

# Enhanced Growth of Preterm Infants Fed a New Powdered Human Milk Fortifier: A Randomized, Controlled Trial

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**ABSTRACT.** *Objective.* A prospective, double-blind, randomized, controlled trial was conducted to evaluate the growth and nutritional status of preterm infants receiving preterm human milk supplemented with a newly formulated powdered human milk fortifier (HMF), study fortifier (SF), or a powdered commercial HMF (CF).

*Methods.* Infants ( $n = 144$ ) with a birth weight  $\leq 1600$  g and gestational age at birth of  $\leq 33$  weeks were enrolled and randomized before 21 days of life. Study day (SDAY) 1 was defined as the day full-strength fortification (4 packets/100 mL) began and the infant reached an intake of at least 100 mL/kg/day. Growth, biochemical indices of nutritional status, enteral intake, feeding tolerance, clinical histories, and morbidity were assessed serially. The primary outcome variable was weight gain (g/kg/day) from SDAYs 1 to 29 or hospital discharge, whichever came first.

*Results.* Infants fed human milk supplemented with SF consistently grew more rapidly from SDAYs 1 to 29 (or hospital discharge), regardless of whether the statistical analyses were performed on all subjects who were randomized into the study and reached SDAY 1 (intent-to-treat) or were limited to those able to adhere strictly to the feeding protocol of the study (subgroup). Using mean values adjusted for study site (least square [LS] means), the weight gain differences were 2.6 and 3.8 g/kg/day for the intent-to-treat and subgroup analyses, respectively. Likewise, the length-gain differences were .14 and .18 cm/week for the intent-to-treat and subgroup analyses, respectively. Infants in the SF group reached a weight of 1800 g at SDAY 18, and those in the CF group at SDAY 25. Mean alkaline phosphatase values among infants in the SF group were higher than for the CF infants (eg, LS means: 327 U/L vs 272 U/L, intent-to-treat analysis), likely reflecting the more rapid linear growth of the SF infants. Mean serum calcium values tended to be lower in the SF group in the intent-to-treat analysis and were significantly lower in the subgroup analysis (LS means: 10.3 mg/dL vs 11.2 mg/dL). Both fortifiers were generally well-tolerated, although an increased number of infants in the CF group exited the feeding protocol because of gastric residuals and abdominal distention.

*Conclusion.* A new powdered HMF was shown to enhance the growth of preterm infants, compared with a commercially available powdered HMF in the United States. *Pediatrics* 2000;106:581-588; *human milk, human milk fortifier, growth, preterm infants, low birth weight.*

ABBREVIATIONS. HMF, human milk fortifier; SF, study human milk fortifier; CF, commercial human milk fortifier; SDAY, study day; NEC, necrotizing enterocolitis; ANOVA, analysis of variance; SD, standard deviation; LS, least square.

Many nutritional as well as immunologic advantages have been ascribed to feeding preterm infants human milk.<sup>1-3</sup> Nonetheless, human milk feeding, in the absence of fortification, does not meet the nutritional goal of supporting intrauterine rates of growth and nutrient retention, particularly among infants weighing  $<1500$  g.<sup>1,4</sup> Specifically, unsupplemented mature human milk provides an insufficient quantity of protein to support the growth and lean body mass accretion of smaller preterm infants. Fortification or supplementation of human milk with energy and protein improves both rates of weight gain and indices of protein status.<sup>5,6</sup> Likewise, the concentrations of calcium and phosphorus in human milk are significantly below that necessary to attain in utero levels of bone mineralization in small preterm infants.<sup>7-9</sup>

Different forms (liquid and powder) of preterm human milk fortifiers (HMFs) have been developed to enhance the nutrient intake of the human milk-fed preterm infant. Although advantages and disadvantages of each have been suggested, the primary advantage of powdered HMF is that there is minimal dilution of human milk. Until recently there was only 1 powdered HMF available on the market in the United States. Specific areas of concern with this commercial powdered HMF include its protein content,<sup>10</sup> the inclusion of highly soluble calcium and phosphorus salts,<sup>11,12</sup> high osmolality,<sup>13,14</sup> difficulties with mixability, and the separation of human milk fat with continuous tube feeding.<sup>15</sup> These concerns may be related to the nutrient content and/or selection of ingredients when mixed with preterm milk.

Kashyap et al<sup>5</sup> reported a linear correlation between protein intake and both weight gain and nitrogen retention among low birth weight infants fed either unsupplemented human milk or human milk supplemented with protein. Likewise, protein intake

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in their study was correlated with serum transthyretin and albumin, suggesting that growth and biochemical indices of protein status of human milk-fed infants are significantly influenced by protein intake and that an increase in the protein content of a powdered HMF may improve the growth and protein status of these infants.

