Abstract

Background: In order to reach nutritional purposes, human milk has to reach 2 g/dL. In his publication Lefeber et al. 2013, journal of pharmacology and clinical research, the authors stated that, when women's milk is fortified up to 2 g/dL, it may increase it's osmolality up to 500 mosm/kg. He also argued that caution should be taken when adding a drug or vitamins into the milk.

Aim: We studied for the first time the impact on a women’s fortified milk osmolality, when adding multi vitamins (ADEC).

Method: The osmolality of 36 pasteurized fortified human milk samples was measured. The amount of milk required as a solvent to maintain osmolality below 500 mosm/Kg was then determined.

Results: The 2mL fortified human's milk reached up to 750mosl/kg when the multi vitamins ADEC was added. The osmolality decreased proportionately with the solution dilution. It is only with 20 mL of milk that the osmolality comes down to its initial rate 430mosm/kg. Stronger the milk’s fortification is, the bigger impact it has on the milk osmolality.

Conclusion: New nutritional recommendations for premature infants are needed. In the meantime, when the fortified milk intake is under 20 mL, it is preferable to extend parenteral intakes with fat-soluble vitamins. Also, we should use enriched women's milk as fortifier and be cautious with fortification « à la carte » or when adding drugs and electrolyte solutions.

Keywords: Human milk osmolality ; Human milk fortifier ; Vitamin D ; Preterm ; Nutrition
Paris, France. A batch of frozen-pasteurized human milk from our human milk bank (Lactarium Regional d’Ille de France) has been used for our study. A bottle (200mL) of unfortified milk (HMF 0) was thaw. We used 2 different methods to fortify human milk. 200 mL bottles of FHM1 and FHM2 were fortified. In the first time, we have used a multi-component human milk fortifier at 4% (HMF) containing whole protein, dextrin and mineral (Suppletine, Milupa, France) and 0.5% of an exclusive whole protein fortified (Nutriprem, Gallia, France) (FHM 1). The second one contained the same multi component HMF at 4% but added with a 1% of an exclusive whole protein fortified (FHM 2). Milk sample were kept 24 hours at +4 °C.

Supplemented with vitamin ADEC

As recommended in the NICU practice guidelines. We then added 0.3mL of vitamin ADEC (Uvesterol ADEC, Crinex, France). This vitamin is a concentrate of vitamin A (oil formula), Ergocalciferol (vitamin D2), alpha tocopherol acetate (vitamin E), and ascorbic acid (vitamin C). One dose of 0.3 mL contains: 1000 UI of vitamin D was add to milk samples of 2, 6, 12 and 20mL out of each type of milk (HFM 0, HMF 1 and FHM 2).

Osmolality determination

The milk osmolality was measured with an advanced micro-osmometer model, Fiske® Modèle 210 (FISKE® associates, Norwood, Massachusetts, USA) in Hospital Cochin, Paris, France. The method used was the freezing point measurement with a precision of 1 mosm/kg. Measures were made in triplicates.

Statistical analysis

Data are expressed as means (SD). Student’s paired t test was used for statistical evaluation. A p value of < 0.01 was taken as level of significance.

Results

The osmolality of our unfortified and fortified milk was respectively 292mosm/kg and 432mosm/kg. The same data was found in another study, multi-component fortifier increase significantly the osmolality of human milk. We also demonstrated that fortified human milk (HMF) containing whole protein, dextrin and mineral, increases significantly more the osmolality compare to exclusive whole protein fortified (Figure 1). When vitamin ADEC is added in 2ml of unfortified human milk, the osmolality increases at 600mosm/kg. It can even reach 738 mosm/L after pasteurization and they had the same value to 30 mosm/L after pasteurization and they had the same value (292 mosm/kg) with standard fortification (431 mosm/kg). Adding fortified human milk with whole protein increases significantly osmolality (471mosm/kg) as demonstrated by Curtis and et al. [9]. However, no study has yet evaluated the impact of multivitamins addition on fortified human milk’s osmolality. As proven by de Curtis and et al. [9] osmolality is similar after storage 24h at +4°C we use 24h +4°C storage milk for more facility to prepare our milk samples. When the milk is being pasteurized the amylase rate remains stable so this could explain an increase of osmolality in pasteurized human milk. In fact Kreisl et al. [11] found a decrease of osmolality from 20 to 500 mosm/kg [10]. Even though the link with necrotizing enterocolitis is not yet well established, digestives disorders can be found. He will forbidden the use of multi-vitamin. The author also said to be careful when adding hyper osmoler drugs or vitamins into the human’s milk. The results on the milk’s osmolality after adding multi component fortifiers such as 4% of Suppletine and protein (0.5% to1% of Nutriprem) were comforting. However, no study has yet evaluated the impact of multivitamins addition on fortified human milk’s osmolality. As proven by de Curtis and et al. [9] osmolality is similar after storage 24h at +4°C.

