POSTPRANDIAL OSMOLALITY OF GASTRIC CONTENTS IN VERY LOW-BIRTH-WEIGHT INFANTS FED EXPRESSED BREAST MILK WITH ADDITIVES

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Abstract. The objective of the study was to evaluate the effect of each additive [$FeSO_4$, multivitamin (MTV), and vitamin E] on the postprandial osmolality of expressed breast milk (EBM) at 0, 30, 45 and 60 minutes. Babies born at Songklanagarind Hospital from 1 August, 2005 to 31 December, 2006 were studied; EBM was collected from mothers with a child born at an estimated gestational age less than 32 weeks or whose babies had a birth weight less than 1,500 grams. The volume of EBM depended on daily needs. The osmolality was determined by the additives in the EBM both before and after administration of each additive and in the gastric contents after gavage feeding at 0, 30, 45 and 60 minutes. Twenty-six infants were enrolled in the study. The median postprandial osmolality of EBM with MTV at 0, 30, 45 and 60 minutes were 413, 386.5, 388 and 383 mOsm/kg, respectively. At no time was the osmolality of $FeSO_4$ or vitamin E-mixed EBM above 400 mOsm/kg.

INTRODUCTION

Advances in obstetric and neonatal care have improved survival rates in very low birth weight (VLBW) preterm infants (<1,500 g) who survive the early neonatal period. Breast milk is the first choice in feeding a preterm infant due to its nutritional and immunologic superiority (Fenton and Belik, 2002; Lin and Stoll, 2006). The American Academy of Pediatrics (AAP) has recommended exclusive breastfeeding during the first 6 months of life and the continuation of breastfeeding for the second 6 months as optimum nutrition in infancy. There are multiple reasons for this recommendation. First, human milk is the optimum nutrient for term and near-term infants with respect to protein, fat, and carbohydrate composition. Second, the anti-infective properties of human milk reduce the incidence of acute illnesses such as infectious diarrhea, pathogenic bacterial fecal flora, necrotizing enterocolitis, otitis media, lower respiratory tract infections, and urinary tract infections in infants. Third, it has been suggested that the incidence of immune-mediated diseases such as diabetes mellitus, Crohn's disease, eczema, asthma, and allergic gastroenteritis is lower among

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breastfed infants. Fourth, psychological and long-term cognitive advantages have been observed in breastfed infants compared with formula-fed infants. It is believed that mother-infant bonding is enhanced during breastfeeding, and there are consistently improved scores on tests of cognitive development directly correlated with the duration of breastfeeding.

Most VLBW infants have immature suck-swallow coordination and require tube feeding. Tube feedings can be administered by intermittent gavage or by continuous infusion (Lin and Stoll, 2006).

Necrotizing enterocolitis (NEC) is one of the most serious and devastating diseases encountered in the neonatal intensive care unit (NICU). It is the most common gastrointestinal malady in neonates. It has a mortality rate of 10% to 50%. NEC accounts for at least 1,000 deaths annually in the United States. The increased incidence of NEC over the past few decades may be attributable to advancements in perinatal care, which have allowed very preterm infants to survive long enough to develop NEC. The incidence varies inversely with birth weight and gestational age. NEC strikes 4% to 13% of all very low birth weight babies (Jesse and Neu, 2006). The pathophysiology of NEC remains poorly understood. Premature infants are at high risk because of developmental immaturity of key functions, in particular gastrointestinal motility, digestive ability, circulatory regulation, intestinal barrier function, and immune defense. Other potential contributing factors include hypoxic-ischemic injury, feeding with formula milk, and colonization by pathological bacteria (Lin and Stoll, 2006).

However, in neonatal practice, various additives (multivitamins, $FeSO_4$, vitamin E) are routinely combined with expressed breast milk (EBM) for therapeutic or nutritional benefits. All enteral nutrition for VLBW infants needs to be supplemented. Breastfed infants drinking less than 500 ml in 24 hours need multivitamins (MTV) to provide 200 to 400 IU of vitamin D daily (Tender, 2004). The American Academy of Pediatrics (AAP) recommends vitamin A administration of 210 to 450 μg/kg per day of retinol (1 μg of equivalent retinol 3.3 IU). Furthermore, VLBW breastfed infants should be supplemented with iron sulfate drops to provide a total dose of approximately 4 mg/kg per day of elemental iron (Tender, 2004). Vitamin E is fat-soluble and functions as a biological antioxidant. It protects the polyunsaturated fatty acids of cell membranes from peroxidation. Infants are relatively vitamin E deficient at birth because of limited placental transfer and 90% of the tocopherol pool is located in adipose tissue. Preterm newborns have less adipose tissue, therefore, they have less vitamin E reserves. Human milk is a good dietary source of vitamin E for both term and preterm infants. Infants given formulas containing relatively large amounts of polyunsaturated fatty acids with little vitamin E and who receive relatively high doses of iron can develop a form of hemolytic anemia. The AAP Committee on Nutrition also suggests premature infants receive approximately 5-25 IU of supplemental oral vitamin E/day (Melhorn and Gross, 1971; Gross and Melhorn, 1974; Trindade, 2007).