Schanler and Abrams<sup>11</sup> reported that preterm infants receiving the powdered HMF containing highly soluble calcium and phosphorus demonstrated poorer fat absorption, compared with similar infants fed powdered HMF containing insoluble calcium and phosphorus. A more recent study by Schanler et al<sup>12</sup> confirmed these findings by demonstrating that when added to human milk, the powdered HMF was associated with a 50% reduction in human milk free fatty acids, suggesting binding of minerals to fatty acids.<sup>12</sup> The data further suggest that the mineral-bound fatty acids were not well-absorbed, resulting in higher fat excretion in infants fed the powdered HMF.

Sankaran et al<sup>15</sup> also suggest that mixing powdered HMF with milk was associated with clumping and adherence to the bottle walls. The separation of fat from the fortified human milk mixture and adherence to feeding equipment is not an uncommon observation in nurseries. Changes in the processing and ingredient composition of the powdered HMF, such as the addition of an emulsifier (phosphatidylcholine), may alleviate these shortcomings and improve nutrient delivery. Further, carbohydrate and protein deliver all of the energy in the current powdered HMF. Substitution of fat for some of the carbohydrate would serve to reduce the osmolality of the fortifier and further reduce an increase in osmolality produced by hydrolysis of the carbohydrate fraction (corn syrup solids) of the powdered HMF by human milk amylase.<sup>14</sup>

Given the aforementioned considerations, a new fortifier was formulated. The objectives of this study were to determine whether a newly formulated powdered HMF would support improved growth and nutritional status in preterm infants, compared with the currently used powdered HMF in the United States.

## METHODS

### Study Population

A prospective, double-blind, randomized, controlled trial was conducted to evaluate growth, nutritional status, feeding tolerance, and morbidity in preterm infants  $\leq 33$  weeks of gestation and  $\leq 1600$  g who received either a newly formulated powdered human milk fortifier (Similac HMF or study fortifier [SF], Ross Laboratories, Columbus, OH) or a commercially available powdered human milk fortifier (Enfamil HMF or commercial fortifier [CF], Mead Johnson, Evansville, IN). Singleton, twin or 2 of 3 triplet infants were eligible to participate. Infants with serious congenital malformations, perinatal asphyxia, those who underwent major surgery, received steroids for  $>8$  days before enrollment, were ventilator dependent at enrollment, or who had a systemic infection at enrollment were not eligible for study entry. The study was approved by the review boards for human subject research at each participating institution. Informed written consent was obtained from a parent of each infant.

### Study Design

Infants were enrolled into the study from June 1997 to November 1998 and randomized to one of the fortifier groups (SF or CF) before 21 days of age and were followed until study day (SDAY) 29 or hospital discharge, whichever came first. SDAY 1 was defined as the day when full-strength fortification (4 packets/100 mL human milk) began and the subject reached an intake of at least 100 mL/kg/day. Study subjects were considered to have completed the feeding protocol if they received fortifier until SDAY 15. Infants were allowed to receive lower concentrations of fortified human milk before SDAY 1 in accordance with usual clinical practice in the participating nurseries. The randomization schedule was blocked for birth weight ( $<1100$  g and  $\geq 1100$  g) and gender. Growth, biochemical indices of nutritional status, enteral intake, feeding tolerance, clinical histories, and morbidity were assessed serially. The primary outcome variable was weight gain (g/kg/day) from SDAYs 1 to 29 or hospital discharge, whichever came first. Additional outcome variables included length and head circumference gains (cm/day) and biochemical indices of nutritional status (serum concentrations of urea nitrogen, albumin, transthyretin, calcium, phosphorus, magnesium, alkaline phosphatase, sodium, potassium, chloride, vitamin A, and vitamin E). Enteral intake, feeding tolerance (eg, incidence of gastric residuals, abdominal distention, emesis, and feedings withheld), and morbidities (suspected and/or confirmed cases of systemic infection or necrotizing enterocolitis (NEC), respiratory status [percent of infants receiving supplemental oxygen, receiving high-frequency/mechanical ventilation, or having an apnea and/or bradycardia episode], and steroid or antibiotic therapy were also assessed.

Daily weights using electronic scales ( $\pm 10$  g) were measured until hospital discharge by nursery personnel. Other anthropometric measurements were performed weekly by specially trained study personnel using standardized procedures. Recumbent length was obtained with a fixed headboard and movable footboard and head circumference using a nonstretchable tape to the nearest .1 cm.

