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Original article

Safety of superfortification of human milk for preterm

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Background: The standard preparation of fortified preterm human milk (FHM), 1 to 2 packs of human milk fortifier (HMF) in 50 milliliters of expressed breast milk (EBM), may fail to meet the theoretical requirements of ill preterm infants. The superfortified preparation of preterm human milk (SHM), 3 packs in HMF in 50 milliliters of EBM, however, leads to concerns about the osmolality of the preparation, as the higher the osmolality, the greater the risk of necrotizing enterocolitis.

Objective: We evaluated the effect of the superfortification of HMF on the osmolality of EBM

Material and method: Twelve samples of EBM were collected from mothers of a gestational age of less than 37 weeks. We measured the osmolality of the preterm human milk (PHM), FHM, and SHM. The SHM was kept at room temperature to measure the osmolality at 10 minutes after fortification.

***Results**:* The means (SD) osmolality of the PHM and FHM were 293.9 (12.7), 335.2 (18.7) mOsm/kg H_2O, respectively. The means (SD) osmolality of SHM immediately after fortification and 10 minutes after fortification at room temperature are 370.6 (17.4) and 369.8 (17.2) mOsm/kg H_2O respectively.

Conclusions: The measured osmolality of SHM was less than 450 mOsm/kg H_2O. This is still within the international reference range for the composition of PHM, except Ca, P, Zn, Cu, vitamin A, B_1, B_2, niacin, and folic acid. Therefore, SHM should be considered for feeding in only high-risk preterm neonates for short-term periods. Adverse effects need to be observed.

Keywords: Human milk, newborn, osmolality, preterm, safety

List of abbreviations

EBM	Expressed breast milk
FHM	Standard preparation of fortified preterm human milk
HMF	Human milk fortifier NEC
	Necrotizing enterocolitis
PHM	Preterm human milk
PRSL	Potential renal solute load
SHM	Superfortified preparation of preterm human milk
VLBW	Very low birth weight

Human milk is species-specific and superior for infant feeding. Extensive research using improved epidemiological methods and modern laboratory techniques documents diverse and compelling advantages for infants, mothers, families, and society from breastfeeding and the use of human milk for infant feeding [1]. Human milk is beneficial in the management of premature infants. Current evidence is clear that despite the many non-nutritional advantages of human milk feeding, the growth of preterm babies is poorer than in infants who have received a standard preterm formula [2, 3]. Premature infants as compared with term infants are at risk of nutrient deficiencies and/or toxicities for many reasons [4], including significant deficiencies of protein and sodium normally received through lactation [5]. Other nutrients such as calcium and phosphorus are too low to meet the greater needs of the premature infant [6, 7]. The exclusive feeding of unfortified human milk in premature infants has been associated with poorer rates of growth and nutritional deficits, during and beyond the period of hospitalization [6, 8]. When fluid restriction is indicated, human milk fortifier (HMF) is

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considered for the enterally fed preterm infant. Very low birth weight (VLBW) infants fed fortified human milk have a significantly lower incidence of necrotizing enterocolitis (NEC), sepsis, and meningitis compared with formula-fed VLBW infant [9].

Systematic reviews reveal that multi-component fortification [10] and protein supplementation [11] of human milk is associated with short-term improvements in weight gain, linear and head growth. However, a high protein intake ($\geq$3 g/kg/day but <4 g/kg/day) accelerates weight gain [12]. Supplementing very preterm infants with selenium is associated with benefit in terms of a reduction in one or more episodes of sepsis [13].

Standard preparation of fortified preterm human milk (FHM), breast milk 25 mL to HMF 1 to 2 packs, is the preferred feeding although actual intakes of protein by preterm infants fed fortified human milk are substantially lower than assumed intakes [14]. Higher caloric densities are possible and are sometimes used for preterm infants under close medical supervision, usually because intake of fluid is restricted. The superfortification, composed of three packs of HMF added to 50 mL of preterm human milk, may eliminate this problem. With milk manipulations, milk osmolarity should be < 400 mOsm/L of milk or an osmolality of <450 mOsm/kg H_2O [15]. Human milk and HMF interacted to induce a rapid increase in osmolality after 10 minutes [16, 17]. The objective of this study was to compare the osmolality between standard and superfortified preparations of HMF mixed with preterm human milk (PHM) to understand the potential uses and safety of superfortification in providing adequate nutrition to preterm infants.

