

Growth in Human Milk-Fed Very Low Birth Weight Infants Receiving a New Human Milk Fortifier

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Key Words

Human milk · Human milk fortifiers · Infant nutrition · Very low birth weight infants · Weight gain · Copper

Abstract

Background/Aims: Human milk fortification has been advocated to enhance premature infants' growth. We, therefore, undertook this study of a new human milk fortifier containing more protein than a reference one. **Methods:** Open, randomized, controlled, multiclinic trial, with weekly growth parameters and safety evaluations in premature infants <1,500 g. **Results:** The 2 groups did not differ in demographic and baseline characteristics. The adjusted daily milk intake was significantly higher in the infants fed reference human milk fortifier ($n = 29$; 154.2 ± 2.1 vs. 144.4 ± 2.5 ml/kg/day, mean \pm SE; $p < 0.05$). Both human milk fortifiers produced increases

over baseline in weight, length, and head circumference, with greater gains observed in the new human milk fortifier-fed infants for the former two parameters (weight gain 26.8 ± 1.3 and 20.4 ± 1.2 g/day, $p < 0.05$; head circumference 1.0 ± 0.1 and 0.8 ± 0.1 cm/week; length 0.9 ± 0.1 and 0.8 ± 0.1 cm/week, respectively). Serum chemistries were normal and acceptable for age. Study events were typical for premature infants and similar in both groups. **Conclusions:** This new human milk fortifier had comparable safety to the reference human milk fortifier and promoted faster weight gain and head circumference growth.

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Introduction

The generally accepted goal of appropriate nutritional management of the very low birth weight (VLBW) infant is the provision of nutrients in sufficient quantity and quality to support a growth rate and tissue composition

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similar to that of the third trimester of intrauterine life [1]. Human milk is generally considered to be the ideal feeding for term infants [2, 3]. It is easily digestible, contains nutrients that are readily bioavailable and appropriate for the human infant, and provides a combination of immunological factors that protect against infection [2, 3]. The nutrient composition of human milk changes during lactation, with preterm human milk containing higher levels of protein, sodium, potassium and chloride than term milk. The levels of these nutrients in preterm milk decline over the first 4 weeks of lactation [4–6].

To enhance the nutritional content of human milk to adequately support growth and bone mineralization in VLBW infants, fortification of human milk with various nutrients has been advocated [7, 8]. At present, the use of fortifiers that provide additional protein, carbohydrate and/or fat, minerals, particularly calcium and phosphorus, and vitamins to human milk has become standard medical practice in many neonatal intensive care units. A number of clinical trials have demonstrated that these fortifiers, including fortifiers based on human milk protein and commercial fortifiers derived from bovine milk protein, when combined with human milk, adequately support growth, bone mineralization, and nutrient absorption and retention in VLBW infants [9–17].

Efforts are currently underway to develop new human milk fortifiers that provide more favorable nutritional support for growth and bone mineralization in the VLBW infant. A new powdered human milk fortifier (HMF) was developed that we hypothesized would allow better growth than a currently available reference HMF. This clinical trial was designed to evaluate the efficacy and safety of this new HMF, in comparison with a reference fortifier, in the growth and nutritional status of VLBW infants.

Methods

Ethical Considerations

The protocol was approved by the Institutional Review Board of each study site. Written informed consent was obtained from the parent(s) or guardian(s) of each infant prior to enrollment in the trial.

Study Design

The study design was a single-blind, prospective, randomized, controlled one, which was conducted in the neonatal intensive care unit at 8 US hospitals. The design was not double-blind because the reference HMF required 4 sachets to be added to 100 ml of human milk to provide the same caloric density as 2 sachets of the new HMF. To have blinded the latter would have required a placebo in 2 sachets, which would have changed the caloric density, and thereby

invalidated all comparisons of growth between the study groups, besides introducing an additional risk and, therefore, invalidating safety comparisons between the study groups if lactose, starch, or another substance was chosen as the placebo. Repackaging the reference HMF so as to have it in only 2 sachets was logistically impossible because of the loss incurred when sachets were opened, emptied, and then their contents repacked into new sachets. The investigators were blinded as to which HMF the infants were receiving. All infants were identified by only a randomization number.

