Early versus Delayed Human Milk Fortification in Very Low Birth Weight Infants–A Randomized Controlled Trial

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Objective To compare the effect of initiating human milk fortification at 2 different feeding volumes on feeding intolerance and the time to reach full feeding volume.

Study design Very low birth weight infants (n = 100) were prospectively randomized to early fortification (EF) (beginning at a feeding volume of 20 mL/kg/d) or delayed fortification (at a feeding volume of 100 mL/kg/d). We employed a standardized feeding protocol and parenteral nutrition guidelines for the nutritional management of all study infants.

Results The median days to reach full feeding volumes were equivalent in the 2 groups (20 vs 20, $P = .45$). No significant difference was observed in the total number of episodes of feeding intolerance (58 vs 57). Two cases of necrotizing enterocolitis (Bell stage ≥2) and deaths occurred in each group. Median daily protein intake (g/kg/d) was higher in EF group in week 1 (3.3 [3.2, 3.5] vs 3.1 [2.9, 3.3], $P < .001$), week 2 (3.6 [3.5, 3.8] vs 3.2 [2.9, 3.4], $P < .001$), and week 3 (3.7 [3.4, 3.9] vs 3.5 [2.8, 3.8], $P = .006$). Cumulative protein intake (g/kg) in the first 4 weeks of life was higher in EF group (98.6 [93.8, 104] vs 89.6 [84.2, 96.4], $P < .001$).

Conclusions Very early human milk fortification may improve early protein intake in very low birth weight infants without increasing frequencies of adverse events. *(J Pediatr 2016;174:126-31)*.

Trial registration ClinicalTrials.gov: NCT01988792.

Achieving adequate extra-uterine growth of very low birth weight (VLBW) infants remains a significant challenge.[1] Attention to nutritional management is crucial in premature infants during this critical period of growth. The primary goal of nutrition in VLBW infants is to simulate in utero growth; however, extra-uterine growth restriction is a significant problem.[2] The policy statement of the American Academy of Pediatrics regarding breastfeeding supports the use of human milk for all term and preterm infants, with pasteurized donor breast milk (DBM) recommended for VLBW infants if mother's milk is unavailable.[3] The use of human milk in premature infants provides many nutritional, immunologic, and developmental benefits, including long-term neurodevelopmental improvements.[4] However, the composition of human milk varies throughout the course of lactation,[5] and the amounts of protein, calcium, and phosphorus necessary to achieve adequate growth of preterm infants are insufficient.[6,7] Hence, the long-term use of unsupplemented human milk may lead to metabolic complications such as hypoproteinemia[8] and osteopenia.[9] Adding human milk fortifiers (HMFs) to human milk is necessary to provide additional calories, protein, minerals, and vitamins to premature infants.[3] The practice of timing of the fortification of human milk varies because of concerns about immature gut mucosa and motility in VLBW infants. Clinicians are sometimes concerned that addition of fortifiers may induce feeding intolerance and delay achieving full volume enteral feeds and optimal nutrition. Early fortification (EF) provides several benefits to infants such as provision of adequate calories, protein, and other nutrients compared with delayed fortification (DF).

In 2004, Berseth et al[10] showed that human milk fortification was safely tolerated when enteral intake reaches at least 100 mL/kg/d feeding volume. Sullivan et al[11] showed that fortification with human milk-based fortifier was safe if initiated at 40 mL/kg/d feeding volume. In a retrospective study, Tillman et al[12] showed that fortification of human milk from the first feeding was safe and did not cause feeding intolerance. To our knowledge, a prospective randomized study has not been published that compares the tolerance of EF vs DF with bovine-based HMF. We hypothesized that early human milk fortification will be as well tolerated as delayed human milk fortification and, hence, will not prolong the days to reach full feeding volume.

BW	Birth weight
DBM	Donor breast milk
DF	Delayed fortification
DOL	Day of life
EF	Early fortification
HMF	Human milk fortifier
NEC	Necrotizing enterocolitis
VLBW	Very low birth weight

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Methods

This prospective, randomized, controlled, clinical study was conducted in tertiary neonatal intensive care units at Regional One Health and Le Bonheur Children's Hospital, Memphis, Tennessee (ClinicalTrials.gov: NCT01988792). Infants with birth weight (BW) <1500 g were considered eligible for the study. Infants were excluded if: (1) they died or were expected to die within 72 hours; (2) they were diagnosed with major congenital or chromosomal abnormalities; or (3) mother could not provide her own milk and refused the use of DBM.

The University of Tennessee Health Science Center Institutional Review Board approved the study. Informed written consent was obtained from parents prior to their enrollment. Infants were randomized to either EF (fortification beginning at 20 mL/kg/d of human milk feeds) or DF (fortification beginning at 100 mL/kg/d of human milk feeds). Fortification was done with a commercially available acidified liquid HMF (Enfamil; Mead Johnson, LLC, Evansville, Indiana). Five mL of liquid HMF was added to 25 mL of human milk to increase caloric density to 24 Kcal/oz.