Material and method

Study population

A prospective, controlled trial was performed in laboratory conditions. Expressed breast milk (EBM) was collected from mothers of infants of less than 37 weeks gestation with birth weights of 600 to 2,499 grams, admitted to the neonatal intensive care unit at Songklanagarind Hospital, the major tertiary care institution in southern Thailand. The study was approved by the Ethics Committee Board of Prince of Songkla University, and written informed consent was obtained from the mothers before the study began.

Study design

A single sample of manually-EBM from each mother of a preterm infant who was enrolled between May and October 2009 had given their consent that the manually-EBM was sterilely collected. The EBM was kept frozen separately at -20°C and labeled with the name of the mother and date. The frozen milk was thawed in warm water before use. Osmolality was determined in triplicate using a Gonotech Osmometer® (Intertrade Ltd. USA) based on freezing point depression. We measured the osmolality of the EBM, the EBM mixed with Enfamil® HMF (Mead Johnson & Company, Evansville, IN 47721, U.S.A.) at a ratio of 50 mL breast milk to two packs HMF (FHM = 24 kcal/fl.oz) and 50 mL breast milk to three packs HMF (SHM = 26 kcal/fl.oz). The SHM was kept at room temperature to measure the osmolality at 10 minutes after fortification.

Statistical analysis

The sample size was calculated by the two-sample mean equation

$$n = 2\sigma^2[Z_{\alpha/2}+Z_{\beta}]^2/(\mu_1-\mu_2)^2$$

where $Z_{\alpha/2}$ was a standard score at a 0.05, the power was 80%, and σ^2 was a variance of a previous study, calculated from a pilot study, where σ = 10. The mean of the osmolality in EBM with HMF with a ratio of breast milk 50 mL to HMF two packs was 325 (μ_2). The theoretical value of osmolality of fortified human milk by three packs in EBM was 342 (μ_1). From the equation above, the total minimum sample size required was found to be six per group. We collected in total 12 samples of EBM.

The R version 2.10.1 was used to compare the osmolality of SHM and FHM. The two groups compared the osmolality using a Student's t-test for continuous outcomes. Unless otherwise indicated, for descriptive statistics, means and SDs are reported for normally distributed data, medians and ranges for non-normally distributed data. Ninety-five percent confidence intervals (CIs) are also reported. All tests were 2-sided with a statistical significance of p <0.05.

Results

Twelve samples of EBM from mothers of preterm infants were obtained as shown in **Table 1**. The mean age of the mothers was 31.9 years. The mean gestational age of the infants was 31.5 weeks (range

29-34 weeks). The mean (SD) birth weight was 1,587 g (467.8). The median postpartum age of the mothers was 15 days (range 7-30 days). The means (SD) osmolality of the PHM and FHM were 293.9 (12.7), 335.2 (18.7) mOsm/kg H_2O, respectively. By adding the values obtained before mixing, the authors were able to calculate the theoretical values of the osmolality of SHM to be 335.2 + 17 = 352.2 mOsm/kg H_2O. The means (SD) osmolality of SHM immediately after fortification and at 10 minutes after fortification at room temperature are 370.6 (17.4) and 369.8 (17.2) mOsm/kg H_2O, respectively (**Table 2**).

The osmolality of SHM was statistically significantly higher than the osmolality of FHM alone (p <0.001) and the measured osmolality of SHM was significantly higher than the theoretical osmolality of SHM (p <0.001). Compared to the osmolality of SHM immediately after fortification and at 10 minutes after fortification, no statistical significance was found (p = 0.78). At no time was the osmolality of SHM above 450 mOsm/kg H_2O (p <0.001).