Subjects

VLBW infants were enrolled in the study if they were medically stable; had a gestational age between 25 and 32 weeks, a birth weight between 600 and 1,500 g, were appropriate for gestational age, had an enteral intake of 150 ml/kg/day of human milk, and were being fed human milk exclusively. Infants were excluded for the following reasons: receiving parenteral nutrition or infant formulas; significant acute or chronic illnesses; systemic infections; major congenital malformations; receiving corticosteroids, diuretics, or mother's milk that was < 14 days postpartum.

Composition of Human Milk Fortifiers

The nutrient composition of the two HMFs evaluated in the study is shown in table 1. All the protein in the new HMF is derived from bovine whey in an amount that provides 1 g of protein/100 ml of supplemented milk (approximately 30% greater than that supplied by the reference HMF). In comparison, the reference HMF contains 60% whey protein and 40% casein. The carbohydrate in the new HMF is 85% glucose polymers and 15% lactose, while in the reference HMF it is 75% glucose polymers and 25% lactose. Other differences between the two fortifiers include levels of calcium, phosphorus, sodium, and copper.

Feeding Schedule and Procedures

Infants were randomized to 1 of 2 HMF groups, the test HMF or the reference standard. The test HMF was the Wyeth Nutritionals International's new HMF and the reference HMF was Mead Johnson's Enfamil® Human Milk Fortifier (Mead Johnson Nutritionals; Evansville, Ind.). Within each feeding group, infants were prospectively stratified into 3 subgroups according to birth weight: 600–900, 901–1,250, and 1,251–1,500 g.

An infant started the study when HMF fortification was introduced, and completed the study when fully weaned from the assigned fortifier and receiving only unsupplemented human milk. The efficacy evaluations were made during the period in which the infant had an enteral intake of ≥ 145 ml/kg/day of mother's milk with full-strength HMF supplementation. The duration of participation varied but the efficacy evaluation period was for a minimum of 2 weeks on full-strength HMF supplementation.

Full-strength HMF supplementation consisted of 4 g (2 sachets) of Wyeth Nutritionals International HMF powder in 100 ml of human milk, or 3.84 g (4 sachets) of Mead Johnson Enfamil® Human Milk Fortifier powder in 100 ml of human milk. Both provided approximately 0.81 kcal/ml.

The protein:energy ratios, assuming preterm human milk varied in energy from 67 to 72 kcal and contained 1.76 g protein/100 ml, ranged from 3.38 to 3.72 and 2.97 to 3.33 g/100 kcal for the new and reference HMFs, respectively [18].

Individual human milk samples from the mothers whose infants participated in this study were not analyzed due to the number of

Table 1. Nutrient composition of study HMFs

Nutrient	New HMF ¹	Reference HMF ¹
Calories	13	14
Protein, g	1	0.7
Fat, g	0.05	<0.1
Carbohydrate, g	2	2.7
Lactose, g	0.3	0.7
<i>Vitamins</i>		
A, IU	700	950
D, IU	260	210
E, IU	3.5	4.6
K, µg	10	4.4
B ₁ , µg	180	151
B ₂ , µg	200	210
B ₆ , µg	200	114
B ₁₂ , µg	0.25	0.18
Niacin, µg	3,000	3,000
Folic acid, µg	25	25
Pantothenic acid, µg	800	730
Biotin, µg	1.2	2.7
C, mg	25	11.6
<i>Minerals</i>		
Calcium, mg	80	90
Phosphorus, mg	40	45
Calcium:phosphorus	2:1	2:1
Copper, µg	—	62
Sodium, mg	15	7
Potassium, mg	20	15.6
Chloride, mg	14	17.7
Zinc, mg	0.2	0.71
Manganese, µg	4	4.7
Magnesium, mg	2	1

¹ 4 g new HMF powder or 3.84 g reference HMF powder (amount for 100 ml human milk; 24 kcal/oz).

infants enrolled in the trial ($n = 90$), the number of investigators involved in this multiclinic study ($n = 8$), and the logistics involved in analyzing multiple milk samples from each mother during the 2-week minimum feeding period for each infant enrolled in this trial.

No vitamins except vitamin E, no mineral supplements except iron, and no corticosteroids or diuretics were permitted during the course of the study.