Parenteral nutrition was initiated on the day of life (DOL) 1. Enteral feedings were initiated at the attending physician's discretion, followed by a standardized feeding protocol that guided the method of feeding and increments of advancement. Infants with less than 800 g BW received trophic feedings (10 mL/kg/d) for 3 days and advanced by 10 mL/kg/d every other day. Infants between 800 and 1000 g BW received trophic feedings for 2 days and advanced by 10 mL/kg/d every day. Infants between 1001 and 1250 g BW received trophic feedings for 2 days and advanced by 10-20 mL/kg/d every day. Infants between 1251 and 1499 g BW received trophic feedings for 1-2 days and advanced by 20 mL/kg/d every day. Parenteral nutrition was decreased as enteral feeding volumes were advanced. Nursing staff fortified human milk at the bedside. Fortified human milk was delivered continuously (3 hours on and 1 hour off) through a nasogastric tube. DBM was used if mother was unable to provide her own breast milk. DBM was weaned to preterm formula when an infant reached 1500 g weight or 34 weeks postmenstrual age.

The primary outcome was the number of days to reach full feeding volume (greater than 140 mL/kg/d enteral volume[13]). Secondary outcomes included frequency of feeding intolerance, necrotizing enterocolitis (NEC), weight velocity at 4 weeks after birth and at 36 weeks postmenstrual age, parenteral nutrition days, and length of stay. We also collected data on daily weight, protein and caloric intake for the first 4 weeks of life, metabolic acidosis (base deficit ≥10 mEq/L on blood gas measurement), late-onset sepsis, ventilator days, postnatal steroid treatment, chronic lung disease, patent ductus arteriosus, severe intraventricular hemorrhage (grade III and IV), periventricular leukomalacia, and retinopathy of prematurity.

The day when the infant regained BW for the first time was considered as date of regained BW. Gestational age was determined by the best obstetrical estimate using last menstrual period and/or dating ultrasound. Feeding intolerance was defined as enteral feedings being held for at least 24 hours secondary to emesis/aspirates or abdominal distension. We defined NEC as stage II or greater using modified Bell criteria.[14] Late-onset sepsis was defined as clinical signs of sepsis associated with positive blood culture after 3 days of age. The duration of total parenteral nutrition days was also recorded. The duration of endotracheal ventilation was defined as total number days infant remained on a ventilator with endotracheal tube. Bronchopulmonary dysplasia was defined as an oxygen requirement at 36 weeks postmenstrual age. A pediatric radiologist evaluated head ultrasounds to identify periventricular leukomalacia and intraventricular hemorrhage. Severe intraventricular hemorrhage on head ultrasound was defined as grade III or grade IV per Papile classification.[15] A pediatric ophthalmologist evaluated eyes to diagnose retinopathy of prematurity.

Daily weight was obtained unclothed and without diaper at a standard time each day using an electronically calibrated scale. Recumbent length and head circumference were measured weekly by nursing staff per unit practice. Weight gain velocity for first 4 weeks (g/kg/d) was calculated using the exponential method[16] and at 36 weeks was calculated by 2-point BW model.[16] The 2013 Fenton growth charts were used to obtain z-scores.[17] We also calculated daily caloric and protein intake for the first 4-week period and also the cumulative protein and caloric intake for first 4 weeks. Protein and caloric calculations were made by assuming human milk's contents.[18] We collected data regarding serum indices of protein, albumin, blood urea nitrogen, alkaline phosphatase, phosphorous, and calcium that were measured weekly as a standard practice in our unit.

A total of 96 infants (48 per group) were required to detect a difference of 7 days to reach full enteral feeding volume to obtain 80% power with a type I error rate of 5% using a 2-sided t test. In our unit, mean ± SD days to reach full enteral feeding volume in VLBW infants was 24 ± 12 days.

Subjects were randomly assigned 1:1 to EF or DF using a blocked stratified randomization approach with block size 4 and stratification by BW (<1000 g, 1000-1499 g). Randomization was performed by computerized software after verification of eligibility and signed consent status. The research coordinator and principal investigators enrolled and assigned the patient after randomization. The proper handling of mother's own milk and appropriate fortification prevented masking of the infants' caregivers and research investigators.

Data were analyzed using an intent-to-treat approach. Data are presented as mean ± SD and compared using a 2-sided t test when normally distributed. Otherwise, median (IQR) was compared using the Wilcoxon rank sum test. For categorical data, Fisher exact test was conducted. To adjust for the study design, a linear mixed model,[19] which is a linear model that contains both fixed and random effects, was performed to test differences in the primary outcome, days to reach full feeding volume. The linear mixed model included treatment and BW as fixed effects and the blocking factor as the random