Discussion

The osmolality of unfortified milk is similar to amniotic fluid 275mosm/kg. High milk osmolality reflects a high concentration of solute particles. In 2010 EPSGHAN published new recommendations for preterm’s enteral nutrition [1]. Especially the increase of protein and caloric intake for immature infants. In order to reach nutritional purposes, human milk has to reach 2 g/dL. In 2013, Lafeber and al states that when women’s milk is fortified up to 2 g/dL, it may increase its osmolality up to 500 mosm/kg [10]. Even though the link with necrotizing enterocolitis is not yet well established, digestives disorders can be found. He will forbidden the use of multi-vitamin. The author also said to be careful when adding hyper osmoler drugs or vitamins into the human’s milk. The results on the milk’s osmolality after adding multi component fortifiers such as 4% of Suppletine and protein (0.5% to1% of Nutriprem) were comforting. However, no study has yet evaluated the impact of multivitamins addition on fortified human milk’s osmolality. As proven by de Curtis and et al. [9] osmolality is similar after storage 24h at +4°C. When vitamin ADEC is diluted in a higher milk volume, the milk osmolality decreases proportionality, until it reaches the initial osmolality at a volume of 20mL of human milk (Figure 1).
different concentrations of fortifiers so it need to be controlled. Agarwal and al. [12] suggest that fat has no effect on milk’s osmolality and that it may be preferable to use a predominantly fat based HMF. Pearson and al suggest that the addition of mineral and vitamin into human milk, can increase osmolality. Srinivasan and et al. [13] demonstrated an increase of the raw milk’s osmolality, with increasing dose of all additives (sodium, iron, dalavat-vitamin ABCD, folic acid) until 951 mosm/kg excepted for caffeine. They did not find a significant increase of the milk’s osmolality when adding fortifier, their osmolality was not more than 400mosm/kg. Consequently they did not test the effect of both fortifiers and vitamins. Our results have proved by adding vitamin ADEC to human milk, will increased the milk’s osmolality (Figure 1). We have demonstrated for the first time that this increase was larger when the milk contained human milk fortifier. This could be responsible for slowing down gastric emptying, causing intestinal distension, frequent stools and more rarely diarrhea. Some authors have showed that there might be a correlation between osmolality superior to 400mosm/kg and necrotizing enterocolitis [12,14]. But for others there is no evidence of mucosal injury. However the safety cut offs to prevent enterocolitis are unclear [2]. Current lipo solubles vitamins are used by NICU with an osmolality as high as 3200 mosm/ml. The addition of vitamins into a small volume of milk, increases significantly osmolality (up to 700 mosm/l for 2ml milk sample). Osmolality decreases proportionally to the increase of milk volume used for administration (Figure 1). When the volume of dilution reaches 20ml, the osmolality of unfortified and fortified milk remains unchanged. Hyperosmolar drug like retrovir have been responsible of netrotizing enterocolitis for preterm baby will it is well tolerate for term baby and special recommendations of dilution have been written [15,16]. Kreissl and et al. [11] concludes that adding therapeutic additives such as iron, multivitamin supplement and calcium-phosphorus capsules may increase osmolality up to 868 mosm/l. Liposoluble oral suspension of vitamin could have the same impact on human milk osmolality. A certain amount of milk (20ml) is required as a solvent to maintain osmolality below 400 mosm/kg. It is not possible for very small premature babies to receive such a volume of dilution, when enteral nutrition has been initiated, in order to administrate orally these vitamins. It may be safer to start this type of oral vitamin when preterm tolerates 20ml of milk. As 800 to 100UI/kg/day of vitamin D is recommended we could suggest to pursue as late as possible intravenous fat-soluble vitamins. We could also prefer to use human milk fortifier with high concentration of vitamin D. The ultimate question could be is this osmolality reflect the one measured in gastric contents in very low-birth-weight infants after gavages feeding. Thatrimontrichai and et al. [17] found no significantly change in osmolality, so we could expect than our results are representative of the effective osmolality of gastric content with fortified milk and vitamins.

