



Physicochemical analysis of pasteurized human milk and commercial premature formulas fortified with phosphorus solution or commercial fortifier

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ABSTRACT

A study was conducted to determine: the most appropriate proportion (1% or 10% v/v) of a phosphate solution (PS), containing 39mg/mL phosphorus, to be added to pasteurized human milk (HM) or commercial premature formula (FM); the final osmolality of such products, and whether precipitation occurs between calcium (Ca) and phosphorus (P) when commercial fortifier (FOR) or PS is added. A significant increase was observed in the concentrations of Ca in the samples of HM and FM containing FOR and a decrease in the samples of HM containing 10% (v/v) PS. The phosphorus levels increased significantly, in both HM and FM, when FOR or PS (1 and 10%) were added. Osmolality showed a significant increase in the solutions of HM with FOR or 10% PS added, and in the solution of FM containing 10% PS. Qualitative analysis of the precipitate formed on addition of 10% PS to FM revealed that it consisted of dicalcium phosphate. It was concluded that the addition of 10% PS to FM causes a fall in the Ca content, due to its precipitation with phosphate, promoting a reduction in the availability of both ions. On the other hand, the addition of 1% PS was demonstrated to cause no alteration in the Ca concentration, but a significant increase in P. These results suggest that the use of 1% PS is a potential means of supplementation of P after these patients are discharged.

Keywords: Pasteurized human milk. Commercial premature formulas. Phosphorus solution. Supplementation. Calcium. Phosphorus.

INTRODUCTION

Premature infants have greater nutritional needs to achieve optimal growth in the neonatal period than at any other time of their life. There are several reasons for this: first, infants born at the beginning of the third trimester of pregnancy often are growth-restricted because of decreased intrauterine nutrient deposition; second, medical conditions, including hypotension, hypoxia, acidosis, infection and surgery, increase metabolic energy requirements and thus nutrient needs and, finally, additional impediments to growth are physiological immaturity of the gastrointestinal tract, including decreased gastrointestinal motility and reduced intestinal enzyme activity, and treatments such as corticoids (Hay et al., 1999).

Nutritional support helps to correct growth restriction at birth and to achieve an appropriate rate of weight gain, which is almost twice that of a term infant (Schanler et al., 1985). Enteral nutrition with either human milk or commercial premature formulas can be provided to premature infants. The American Academy of Pediatrics (2005) recommends human milk as the food of choice for all infants, though supplementation with human milk fortifiers is required to meet the nutritional needs of premature newborns. Although human milk is the preferred food for premature infants, premature formula can be an appropriate option, especially if there is an inadequate milk supply from the mother, volume restrictions limiting the intake of the infant, or nutrient limitations of the human milk, as unfortified human milk may not supply sufficient quantities of nutrients such as phosphorus, calcium, protein, iron and vitamins. For an inadequate supply, fortified mother's milk is either mixed or alternated with premature formula (Gartner et al., 2005).

A major concern with human milk fortification is that the added nutrients may affect the protective benefit derived from mother's milk against infection (Gartner et al., 2005). However, there are limited data addressing this

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issue, including the vulnerability of infants to the adverse effects of oral administration of hypertonic substances, such as fortifiers. In fact, few reports have been published on the osmolality of such substances in the care of sick infants. A variety of commercial fortifiers is available globally and they vary widely in cost. In light of the above concerns, a study was undertaken at University Hospital at the University of São Paulo to determine: the concentration of dissolved phosphorus that would be most appropriate to be added to pasteurized human milk and to a premature formula; if the addition of such solutions could alter the osmolality of the final solution, and if these alterations could induce precipitation.

MATERIAL AND METHODS

Samples

First, 10 mL of premature formula (FM) was reconstituted. Then, a solution of sodium and potassium phosphates (PS) containing 39 mg/mL of phosphorus was prepared. Finally, samples of human milk (HM) and FM were supplemented with PS, to final concentrations of 10 and 1 % (v/v), and with a commercial fortifier (FOR) (FM 85, Nestle®, containing 8.6 mg P/g; 14 mg Ca/g and other components such as vitamins, proteins and carbohydrates) at a concentration of 5% (w/v), as recommended by the manufacturer. All samples were kept between 2 and 8°C until analysis.