Clinical Evaluations

Baseline anthropometric measurements consisting of weight, length, and occipitofrontal head circumference (OFC) were obtained immediately prior to the start of HMF fortification (baseline); thereafter, weight (g) was measured daily, and length (cm) and OFC (cm) were measured weekly during the study. Anthropometric devices were standardized across all study sites. An O'Leary Length BoardTM for premature infants (Ellard Instrumentation Ltd., Seattle, Wash.) was used for length measurements, a flexible metal tape (Lufkin) for

OFC measurements, and an electronic digital scale for weight measurements.

Blood samples were obtained by venipuncture immediately prior to baseline and following the last feeding of human milk plus full-strength fortifier supplementation (the feeding prior to the initiation of weaning from the HMF). All blood analyses were performed by two central laboratories. Corning Nichols Institute, San Juan Capistrano, Calif., performed vitamin D determinations; all other clinical chemistries were done in the laboratory of Dr. Stanley Zlotkin, Hospital for Sick Children, Toronto, Ont., Canada. Urea, calcium, phosphate, alkaline phosphatase and sodium were assayed using a Kodak Ektachem 700 Analyser (Kodak Clinical Products Division, Rochester, N.Y.). Serum 25-hydroxy vitamin D and 1,25-dihydroxy vitamin D levels were determined using a radioreceptor binding assay [19]. Serum copper was analyzed using graphite furnace atomic absorption spectrophotometry [20]. Serum ceruloplasmin was measured using a human ceruloplasmin turbidimetric kit (The Binding Site, Birmingham, UK) [21] with the test procedure modified to allow the use of small volumes of serum.

In a multiclinic trial such as this, which was designed to assess safety according to accepted regulatory guidelines throughout the world, safety was assessed by comparing the differences between study groups in the overall incidence of study events, the possible relationship of these study events to the individual study groups, individual study events incidence, discontinuations, and the reason for these discontinuations. The definition of a study event, as used in this multiclinic trial, is identical to that used by the Federal Drug Administration for an adverse event; therefore, in this trial, a study event was any untoward medical occurrence in an infant to whom either of these HMFs was given, regardless of whether the event was believed to have a causal relationship to the HMF feeding [18]. These events would, therefore, encompass any sign, symptom, clinical laboratory value, and/or diagnosis considered by an investigator to be abnormal. In order to get the worst possible safety scenario, various study events, such as apnea, bradycardia, or vomiting were not defined but were left to the clinical assessment of the individual investigator. If an investigator assessed an infant as experiencing bradycardia, this was accepted as a study event and not further assessed; therefore, the safety database is a direct reflection of the safety assessments of the two HMFs by the investigators. The investigator also indicated if he or she believed the study was related to the HMF being fed.

The infants were monitored for symptoms, physical examination signs, clinical morbidities, and/or changes in clinical chemistries throughout the entire study period.

Statistical Methods

The sample size of 60 evaluable infants was designed to provide sufficient power of 0.99 for the test of the expected mean difference between feeding regimens for weight gain of 4.2 g/day at the 0.05 level of significance [22]. Data were analyzed in two populations, the infants who completed the study (evaluable population) and all infants who were randomized (intent-to-treat population). The primary population for the efficacy analyses were the infants who completed the efficacy period. The intent-to-treat population was then analyzed to confirm the validity of the evaluable population. All reported values are means and standard error of this value.

Anthropometric data were analyzed using a repeated measures analysis of variance. The laboratory data were analyzed using covariance analysis adjusting the final observations for the variability of

Table 2. Growth characteristics of infants who completed the study (mean \pm SE)

Variable	New HMF	Reference HMF	p value
n	35	29	
Gestational age, weeks	29.3 \pm 0.3	29.0 \pm 0.3	
Postnatal age, days			
Enrollment	15.3 \pm 1.5	14.1 \pm 1.3	
Baseline	21.2 \pm 1.5	17.7 \pm 1.3	
Final	42.0 \pm 1.9	39.8 \pm 2.5	
Duration	20.8 \pm 1.2	22.2 \pm 2.0	
Weight, g			
Birth	1,257.5 \pm 30.3	1,193.5 \pm 36.6	
Baseline	1,419.5 \pm 38.6	1,255.2 \pm 36.2	0.0033
Final	1,993.8 \pm 60.5	1,711.3 \pm 65.5	0.0153
Study gain			
g/day	26.8 \pm 1.3	20.4 \pm 1.2	0.0007
g/kg/day	19.7 \pm 0.98	16.8 \pm 0.96	0.0436
Length, cm			
Birth	39.0 \pm 0.4	38.0 \pm 0.7	
Baseline	40.6 \pm 0.3	39.4 \pm 0.4	
Final	42.9 \pm 0.4	41.6 \pm 0.5	
Study gain, cm/week	0.9 \pm 0.1	0.8 \pm 0.1	
OFC, cm			
Birth	27.1 \pm 0.3	27.1 \pm 0.3	
Baseline	28.5 \pm 0.3	27.8 \pm 0.3	
Final	31.0 \pm 0.3	30.0 \pm 0.3	0.0043
Gain, cm/week	1.0 \pm 0.1	0.8 \pm 0.1	0.0354