**Conclusion**

The use of human milk fortifiers with an additive therapy, like fat-soluble vitamins, increases significantly osmolality of human milk. This increase is significantly more important than the one expected with human milk fortified alone. Like fortification, vitamins are necessary in order to optimize the infant’s growth and development. Rational policies for the use of additives with human milk will allow safety administrations in neonatal units unfortunately with fortification “à la carte” or with additive in human milk [18].

**References**

1. Agostoni C, Buonocore G, Carnielli VP, De Curtis M, Darmaun D, et al. (2010) Enteral nutrient supply for preterm infants: commentary from the European Society of Paediatric Gastroenterology, Hepatology and Nutrition Committee on Nutrition. J Pediatr Gastroenterol Nutr 50(1): 85-91.

2. Rochow N, Landau-Crange E, Fusch C (2015) Challenges in breast milk fortification for preterm infants. Curr Opin Clin Nutr Metab Care 18(3): 276-284.

3. Voyer M, Mitanche D, Magy JF. Rigourd V, Kieffer F (2004) Nutrition entérale du prématuré: quelle(s) supplémentation(s) pour le lait de femme? J Gynecol Obstet Biol Reprod 33(1): 117-122.

4. Cooke RJ (2006) Adaptable fortification of human milk fed to preterm infants. J Perinatol 26(10): 614-621.

5. Money R, Fewtrell MS, Abbott RA and all (2004 ) Neurodevelopment in children born small for gestational age: randomized trial of nutrient-enriched standard formula and comparison with a reference breastfed group. Pediatrics 113(3 Pt 1): 515-521.

6. Grant WB (2010) Vitamin D supplementation of mother and infant could also reduce risk of sepsis in premature infants. Early Human Dev 86(2): 133.

7. Dutau G, Lavaud F (2012) Vitamine-D, immunity, asthma and symptoms of atopy. Rev Pr Allergologie 52: 10-18.

8. Salle BL, Delvyn E, Clariu O, Hascoet JM, Levy E (2007) Is it justifiable to administrate vitamin A, E and D for 6 months in the premature infants? Arch Pediatr 14(12): 1406-1412.

9. De Curtis M, Candusso M, Peltain C, Rigo J (1999) Effect of fortification on the osmolality of human milk. Arch Dis Child Fetal Neonatal Ed 81(2): F141-F143.

10. Lafeber H (2013) Fortification of human milk fed to preterm infants: are our guidelines safe? J Pediatr Gastroenterol Nutr 57(4): 414.

11. Kreissl A, Zwiauer V, Reppa A, Binder C, Haninger N, et al. (2013) Effect of fortifiers and additional protein on the osmolality of human milk: is it still safe for the premature infant? J Pediatr Gastroenterol Nutr 57(4): 432-437.

12. Agarwal R, Singal A, Aggarwal R, Deorari AK, Paul VK (2004) Effect of fortification with human milk fortifier (HMF) and other fortifying agents on the osmolality of preterm breast milk. Indian Pediatr 41(1): 63-67.

13. Pearson F, Johnson MJ, Leaf AA (2013) Milk osmolality: does it matter? Arch Dis Child Fetal Neonatal Ed 98(2): F166-F169.

14. Musara C, Pote W (2014) Application of osmometry in quality analysis of milk. J Food Sci Technol 51(3): 606-610.
15. De Carolis MP, Lacerenza S, De Luca D, Bersani I, Costa S, et al. (2010) Is neonatal antiretroviral therapy a risk factor for NEC occurrence? Turk J Pediatr 52(1): 108-110.

16. Srinivasan L, Bokiniec R, King C, Weaver G, Edwards AD (2004) Increased osmolality of breast milk with therapeutic additives. Arch Dis Child Fetal Neonatal Ed 89(6): F514-F517.

17. Thatrimontrichai A, Janjindamai W (2009) Post prandial osmolality of gastric contents in very low-birth-weight infants fed expressed breast milk with additives. Southeast Asian J Trop Med Public Health 40(5): 1080-1086.

18. De Halleux V, Close A, Stalport S, Studzinski F, Habibi E, et al. (2007) Intérêt de la supplementation du lait maternel "à la carte". Archives de Pédiatrie 14(Suppl 1): S5-S10.