Analysis

Before analysis, the samples were left on the bench to warm naturally and vortexed prior to use. A 1 mL aliquot of each sample was centrifuged at 7000 r.p.m. for 10 minutes. The supernatant was removed and the osmolality was measured by vapor point depression at the Department of Chemistry of the University of São Paulo, using a model 5500 osmometer (Wescor Inc, Logan, Utah, USA). Calibration of the osmometer with standard solutions was performed after every 30 measurements.

Samples were analyzed for calcium by the ortho-cresolphthalein complexone method and for inorganic phosphorus by the phosphomolybdate/UV method, using an automated biochemical analyzer (Advia 1200, Siemens, NY, USA). The precipitate was analyzed as described in the US Pharmacopoeia, (2006). All samples were analyzed in triplicate by a single investigator (ABS), blinded in relation to all the analyses.

Statistical Analysis

Analysis of variance (ANOVA) followed by the Tukey-Kramer test was used to analyze data. All analyses

were performed with GraphPad InStat software (1998). In all experiments, $p < 0.05$ was the criterion for statistical significance.

RESULTS

Table 1 shows the results obtained from the analysis. Values were presented as the mean and standard deviation. There was a statistically significant ($p < 0.05$) increase in calcium concentration in the samples of HM and FM supplemented with FOR and a decrease in FM containing 10% PS. With regard to the phosphorus concentration, there was a statistically significant ($p < 0.05$) increase in all supplemented solutions. Also, there was a statistically significant ($p < 0.05$) increase in the osmolality of the solutions of HM to which FOR and 10% PS had been added and FM with 10% PS added. Qualitative analysis of the precipitate formed when 10% PS was added to FM revealed that it was constituted by dicalcium phosphate.

DISCUSSION

‘Preterm’ refers to a birth that occurs before 37 completed weeks (less than 259 days) of gestation. A very preterm birth is generally defined as less than 32 weeks of gestation. In the United States, about two percent of live births occur at less than 32 weeks and 12 percent before 37 weeks of gestation. In Brazil, 8.2 percent in 2004 were born before 37 weeks, according to DATASUS (Brazilian Ministry of Health, 2008). These patients need mineral supplementation during hospitalization, which prevents a decrease in linear growth and increases bone mineralization during and beyond the neonatal period (Abrams et al., 1989). One study showed that supplementation with calcium and phosphorus resulted in normalization of biochemical indices of mineral status, including serum phosphorus, calcium and alkaline phosphatase activity and urinary excretion of calcium and phosphorus (Schanler & Garza, 1988). Another study indicated that a continuation of the supplementation with Ca and P is justified in very low birth weight (VLBW) infants with a body weight of more than 2000 g. There was no evidence of adverse effects of Ca and P supplementation in VLBW infants with a body weight lower than 1500 g, who might therefore also benefit from supplementation (Hettrich et al., 1995).

The aim in this study was to determine whether the addition of a solution of phosphorus and a commercial fortifier to human milk and premature formula would change some characteristics of these products. In fact, the results showed that addition of PS to FM in a proportion of 10% (v/v) resulted in the precipitation of dicalcium phosphate, leading to a decrease not only of free calcium but also of phosphorus. Moreover, the addition of 10% PS raised the osmolality of the final product to more than 400.0 mOs/L, which could promote diarrhea in the infants.