the initial observations, including the differences in weights between the 2 groups at the start of the study. The feeding regimen was the main effect to be tested for a significant mean difference for every variable of interest.

Results

Baseline Demography

Of the 90 infants enrolled, 47 were randomized to the new HMF group and 43 to the reference HMF group. Sixty-four infants completed the study (35 in the new HMF group, 29 in the reference HMF group). The enrollment was terminated when 60 infants completed the 2-week minimum of feeding human milk plus the new or reference HMF. The 4 infants who were in the study when the 60th infant completed this feeding interval were allowed to complete the study, therefore giving 64 infants who completed the study.

Between the 2 HMF groups, neither the 90 enrolled infants nor the 64 infants who completed the study differed in demographic or baseline characteristics (table 2) nor in the medical interventions prior to (table 3) or after

(table 4) study enrollment, except that the mean baseline weight was greater in the group receiving the new HMF.

Forty-seven (100%) of the infants receiving the new HMF received at least 1 medication while in the study, while 42 (92.7%) of those infants receiving the reference HMF did. Forty-nine different medications were administered to at least 1 infant in 1 of these groups, but the incidence of usage of the individual medications was not different between the 2 feeding groups.

Gestational ages (GAs) were not statistically different between the groups, although there were 5 infants who received the new HMF who had a GA of 32 weeks, while none who received the reference HMF was of this GA (table 5).

Milk Intake

Infants given the new HMF had a daily intake and an adjusted daily intake volume that was significantly higher than those fed the reference HMF (table 6). Based on the adjusted daily intake and with full supplementation, the calculated protein intake was approximately 4.33 g/kg/day in the new-HMF-fed infants, and 4.16 g/kg/day in

Table 3. Medical interventions prior to study enrollment

Interventions	New HMF		Reference HMF	
	n	HRS	n	HRS
Phototherapy	44	6.30 ± 4.95	39	5.75 ± 3.04
CPAP	28	10.53 ± 10.84	30	11.03 ± 12.69
O ₂ usage	15	10.41 ± 10.09	17	8.05 ± 8.01
Mechanical ventilation	16	8.26 ± 6.60	14	3.33 ± 5.35
Catheterization				
UA	10		12	
UV	4		5	
ET usage	9	3.08 ± 2.02	6	1.14 ± 0.90
Nasal cannula	3	10.20 ± 6.61	5	6.00 ± 5.54

CPAP = Constant positive airway pressure; UA = umbilical artery; UV = umbilical vein; ET = endotracheal tube.

Table 4. Medical interventions instituted after study entry (mean ± SD)

Interventions	New HMF		Reference HMV	
	n	HRS	n	HRS
O ₂ usage	13	11.10 ± 8.33	10	5.7 ± 5.70
CPAP	11	5.60 ± 6.26	5	8.75 ± 5.91
Nasal cannula	5	8.50 ± 4.51	1	1.0 ± 0
Phototherapy	4	6.00 ± 2.65	3	1.5 ± 0.71
Mechanical ventilation	1	2.00 ± 0	0	0
ET usage	1	2.00	0	0

CPAP = Constant positive airway pressure; ET = endotracheal tube.

Table 5. Gestational ages of infants who completed the study

Week	New HMF (n = 35)*	Reference HMF (n = 29)
26	1 (2.86%)	1 (3.45%)
27	4 (11.43%)	5 (17.24%)
28	7 (20.00%)	3 (10.34%)
29	8 (22.86%)	7 (24.14%)
30	7 (20.00%)	9 (31.03%)
31	3 (8.57%)	4 (13.79%)
32	5 (14.29%)	0

* No significant difference between the 2 groups at $p > 0.05$.

those receiving the reference formula. The infants receiving the new HMF also received full-strength HM supplementation for fewer days than those receiving the reference HMF, although this was not statistically different (table 6).

