and by the mother's own milk is required to provide the necessary amount of nutrients for an adequate growth and development, and to obtain that, the use of additives is needed.[5,8,16,17]



HM = human milk; SH = homologous supplement.

Figure 3 - Comparison of the osmolality of the 100 mL samples of human milk with the evaporated homologous supplement (liquid), the lyophilized homologous supplement (powdered) and FM85®. The columns represent the median values, and the bars, the median standard error. In the comparison between the groups (ANOVA test with Tukey post-test), there was no significant difference (p = 0.074)*



HM = human milk; SH = homologous supplement.

Figure 4 - Comparison of the caloric content of the 100 mL samples of human milk with the evaporated homologous supplement (liquid), the lyophilized homologous supplement (powdered) and FM85®. The columns represent the median values, and the bars, the median standard error. In the comparison between the groups (ANOVA test with Tukey post test), there was a significant difference (p = 0.001)*

In Brazil, the commercial additive commonly used is the FM85®, which supplements the banked human milk with protein, carbohydrate, vitamins, minerals and oligoelements, justifying its choice for the comparison with the additives proposed in this study.

The number of samples in each group was based in a previous study, which took into consideration the small variation of nutrients of mature milk in different mothers.[12] The volume of pooled human milk used for the addition of the supplements in the liquid and powdered forms – 80 and 100 mL, respectively, in each sample – was calculated by previous analysis with the objective to reach the lowest volume of modified human milk, to serve as an additive capable of offering nutrients in acceptable quantity, quality and cost when added to 100 mL of human banked milk. The additive in the liquid form is added to a lower volume of pooled milk when compared to the additive in its powdered form. The reconstitution of the powdered additive in 100 mL of human banked milk provides a higher volume of dilution with higher nutritional and bioactive values than the additive in the liquid form.[11] However, the lyophilization technique requires more investment, the acquisition of an evaporator, a refrigerated centrifuge and a lyophilizator, while the additive in its liquid form, once it depends only on the 2 previous pieces of equipment, is considered of low cost and easy operation. The only and initial cost to equip the milk bank and produce the liquid and powdered supplements is of approximately R$ 25,000.00 and R$ 52,000.00, respectively. Considering that the cost of the box containing 70 flacons of the commercial additive is R$ 90.98 in the pledge number 801-790 of UFMS and that a flacon supplements 20 mL of human milk, the cost to feed a child weighing 1,000 g, with a daily intake of 150 mL/kg for 30 days, will be of R$ 682.35. To feed 10 children per day with this volume for 1 year, the expense will be R$ 81,840.00. Therefore, one unity of neonatal therapy which assists 10 children a month, using the proposed volume, would have spent in 1 year three times the value of the initial cost of the liquid additive and one time and a half that of the powdered additive.

The samples of the powdered additive produced a median of 5.4 g of powder, and this quantity was used to supplement 100 mL of banked human milk.

Evaporation and lyophilization concentrate the nutrients, and it is necessary to control fat and lactose content, avoiding gastrointestinal alterations in VLBWI.[12] Justifying the need for lactose and fat removal.

Lactose providing should be around 3 to 12 g for each 100 kcal and should not surpass 8 g/100 mL.[17] Groups I, II and III provided respectively 9.88 g/100 kcal, 10.03 g/100 kcal and 12.3 g/100 kcal of carbohydrates.

In relation to the amount of calories, group I offers 101.7 to 135.6 kcal/kg/day; group II, 108.4 to 144.5 kcal/kg/day; and group III, 122.4 to163.3 kcal/kg/day, if volumes of 150 to 200 mL/kg/day are used. Though groups I e II provided lower caloric levels to the ones provided by group III, there is an offer of caloric content according to the nutritional recommendations for VLBWI.[18]

The fat content provided by the samples for groups I and II was of approximately 5.53 g/100 kcal and for group III was 5.16 g/100 kcal. The total lipid recommended is based on the amount of human milk and may vary from 3.8 to 11 g/kg/day, and the proximity of the maximum value in a diet can lead to steatorrhea. If we use a volume of diet that varies between 150 to 200 mL/kg/day, we will be offering an approximate amount of 5.6 g/kg/day to 11.3 g/kg/day.[18]