Table 1 - Calcium and phosphorus concentration and osmolality of pasteurized human milk (HM) and formula (FM) supplemented with phosphate solution (PS) at 1 and 10% (v/v) or with 5% (w/v) commercial fortifier (FOR)

| HM/FM | Calcium concentration | Phosphorus concentration | Osmolality | Precipitate analysis |
|---------|-----------------------|--------------------------|----------------|----------------------|
| (mg/dL) | (mg/dL) | (mg/dL) | (mOsmol/kg) | |
| HM | 28.53±0.13 | 6.23±0.21 | 265.25±27.75 | - |
| HM 1 | 28.40±0.01 | 55.67±0.55* | 297.67±8.14 | - |
| HM 10 | 25.67±0.47 | 393.30±5.90* | 449.00±5.57* | - |
| HM FOR | 46.43±4.31* | 30.87±2.06* | 336.00±19.47* | - |
| FM | 43.03±8.94 | 16.27±0.55 | 239.50±19.36 | - |
| FM 1 | 42.80±0.61 | 63.83±0.55** | 282.50±14.84 | - |
| FM 10 | 27.77±3.01** | 364.40±3.95** | 425.83±27.07** | CaHPO ₄ |
| FM FOR | 51.48±0.88** | 45.20±0.98** | 275.50±31.05 | - |

* p< 0.05 in relation to HM values. ANOVA followed by Tukey-Kramer test

** p< 0.05 in relation to FM values. ANOVA followed by Tukey-Kramer test
CaHPO₄ : dicalcium phosphate

Growing preterm infants need more calcium (Ca) and phosphorus (P) than term-born infants (American Academy of Pediatrics Committee on Nutrition, 1985). To achieve foetal mineral retention, preterm infants require an intake of 4.6 to 5.5 mmol Ca/kg/d (184-220 mg/kg/d) and 3.9 to 4.5 mmol P/kg/d (120.9-139.5 mg/kg/d) (American Academy of Pediatrics Committee on Nutrition 1985; Koo & Tsang, 1993; Demarini, 2005). These amounts were estimated by assuming an average intestinal absorption of 65% for Ca and 80% for P. Preterm infants fed with human milk receive less P and Ca than they need and show maximal tubular reabsorption of P, leading to undetectable urinary concentrations of P and paradoxical hypercalciuria (Schilling et al., 1982). Conversely, feeding a formula containing relatively low amounts of Ca leads to very low urinary Ca excretion and paradoxical hyperphosphaturia (Demarini, 2005; Manz et al., 1989). Thus, the shortfall may be met by using human milk fortified with supplementary solution or with specially designed formulas for premature infants with 1% PS, which yields 39 mg P/mL, corresponding to 1.3 mmol/mL; so neonates who need 3.9 mmol P/kg/d have to receive 3 mL of 1% PS incorporated into lactic products and administered over 24 h. The calcium solution must be administered between nursing periods, to guard against precipitation, as demonstrated in this study. In fact, in our institution, during the preparation of the discharge of these children, the mother is advised to buy a source of calcium, such as calcium citrate, to supply the needs of the premature child.

It is possibly to conclude that the addition of 10% (v/v) of PS to FM causes a decrease in its calcium concentration, due to precipitation of Ca²⁺ by the phosphate, promoting a reduction in the availability of either ion. On the other hand, the addition of 1% PS has been shown to cause no

alteration in the calcium concentration, but a significant increase in that of phosphorus. These results suggest that 1% PS could be used as an alternative form of phosphorus supplementation for premature infants. Future clinical trials would be necessary to find out if this solution was capable of supplementing the premature after discharge from hospital.

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RESUMO

Análise físico-química do leite humano pasteurizado e de fórmula comercial para prematuros suplementados com solução de fósforo ou fortificante comercial