Growth Characteristics

Both HMFs produced increases over baseline in weight, length and OFC; however, greater gains were observed in the new-HMF-fed infants in both the evaluable infants (infants who completed study; table 2) and intent-to-treat populations (all infants entered into trial; data not shown). The increases in weight and OFC were significantly greater in the new HMF treatment group than in the reference HMF group ($p < 0.05$).

Laboratory Results

All values observed for the clinical chemistries, vitamin D, copper, and ceruloplasmin measurements were within the normal range and were acceptable for the age of the infants in the study (table 7). No statistically significant differences between the treatment groups were observed for clinical chemistries (sodium, calcium, phosphorus, blood urea nitrogen, and alkaline phosphatase), except that the new-HMF-treated group had a significantly higher ($p = 0.0409$) mean blood urea nitrogen that was,

Table 6. Human milk intake of infants who completed the study (mean \pm SE)

Variable/time interval	New HMF (n = 35)	Reference HMF (n = 29)
Human milk intake		
Daily, ml/day	248.1 \pm 7.1*	228.9 \pm 8.1*
Adjusted daily, ml/kg/day	144.4 \pm 2.5*	154.2 \pm 2.1*
Full-strength supplementation, days	20.8 \pm 1.2	22.2 \pm 2.0

* Difference between the 2 groups at $p < 0.05$.

Table 7. Laboratory results (mean \pm SE)

Variable (normal range)	New HMF	Reference HMF	p value
n	29	24	
Sodium (130–145 mEq/l)			
Baseline	136 \pm 1.2	132 \pm 1.9	
Final	134 \pm 1.8	131 \pm 1.7	
Calcium (1.95–2.80 mmol/l)			
Baseline	2.7 \pm 0.1	2.7 \pm 0.1	
Final	2.6 \pm 0.1	2.6 \pm 0.1	
Phosphorus (1.0–2.4 mmol/l)			
Baseline	2.0 \pm 0.1	2.0 \pm 0.1	
Final	2.1 \pm 0.1	2.2 \pm 0.1	
Alkaline phosphatase (25–500 U/l)			
Baseline	400 \pm 29	352 \pm 34	
Final	290 \pm 20	288 \pm 29	
Urea nitrogen (2–28 mg/dl)			
Baseline	2.1 \pm 0.2	2.7 \pm 0.3	
Final	2.1 \pm 0.2	1.7 \pm 0.1	0.0409
25-OH vitamin D (10–50 mg/dl)			
Baseline	31 \pm 1.8	30 \pm 2.6	
Final	44 \pm 3.4	50 \pm 4.2	
1,25-(OH) ₂ vitamin D (21–85 pg/ml)			
Baseline	69 \pm 5.0	72 \pm 4.4	
Final	69 \pm 6.1	45 \pm 3.6	0.0022
Copper (12.1–100.6 mg/dl)			
Baseline	40 \pm 4.2	40 \pm 3.7	
Final	58 (6.3)	47 \pm 4.4	
Ceruloplasmin (70–450 mg/dl)			
Baseline	169 \pm 14	211 \pm 21	
Final	200 \pm 16	230 \pm 24	

however, within the accepted range. There were also no differences between the groups for the other serum parameters analyzed (25-hydroxy vitamin D, 1,25 dihydroxy vitamin D, serum copper, and ceruloplasmin) except for the new-HMF-treated group having a significant-

Table 8. Reasons for discontinuations

	New HMF	Reference HMF
Study events	2	2
Formula intolerance	2	2
Family/MD request	2	2
Protocol violations		
Increased O ₂ requirements	2	
HM < 14 days of age		1
NPO > 72 h	1	2
Intake > 150 cm ³ /kg/day	2	
Continuous feeds (apnea/bradycardia)		1
Formula initiated	1	5
Insufficient HM		1
Received multivitamins/folate	1	1
Intravenous lipids	1	

HM = Human milk; NPO = nothing per os.

ly higher mean 1,25-dihydroxy vitamin D level ($p = 0.0022$). The 1,25-dihydroxy vitamin D values, however, were within the normal range for both groups.