These additives have the potential to increase the osmolality of EBM. The administration of hyperosmolar feeds is thought to be associated with NEC (Book *et al*, 1975; Willis *et al*, 1977; Le Guennec *et al*, 1983). This concern led to recommendations that enteral feeds for neonates should not have an osmolality above 400

mOsm/kg (Committee of Nutrition, 1976; Srinivasan *et al*, 2004).

Our review of the literature showed previous studies regarding this subject are limited to *in vitro* and animal studies, which led us to conduct our own study of the osmolality of EBM with additives used in VLBW infants.

MATERIALS AND METHODS

The present study was performed in the NICU at Songklanagarind Hospital, Thailand between 1 August, 2005 and 31 December, 2006. Manually EBM from mothers of VLBW infants who had given their consent for the study was sterilely collected. The EBM was kept frozen at -20ºC and labeled with the name of the mother and date. The frozen milk was thawed in warm water before use. The volume of EBM depended upon daily needs (150-200 ml/kg/day).

The osmolality was determined by the additives in the EBM both before and after administration of each additive and in the gastric content after gavage feeding at 0, 30, 45 and 60 minutes using a Gonotech Osmometer® (Intertrade, USA) based on freezing point depression. The additives evaluated were: $FeSO_4$ 4 mg of elemental iron/kg/day (Pediron® drops : THAIPHARMED 1942, Bangkok; containing $FeSO_4$ 50 mg (elemental iron 15 mg) in 0.6 ml], multivitamins 0.5 ml/day (Multivim® drops: B ED HUNG, Bangkok; containing Vitamins A, D_3, C, B_1, B_2, B_6, B_{12}, nicotinamide and dexpantinol), vitamin E 0.5 ml/day (Pharmacy Department, Songklanagarind Hospital, 50 IU/ml).

Statistical analysis

Sample size was calculated by the equation:

$N = (Z^2a/_2 . SD^2)/d^2$

$Z^2a/_2$ is a standard score set at 0.05

SD^2 is the variance calculated from a pilot study, where $SD^2 = 21$, and d was defined as an acceptable error equal to 15. From the equation above, the total minimum sample size required was 8.

Data was analyzed as median (minimum and maximum) and mean (SD). STATA software version 7 was used to compare the osmolality of EBM and the osmolality of the mixture of EBM at 0, 30, 45, 60 minutes. The present study was approved by the Ethics Committee Board of the Prince of Songkla University, and written informed consent was obtained from the mothers before the study began.

RESULTS

Twenty-six infants were enrolled in the study. We gave these infants additive-mixed milk. The infants, divided into three groups, were fed ferrous sulfate, vitamin E or multivitamins which were 9, 9 and 8, respectively.

The median gestational age of the infants was 30 weeks (range 28-33 weeks). The male infants in this study totaled 10 infants. The median birth weight was 1,246 g (range 690-1,500 g). The median postnatal age of the infants was 30 days (range 14-52 days). The median milk volume was 23 ml (range 18-30 ml). The mean milk volume was 24 ml (SD 6.8 ml).

The median osmolality of EBM was 311.5 mOsm/kg (range 263-360 mOsm/kg). We measured the osmolality of each additive. The median osmolalities of ferrous sulfate, vitamin E and multivitamins were 3,821, 1,182 and 8,512 mOsm/kg, respectively. The osmolality of EBM with each of the additives at 0, 30, 45 and 60 minutes is shown in Table 1.

Tabel 1
The osmolality of additives and changes in osmolality (mOsm/kgH_2O) of EBM after supplementation with additives (n = 26).