Discussion

The use of HMF for human milk-fed premature infants has increased in neonatal centers. Previous studies of human milk fortification in preterm babies have demonstrated beneficial increases in weight gain, calcium, and phosphorus retention. Actual protein intakes were significantly and consistently lower than assumed protein intakes. The range of differences in mean intakes was 0.5 to 0.8 g/kg/day [14]. Extra-uterine growth restriction is common in VLBW infants nevertheless they fed fortified human milk. A significant positive association between energy intake and reduced risk of growth restriction at discharge in VLBW infant, especially extremely low birth weight infants (ELBW; birth weight less than 1,000 g). Therefore, we add three packs of HMF in 50 mL EBM (SHM), to increase protein and other nutrients. The protein in one pack of HMF is 0.25-0.28 g and is increased in PHM to 0.4-0.5 g/100 kcal [18]. With milk manipulations, milk osmolality increased after mixing additives more than the theoretical calculation stated [19]. The amylase activity of human milk is an alpha amylase that hydrolyses starch and dextrins [20]. Therefore, the fortification of human milk may lead to oligosaccharide production, depending on the carbohydrate composition of the HMF. Thus, the rise of osmolality observed in human milk supplemented with HMF can be explained by the fact that polysaccharides, present in the HMF, are broken down into their constituent mono and disaccharides [17]. The administration of hyperosmolar

Table 1. Demographic Data

Demographic Data	Mean±SD
Maternal age (years)	31.9±6.1
Gestational age (weeks)	31.5±2.3
Birth weight (grams)	1,587.0±467.8
Age at study (days), median (range)	15 (7-30)
Weight at study (days)	1,752.5±453.6
Gender, n (%)	
Male	8 (66.6%)
Female	4 (33.6%)

Table 2. The measured osmolality (mOsm/kg H_2O) of human milk before and after supplementation with HMF at various concentration (n=12)

	Mean (SD)	Median (range)
PHM	293.9 (12.7)	294.5 (270-320)
FHM	335.2 (18.7)	332.0 (304-359)
SHM	370.6 (17.4)	367.5 (342-393)
SHM 10 min	369.8 (17.2)	370.0 (348-399)

feeds is thought to be associated with NEC. The American Academy of Pediatrics (AAP) led to recommendations that enteral feeds for neonates should not have osmolality above 450 mOsm/kg [15]. The study has added to the present knowledge by showing that after fortification, the SHM induces a rapid and clinically significant increase in osmolality. After 10 minutes at room temperature, the osmolality of the SHM does not change significantly.

Regarding concerns about safety of SHM, we considered macronutrients and micronutrients in PHM, FHM, and maximal nutritional intakes of preterm milk from FDA and ESPGAHAN, We also calculated the theoretically nutritional values of SHM and measured the osmolality of SHM as shown in **Table 3** [18, 21, 22]. All were within the international reference ranges for the composition of preterm human milk, except for calcium (Ca), phosphorus (P), zinc (Zn), copper (Cu), vitamin A, vitamin B_1, vitamin B_2, niacin, and folic acid. Vitamin B_1 and niacin which were in the excess upper limits both FDA [22] and ESPGAHAN [21] recommendations, but adverse effects were not reported in infants [22]. This is because excess amounts of water-soluble vitamins are easily excreted by the body in the urine.

Table 3. Macronutrients and micronutrients in preterm human milk (PHM), standard fortified preterm human milk (FHM), theoretical values of superfortified preterm human milk (SHM), measured osmolality of SHM, and maximal nutritional intakes of preterm milk from FDA and ESPGAHAN