Safety Results

There was no difference between the study groups in: the overall incidence of study events; the relationship of study events to HMF (new HMF = 47, reference HMF = 43), and the incidence of individual study events.

Gastrointestinal events had an overall incidence of 27% with diarrhea, vomiting, and gastric residuals being the most common events with each fortifier. Respiratory events were recorded for approximately 30% of the infants. The main respiratory event was apnea (reference HMF 13/43, new HMF 13/47). All other respiratory events were found in 2 infants or less. Cardiovascular events were recorded for 40% of the reference-HMF-fed and 36% of the new-HMF-fed infants. All other cardiovascular events, again, occurred in only 1 or 2 infants.

Approximately 28% of the 90 infants enrolled were discontinued for the following reasons: study events ($n = 4$); infant sent to step-down unit at another hospital ($n = 1$); family and/or physician request ($n = 4$), and protocol violations ($n = 19$). The reasons for discontinuations were similar in both groups, except that the number of infants that required supplementation with formula was greater in the reference HMF ($n = 5$) than in the new HMF group ($n = 1$) (table 8).

Discussion

The levels of calcium (30 mg/dl) and phosphorus (15 mg/dl) in preterm human milk and term human milk are similar and remained relatively constant throughout lactation [4–6]. These levels are insufficient to support bone growth and mineralization in the VLBW infant, since infants <1,800 g and gestational age 26–32 weeks have been estimated to require 160–180 mg/kg/day of calcium and 70–90 mg/kg/day of phosphorus [23–25]. Inadequate enteral intake of calcium and phosphorus in VLBW infants fed unsupplemented human milk has been associated with hypophosphatemia, hypercalciuria, elevated serum alkaline phosphatase activity, low net mineral retention, low rates of bone mineralization, and reduction in linear growth [17, 26].

A new HMF has been designed to more effectively meet the nutritional needs of VLBW infants and to have a nutrient composition that theoretically would make it more digestible than currently available fortifiers. The 100% bovine whey protein in the new HMF has a nutrient composition that may make it more digestible than the 60% bovine whey/40% casein contained in the reference HMF. This hypothesis remains to be evaluated with a direct comparison of such HMFs, although in a previously reported study in which a casein- or whey-predominant formula and human milk were evaluated, it was documented that the whey-predominant formula resulted in an amino acid pattern more similar to that found in infants fed human milk [27]. Other investigators have also shown that bovine protein is an excellent protein source for fortification, with growth rates, protein status and biochemical profiles in bovine protein fortifier-fed infants being similar to those in human milk protein fortifier-fed infants [10, 24, 28, 29].

The lower lactose concentration in the new HMF as compared with the reference HMF (15 versus 25%, respectively) may increase the overall carbohydrate tolerance and percentage of ingested lactose that is absorbed,

since preterm infants may have transiently impaired lactose digestion secondary to reduced lactase activity [25]. Any clinical effect resulting from this difference in lactose load was not great enough to be detected in this study. If the malabsorption of lactose had been significant enough, there should have been a difference in diarrhea incidence between the 2 HMF groups, resulting from the osmotic effects of the malabsorbed disaccharide beyond the ileocecal valve in the HMF group receiving the larger lactose load; however, no such difference in diarrhea incidence was documented. It is not likely that this 10% difference in lactose concentration was a significant factor in the greater increase in weight and OFC seen in the group receiving the new HMF, since the calorie difference, based on this factor, was <1 kcal/100 ml HM consumed.

The new HMF contains calcium and phosphorus as the highly bioavailable organic salts calcium gluconate and calcium glycerophosphate. In a clinical trial comparing two sources of calcium, VLBW infants fed human milk supplemented with a calcium gluconate-calcium glycerophosphate preparation had significantly greater bone mineralization plus greater net absorption and retention of calcium and phosphorus than VLBW infants given milk supplemented with calcium phosphate [26].

Copper fortification of the new HMF was not considered to be necessary, since preterm human milk contains sufficient copper [29], and absorption of copper from human milk is adequate to meet the needs of the preterm infant [30]. The copper content of preterm human milk ranges from an initial value of 0.8 to 0.6 mg/l at 4 weeks postpartum [29]. The reference HMF is supplemented with approximately 15.5 μ g of copper per packet (table 1). Since copper status was similar in both study groups, we believe this confirms that copper supplementation of the new HMF is unnecessary.