	Osmolality (mOsm/kgH_2O)		
	Ferrous sulfate	Multivitamins	Vitamin E
Before feeding	344 (304-378)	426 (367-506)	315 (273-364)
After feeding			
0 minute	347 (302-370)	413.5 (354-533)	316 (279-354)
30 minutes	342 (299-393)	386.5 (347-467)	312 (285-352)
45 minutes	366 (298-370)	388 (345-481)	314 (279-349)
60 minutes	354 (301-378)	383 (325-458)	315 (280-340)

Table 2
Neonates with an osmolality less than and greater than 400 mOsm/kg of mixed EBM.

	Osmolality < 400 mOsm/kg (percentage)	Osmolality > 400 mOsm/kg (percentage)	Total (percentage)
Ferrous sulfate			
After feeding 0 minute	9 (100%)	0	9 (100%)
After feeding 30 minutes	9 (100%)	0	9 (100%)
After feeding 45 minutes	9 (100%)	0	9 (100%)
After feeding 60 minutes	9 (100%)	0	9 (100%)
Multivitamins			
After feeding 0 minute	3 (37.5%)	5 (62.5%)	8 (100%)
After feeding 30 minutes	5 (62.5%)	3 (37.5%)	8 (100%)
After feeding 45 minutes	5 (62.5%)	3 (37.5%)	8 (100%)
After feeding 60 minutes	5 (62.5%)	3 (37.5%)	8 (100%)
Vitamin E			
After feeding 0 minute	9 (100%)	0	9 (100%)
After feeding 30 minutes	9 (100%)	0	9 (100%)
After feeding 45 minutes	9 (100%)	0	9 (100%)
After feeding 60 minutes	9 (100%)	0	9 (100%)

Only the MTV-mixed EBM had an osmolality above 400 mOsm/kg, the osmolality of the $FeSO_4$ or vitamin E-mixed EBM were not above 400 mOsm/kg (Table 2).

DISCUSSION

The risk of hyperosmolar solutions to the neonatal intestine have been documented in both animal and human studies (Ernst *et al*, 1983; AAP, 1997). In this study, the median osmolality in EBM was 311.5 mOsm/kg. The osmolality of each additive is shown in Table 3.

The purpose of this study was to deter-

Table 3
Osmolality of commonly prescribed medications in the NICU (mOsm/kg).

	Ferrous sulfate	Vitamin E	Multivitamins
Srinivasan *et al* (1985)	3,200	-	2,333
Ernst *et al* (1983)	5,010	3,990	11,180
Mutz *et al* (1985)	5,079	605	6,023
Songklanagarind Hospital	3,821	1,182	8,512

Table 4
The osmolality and volume of EBM, MTV and sterile water.

	Osmolality (mOsm/kg)	Volume of dose (ml)
Human milk	311.5	V_B
Multivitamins	8,512	0.5
Sterile water	0	V_W

mine the osmolality of additives when added to EBM. The osmolality of the intestinal contents is the result of multiple factors such as diet tonicity, permeability of the mucosa to bulk water movement in the osmotic gradient, dilution of food by digestive secretions, rate of gastric emptying and digestion and absorption rate of nutrients.

In light of these concerns, recommendations have been made that enteral feeds for neonates have osmolalities no greater than 400 mOsm/kg (Committee of Nutrition, 1976). In this study, only postprandial osmolality in VLBW infants fed EBM with MTV exceeded 400 mOsm/kg.

Billeaud *et al* (1982) reported the osmolality of gastric contents of human milk and hypotonic formula remained close to 280-290 mOsm/kg throughout the test but the osmolality of gastric contents of elemental formula (hypertonic diet about 600 mOsm/kg) fed to low birth weight infants was 470 mOsm/kg at 45 minutes and 345 mOsm/kg at 180 minutes. In this study, the osmolality of hyperosmolar milk (MTV-mixed EBM) decreased throughout the test after feeding. The postprandial osmolality may have been diluted by digestive secretions.

We have been giving therapeutic additives to infants after they reach full feedings, but this study suggests we should add additives when infants can feed on EBM because it has an osmolality less than 400 mOsm/kg. The osmolality of the mixture of EBM and additives was calculated using the following equation:

$$O_M = \frac{O_A(V_A) + O_B(V_B)}{V_{A+B}}$$

Where: O_M is the osmolality of the additive and EBM mixture; O_A is the osmolality of the additive; O_B is the osmolality of the EBM; V_A is the volume of the additive in milliliters; V_B is the volume of EBM in milliliters.