Foi realizado um estudo para verificar: a concentração, 1 ou 10% (v/v), mais apropriada de solução de fósforo (SP), para ser adicionada nos produtos lácteos; a osmolalidade final de tais produtos suplementados; a ocorrência de precipitação entre o cálcio (Ca) e o fósforo (P) com a adição de um fortificante comercial (FOR) ou SP. Verificou-se aumento significativo nas concentrações de Ca nas amostras de leite materno pasteurizado (LM) e fórmula comercial (FL) contendo FOR e diminuição nas amostras de FL contendo 10% (v/v) de SP. Quanto aos níveis de fósforo, houve aumento significativo tanto no LM quanto na FL, adicionados de FOR ou SP a 1 e 10%. Em relação à osmolalidade, houve aumento significativo nas soluções de LM contendo FOR e de SP a 10% e na solução de FL a 10% de SP. A análise qualitativa do precipitado formado pela adição de 10% SP na FL revelou que o mesmo é constituído de fosfato bicálcico. Concluiu-se que a adição de SP na concentração de 10% em FL acarreta diminuição do Ca devido à precipitação do mesmo com o P, promovendo uma baixa na oferta de ambos os íons para o prematuro. Por outro lado, a adição de SP a 1% demonstrou que não houve diminuição dos níveis de Ca, mas aumento significativo nos níveis de P. Estes resultados sugerem que o uso da SP a 1% é uma alternativa de suplementação de fósforo na alta hospitalar destes pacientes.

Palavras-chave: Leite humano pasteurizado. Fórmula láctea para prematuros. Solução de fósforo. Suplementação. Cálcio. Fósforo.

REFERENCES

- Abrams SA, Schanler RJ, Tsang RC, Garza C. Bone mineralization in former very low birth weight infants fed either human milk or commercial formula: one year follow-up observation. *J Pediatr.* 1989; 114:1041-4.
- American Academy of Pediatrics Committee on Nutrition. Nutritional needs of low-birth-weight infants. *Pediatrics* 1985; 75:976-86.
- American Academy of Pediatrics. Breastfeeding and the use of human milk. *Pediatrics* 2005; 115:496-506
- Brasil. Ministério da Saúde. DATASUS. Sistema de informações sobre nascidos vivos. [citado 2008 maio 15]. Disponível em: <http://tabnet.datasus.gov.br/cgi/idb2006/d16.pdf>.
- Demarini S. Calcium and phosphorus nutrition in preterm infants. *Acta Paediatr.* 2005; 94(Suppl 449):87-92.
- Gartner LM, Morton J, Lawrence RA, Naylor AJ, O'Hare D, Schanler RJ, Eidelman AI. Breastfeeding and the use of human milk. *Pediatrics* 2005; 115:496-506.
- Hay Jr WW, Lucas A, Heird WC, Ziegler E, Levin E, Grave GD, Catz CS, Yaffe SJ. Workshop summary: Nutrition of the extremely low birth weight infant. *Pediatrics* 1999; 104:1360-8.
- Hettrich B, Kalhoff H, Rudloff S, Diekmann L, Stock GJ, Manz F. Supplementation of premature infant nutrition with calcium and phosphorus for improving mineral supply of premature and small-for-gestational age newborn infants. *Klin Padiatr.* 1995; 207(6):334-40.
- Koo W, Tsang R. Calcium, magnesium, phosphorus, and vitamin D. In: Tsang R, Lucas A, Uauy R, Zlotkin S. editors. *Nutritional needs of the preterm infant.* Baltimore: Williams & Wilkins; 1993.
- Manz F, Diekmann L, Stock GJ. Effect of calcium supplementation on calcium and phosphorus balance and renal net acid excretion in preterm infants fed a standard formula. *Acta Paediatr Scand.* 1989; 78:525-31.
- Schanler RJ, Garza C, Nichols BL. Fortified mothers milk for very low birth weight infants: results of growth and nutrient balance studies. *J Pediatr.* 1985; 107:437-45.
- Schanler RJ, Garza C. Improved mineral balance in very low birth weight infants fed fortified human milk. *J Pediatr.* 1988; 112:452-6.
- Schilling R, Haschke F, Kovarik J, Woloszczuk W. Phosphorus and calcium metabolism of premature infants fed human milk and formulated milk. *Padiatr Padol.* 1982; 17(4):667-74.
- United State Pharmacopeia, 29th.ed. Rockville: United States Pharmacopeial Convention, 2006 p.359-60.