The infants given new HMF demonstrated a statistically significant greater increase in weight and OFC than the infants given reference HMF, observations that were documented by both the evaluable and intent-to-treat population analyses. Both HMF feeding regimens produced increases over baseline in weight, length and OFC in the 64 completed (evaluable) infants.

The greater increase in weight and OFC in the new-HMF-fed infants occurred even though they maintained a daily intake volume of fortified human milk and an adjusted daily intake volume that was significantly lower than the infants fed the reference HMF ($p < 0.05$). Calculations based on the average daily intake volumes of supplemented mother's milk and on the protein concentration of preterm human milk (approximately 2 g protein/

100 ml) indicated that the new-HMF-fed infants ingested 2.89 g/kg/day of human milk protein, and the reference-HMF-fed infants ingested 3.08 g/kg/day of human milk protein. Full-strength supplementation provided an additional 1.44 g of protein with the new HMF powder and 1.08 g of protein with the reference HMF. Therefore, the adjusted daily mean total protein intake was approximately 4.33 g/kg/day in the new-HMF-fed group and 4.16 g/kg/day in the reference-HMF-fed group. The growth differences documented in this study may also be due to the different protein energy ratios that will exist if these HMFs are added to human milk that contains 67–72 kcal and 1.71–2.17 g protein/100 ml. Since the new HMF provides 1 g protein/13 kcal compared to the reference HMF's 0.7 g protein/14 kcal, the protein ratios are greater with the new HMF throughout the premature human milk protein levels cited above with a difference of approximately 0.4 being maintained throughout this range. These results may indicate that the new HMF provides a nutrient mixture that, on a per volume basis, is better than the reference HMF in supporting growth in VLBW infants.

The significantly higher mean serum urea nitrogen found in the new-HMF-treated group was within the normal range, acceptable for the age of the infants in the study and was, therefore, not considered to be clinically significant. Serum urea nitrogen is a sensitive biological marker of protein intake and is significantly correlated with protein intake in low birth weight and term infants [10, 31]. Protein intake differences explained 18% of the variation in urea nitrogen in a group of exclusively breast-fed infants at the age of 2 months [31]. In a study involving 36 VLBW infants treated with different human and bovine milk protein fortification regimens, the infants receiving the fortification regimen that provided a significantly higher protein intake had a significantly higher serum urea nitrogen. The magnitude of the differences in protein intake, while statistically significant, were not large and ranged from 0.1 to 0.3 g/100 ml [10]. These observations demonstrate that small differences in protein intake can result in significant differences in serum urea nitrogen. Consequently, the higher serum urea nitrogen level in the group of new-HMF-fed infants is most likely a result of the slightly higher protein intake.

No statistically significant differences between the feeding groups were observed for serum levels of calcium, phosphorus, alkaline phosphatase, and 25-hydroxy vitamin D. The 1,25-dihydroxy vitamin D level was significantly higher in the new-HMF-fed group than in the group fed the reference HMF, but the values for both

parameters were within the normal range. There is no obvious explanation for the significant decrease in 1,25-dihydroxy vitamin D levels observed in the reference-HMF-fed infants, since renal disease was not present in these infants. Serum 1,25-dihydroxy vitamin D levels are considered by some to be indicative of calcium homeostasis, so that increased levels might indicate an increased need for calcium in the infants fed the new HMF. Although this is a viable hypothesis, the similar serum calcium, phosphorus, alkaline phosphatase, and 25-dihydroxy vitamin D argue against this position.

Both feeding groups demonstrated a comparable incidence of total study events that were similar with respect to type and incidence of study events across body systems, indicating that both HMFs were well tolerated and safe.

In this single-blind, randomized, controlled multicenter study, both the new HMF and the reference HMF supported growth at intrauterine growth rates in these VLBW infants. The increases in weight and OFC were significantly greater in infants fed human milk fortified with the new HMF. The safety profile of the new HMF was comparable to that of the reference HMF.

Conclusion

We conclude from this study that the new HMF is safe and provides acceptable or superior growth in this premature infant population in comparison to the reference HMF.

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