The supply of amino acids in a proper quantity, even with a low caloric intake, allows the non-use of the endogenous protein, increasing the protein synthesis and decreasing the difference between proteolysis and protein synthesis. It is necessary 3.8 to 4.0 g/kg/day to attain intra-uterine nutrient accretion rates.[18] A diet with the liquid supplement will provide 2.7 g/100 kcal (group I); with the powdered supplement it provides 3.4 g/100 kcal; and with the commercial supplement, 2.4 g/100 kcal. All provide quantity within the reference for nutrition of VLBWI and extremely low birth weight infant (ELBWI).[18-20] The offer of 160 mL/kg/day of fortified human milk supplemented with homologous powdered additive (group II) will provide the VLBWI with 3.8 g/kg/day of homologous protein, meeting the nutritional needs of this population with a lower health risk in the long-term.[2,3]

Sodium is necessary to maintain extracellular tonicity, influencing the adequate hydric balance, which is a prerequisite for growth and development.[21] Considering that the offer of 160 mL/kg/day of the diets (groups I and II) will offer approximately 2.4 mEq/kg/day and that the need for sodium in VLBWI according to the nutrition committees is 4 to 5 mEq/kg/day, there is a need for a biochemical control of this mineral for newborns with a gestational age lower than 32 weeks.[3,16]

The quantity of calcium in groups I and II is under the nutritional recommendations.[3,16,18] There is a demand for extra offer of approximately 75 mg of calcium and 35 mg phosphorus for each 100 mL of milk, which may be manipulated under the form of calcium glycerophosphate and glycine chelated calcium and added to the proposed additives, so that, while offering 150 mL/kg/day of these supplemented diets, they will provide approximately 180 mg/kg/day calcium and 87 mg/kg/day phosphorus, values that reach the nutritional recommendations of this age group (70 to 140 mg/kg/day calcium and 50 to 90 mg/kg/day phosphorus)[16]; and that, when offered a volume equal to 200 mL/kg/day, would reach the amounts recommended by the American Academy of Pediatrics, which are 200

to 250 mg/kg/day calcium and 110 to 125 mg/kg/day phosphorus.[3] The addition of these minerals did not show a significant increase in the osmolality of the supplement when compared to the initial osmolality of the formula and also proved inferior to the human milk pool supplemented with FM85®.

The osmolality of the groups I, II e III is in tolerable levels.[22] Hypertonic diets are related to acute necrotizing enterocolitis.[23] However, the easy absorption of the nutrients from human milk, associated to the presence of immunoglobulin, epidermal growth factors and enzymes that hydrolyze platelet activating factors, indicate that the osmolality of human milk with the additives I and II offer lower risk to the newborn, besides offering protein from a homologous origin.[23]

The modifications of the banked human milk proposed in this study and its reconstitutions provided food presenting macronutrients in analogous amounts to the ones offered by the mother's own milk in the first lactation weeks. Considering that evaporation does not totally destroy immunological factors and once lyophilization is a method of nutrients' conservation, there is a probable preservation of the enzymatic and immunological functions in the production of the additives from groups I and II,[11,24] which make them better than the commercial additive in terms of quality.

The acquisitions of the evaporator, the refrigerated centrifuge and the lyophilizator by the milk banks are the initial and only investment necessary to equip and develop the methodology proposed. Thus, in equipped milk banks, which have a high number of donors, in developing countries, considering the high cost of the industrialized additives, the additives proposed in the liquid and powdered forms seem to be well indicated as additives to banked human milk or to the mother's own milk to nourish VLBWI. That is because they offer significant amounts of protein from homologous origin and allow newborns to meet the nutritional recommendations of macronutrients elected by the international committees on nutrition, authorizing the routinely use of these additives in neonatology services, considering the additions of calcium and phosphorus in the quantities proposed in this study.

The homologous supplements proposed in this study may be controllably improved regarding its energy content, with the choice of banked human milk with a higher energy and protein content to be added to liquid or powdered homologous supplement, or by adding, in controlled manner, 1 g, e.g., of fat extracted from the same human milk resulting from the skimming process. There will be, thus, an increase in calories and polyunsaturated fatty acids, which will provide a better growth and development in the long-term.[25] Another way would be to increase the caloric content by adding glucose polymers to the homologous supplement, without much interference in the osmolality and with a good absorption.[22]

In a clinical study, not yet submitted for publication, on the growth of VLBWI fed with the different diets proposed, it was observed that there are benefits in head circumference growth for the groups fed with human milk supplemented with modified human milk. This finding probably indicates that the better quality of amino acids provided by the homologous supplement, with a higher supply of some over others, could lead to better brain growth.[26]

Longitudinal studies in different care centers for preterm newborns, which evaluate growth, gastrointestinal tolerability and development in the long-term of newborns feed with milk from milk banks added by homologous supplement, should be performed to prove the benefits of these diets.

Acknowledgements

We thank Elisabete Kamiya for her support at the milk bank, and Ana Maria Miguel Figueiredo e Baltazar Nunes for the osmolality analysis.

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