Nutrient per 100 kcal	PHM [18]	FHM [18]	FDA (2002) [22]	ESPGAHAN (2010) [21]	SHM
Protein, g	2.1	3.1	3.6	4.1	3.5
Fat, g	5.8	6.1	5.7	6	6.2
CHO, g	9.9	8.75	12.5	12	8.3
Ca, mg	37.0	143.3	185	130	184.2†
P, mg	19.1	78.4	109	80	101.1†
Mg, mg	4.6	5.1	17	13.6	5.3
Iron, mg	0.2	2.0	3	2.7	2.6
Zinc, mg	0.5	1.3	1.5	1.8	1.6†
Mn, mcg	0.9	13.2	25	25	18.0
Cu, mcg	96.0	135	250	120	149.8†
Na, mg	37.0	50.9	63	105	56.1
K, mg	85.0	107.1	160	120	115.5
Cl, mg	82.0	84.6	160	161	85.4
Vitamin A, IU	581.1	1,672.1	1,254	2,464.2	2,090.3†
Vitamin D, IU	3.0	190	270	1,000 IU/day	262.0
Vitamin E, IU	1.6	7.1	12	10	9.2
Vitamin K, mcg	0.3	5.8	25	25	7.8
Vitamin B_1, mcg	31.0	213.4	250	275	283.5‡
Vitamin B_2, mcg	72.0	335	620	365	436.0†
Vitamin B_6, mcg	22.1	162.1	250	273	216.0
Vitamin B_{12}, mcg	0.1	0.3	0.7	0.7	0.4
Niacin, mcg	2,24.0	3,936.8	5,000	5,000	5,364.6‡
Folic acid, mcg	5.0	35.4	45	90	47.1†
Pantothenic acid, mcg	269.0	1,136.9	1,900	1,900	1,470.1
Biotin, mcg	0.6	4.1	37	15	5.5
Vitamin C, mg	15.9	28.6	37	42	33.5
PRSL, mOsm	18.7	27.6	32 (FHM), 27 (SHM)		31.1†
Osmolality, $mOsmH_2O$	286	325			371§

FHM= the standard preparation of fortified PHM; PHM= preterm human milk; PRSL=the potential renal solute load; SHM= the superfortified preparation of PHM immediately after fortification

† above upper limit either FDA and ESPGAHAN recommendations

‡ above upper limit both FDA and ESPGAHAN recommendations

§ measured osmolality of SHM

Table 4. Adverse effects of overdose of minerals and vitamins in neonate

Nutrient	Adverse effects of overdose
Calcium	hypercalciuria and nephrocalcinosis [26]
Phosphorus	not been reported in infants [22]
Zinc	no noxious effects [22]
Copper	severe liver damage, cirrhosis [27]
Vitamin A	Anorexia, bulging fontanelles, drowsiness, increased intracranial pressure, irritability, vomiting [28]
Vitamin B_1, Vitamin B_2, Niacin	not been reported in infants [22]
Folic acid	Enlargement of the spleen and/or liver [29]

Under-consumption of food and nutrients among pregnant women in a developing country is due to poor education, poverty, and food availability. A study shows the mean intake of calcium, phosphorus, iron, and vitamin B_1 in the third trimester of pregnancy were lower than 50% of the recommended levels [23]. Osteopenia of prematurity is a common problem encountered in premature infants. Calcium and phosphorus are maximally acquired by the fetus during the third trimester of pregnancy, so premature infants are born with significantly lower mineral stores compared to term infants. The mean vitamin A concentration in Thai woman's term human milk, international term and preterm human milk are 1,078, 2,252, and 3,899 IU/L, respectively [18, 24].

Water-soluble waste products that require excretion by the kidneys are collectively referred to as the potential renal solute load (PRSL). Preterm infants consuming high PRSL formula may be predisposed to hypertonic dehydration [25]. The following equation may be used to calculate an approximation of the PRSL that will result from an infant formula [18].

$$PRSL = [protein(g) \times 5.714] + [Na(mOsm) + K(mOsm) + Cl(mOsm) + P(mOsm)]$$

Although SHM was used in the short term, monitor adverse effects should be carried out (**Table 4**) in preterm infants including frequent physical examinations and laboratory measurements. The SHM has an important alteration for the preterm feeding. At a more fundamental level, examination of the consequences of multinutrient fortification on the biological properties of breast milk itself results in evolutionary changes to add more optimal fortification strategies that minimize risks and optimize benefits.

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