The following is an example of the use of the equation to determine the volume of diluent necessary to deliver the medications below in order to achieve an acceptable final osmolality. In Table 4, we use V_B and V_W instead of volume of human milk and water, respectively. At Songklanagarind Hos-

pital, the mean volume and osmolality in EBM were 24 ml and 311.5 mOsm/kg, respectively. We start MTV after VLBW infants can feed more than 45 ml of EBM per meal. The MTV needs to be diluted in the EBM.

$$400 = \frac{8{,}512(0.5) + 311.5(V_B)}{(V_B+0.5)}$$

$V_B =$ 45.8 ml

The multivitamin should be diluted with 10 ml sterile water before feeding.

$$400 = \frac{8{,}512(0.5) + 0\ (V_W)}{(0.5+V_W)}$$

$V_W =$ 10 ml

The multivitamin should be diluted with 5 ml sterile water before mixing with at least 25 ml EBM

$$O_M = \frac{8{,}512(0.5) + 0\ (4.5)}{(0.5+4.5)}$$

$O_M =$ 815.2 mOsm / kg

$$400 = \frac{815.2(5) + 311.5(V_B)}{(V_B+5)}$$

$V_B =$ 25.5 ml

The postprandial osmolality of gastric contents in VLBW infants fed with expressed breast milk with MTV may exceed 400 mOsm/kg, however the EBM with $FeSO_4$ or vitamin E, did not exceed 400 mOsm/kg.

The multivitamin oral preparation evaluated in this study, should be given diluted with expressed breast milk or distilled water before feeding; extreme caution should be taken with infants who are at risk for NEC. Drug manufacturers should include information about osmotic strength on the product label and should work towards producing oral formulations of lower osmolarity. The osmolality of therapeutic additives used in the neonatal intensive care should be measured

REFERENCES

American Academy of Pediatrics (AAP) Working group on breast feeding: Breast feeding and the use of human milk.*Pediatrics* 1997; 100: 1035-9.

Billeaud C, Sentarre J, Rigo J. Osmolality of the gastric and duodenal contents in low birth weight infants fed human milk and various formula. *Acta Paediatr Scand* 1982; 71: 799-803.

Book LS, Herbst JJ, Atherton ST, *et al*. Necrotizing enterocolitis in low birth weight infants fed an elemental formula. *J Pediatr* 1975; 87: 602-5.

Committee on Nutrition, American Academy of Pediatrics. Commentary on breast feeding and infant formulas, including proposed standards for formulas. *Pediatrics* 1976; 57: 278-85.

Ernst JA, William JM, Glick MR, *et al*. Osmolality of substance used in the intensive care nursery. *Pediatrics* 1983; 72: 347-52.

Fenton TR, Belik J. Routine handling of milk fed to preterm infants can significantly increase osmolality. *J Pediatr Gastroenterol Nutr* 2002; 35: 298-302.

Gross S, Melhorn DK. Vitamin E-dependent anemia in the premature infant. *J Pediatr* 1974; 85: 753-9.

Jesse N, Neu J. Necrotizing enterocolitis: relationship to innate immunity, clinical features, and strategies for prevention. *NeoReviews* 2006; 7: 143-50.

Le Guennec JC, Pare C, Billon B. Hyperosmolar formulas in necrotizing entercolitis. *Am J Dis Child* 1983; 37: 506.

Lin PW, Stoll BJ. Necrotising enterocolitis. *Lancet* 2006; 368: 1271-83.

Melhorn DK, Gross S. Vitamin E-dependent anemia in the premature infant. I. Effect

of large doses of medicinal iron. *J Pediatr* 1971; 79: 569-80.

Mutz AE, Obladen MW. Hyperosmolar oral medication and necrotizing enterocolitis. *Pediatrics* 1985; 75: 371-2.

Srinivasan L, Bokiniec R, King C, *et al*. Increased osmolality of breast milk with therapeutic additives. *Arch Dis Child Fetal Neonatal Ed* 2004; 89: F514-7.

Tender JA. Preterm infant nutrition. *Pediatr Rev* 2004; 25: 328-9.

Trindade CE. Microelements and vitamins in the nutrition of very low-birthweight preterm infants: A Brazilian perspective. *NeoRev* 2007; 8: 3-13.

Willis DM, Chabot J, Radde IC, *et al*. Unsuspected hyperosmolality of oral solutions contributing to necrotizing enterocolitis in very low-birth-weight infants. *Pediatrics* 1977; 60: 535-8.