Enteral intake was collected from enrollment to SDAY 29. Intake of human milk (including donor/banked human milk), fortified human milk, or other enteral feeding (including supplements [eg, vegetable oils and glucose polymers] was recorded. It should be noted that only a small percentage of infants received donor/banked human milk during the study ( $<4\%$ ). The estimated nutrient composition of mature preterm human milk mixed with each of the 2 fortifiers at a concentration of 4 packets/100 mL of milk is presented in Table 1. Although the SF delivered the same amount of energy as the CF, it contained a source of fat and an emulsifier (phosphatidylcholine [lecithin]) and higher levels of protein, minerals, and some vitamins. The ingredient source of some nutrients differed between the 2 fortifiers. Most notably, in addition to the contribution of calcium and phosphorus present in the whey protein concentrate and nonfat dry milk, calcium was added to the SF in the form of calcium phosphate tribasic and calcium carbonate. In contrast, in addition to the calcium and phosphorus inherent in the whey protein concentrate, calcium was added to the CF in the form of calcium gluconate and calcium glycerophosphate.

Blood samples were drawn from each infant by venipuncture or, if necessary, by heelstick on SDAYs 1, 15, and 29. Serum electrolytes were analyzed at the hospital site. Other biochemical determinations for this study were performed at a single laboratory. Serum alkaline phosphatase, calcium, phosphorus, magnesium, and urea nitrogen were analyzed using a Clinical Chemistry Analyzer (Abbott Spectrum EPx, Abbott Park, IL) with a phosphodiode array detector. Serum albumin and transthyretin were analyzed using a Behring Nephelometer 100 (Dade Behring, Newark, DE). Vitamins A and E were measured by high-performance liquid chromatography according to the method described by Nomura et al.<sup>16</sup>

### Statistical Methods

Study data were analyzed on an intent-to-treat basis, including all enrolled infants who reached SDAY 1. Because of the anticipated protocol deviations in this high-risk patient population, a subgroup analysis was prospectively planned to analyze results from infants who strictly adhered to the study protocol. This subgroup of infants was selected based on the following criteria:

**TABLE 1.** Approximate Nutrient Composition of Human Milk and Human Milk Supplemented With SF or CF (per 100 mL Fortified Human Milk)

Nutrients	Human Milk*	Human Milk + SF	Human Milk + CF
Energy, kcal	67	79	79
Protein, g	1.4	2.3	2.0
Source	—	Whey protein concentrate/nonfat dry milk	Whey protein concentrate/sodium caseinate
Fat, g	3.9	4.1	3.9
Source	—	Medium-chain triglycerides (MCT oil)	None added
Carbohydrate, g	6.6	8.2	9.1
Source	—	Corn syrup solids	Corn syrup solids
Vitamins			
A, IU	389	983	1301
D, IU	2.0	119	206
E, IU	1.0	4.2	5.5
K, $\mu\text{g}$	.21	8.3	4.5
Thiamin (B1), $\mu\text{g}$	21	247	167
Riboflavin (B2), $\mu\text{g}$	48	453	251
B6, $\mu\text{g}$	15	220	125
B12, $\mu\text{g}$	.05	.67	.22
Niacin, mg	1.50	3.6	3.1
Folic acid, $\mu\text{g}$	3.3	25.6	27.5
Pantothenic acid, mg	1.8	1.6	.9
Biotin, $\mu\text{g}$	.6	26.2	3.2
C, mg	11	35	22
Minerals			
Calcium, mg	25	138	112
Phosphorus, mg	13	78	56
Source of calcium and phosphorus	—	Calcium phosphate tribasic, calcium carbonate	Calcium gluconate, calcium glycerophosphate
Magnesium, mg	3.2	9.8	4.0
Zinc, mg	.34	1.3	1.0
Manganese, $\mu\text{g}$	.6	7.5	5.0
Copper, $\mu\text{g}$	64	228	122
Sodium, mg	25	39	31
Potassium, mg	57	117	71
Chloride, mg	55	90	71
Iron, mg	.12	.46	.12
Selenium, $\mu\text{g}$	2.0	2.4	1.9

\* Milk nutrient composition values from Ross Products Division, 1998.<sup>27</sup> Where possible, milk composition literature values were used from studies that reported data on milk samples collected from mothers who delivered prematurely.

completed the study protocol for at least 15 days from SDAY 1; received <20% of total energy from sources other than fortified human milk over any 1-week period during the study (SDAYS 1–29); received no postnatal steroids; had feedings withheld for <3 days; and did not develop a condition or disease that could affect growth. The fortifier feeding groups were also assessed for comparability of demographic variables and exit status using analysis of variance (ANOVA), log-rank test, or Cochran Mantel-Haenszel test.

The primary outcome variable for this study was weight gain (g/kg/day) from SDAYS 1 through 29 or hospital discharge, whichever came first. Weight gain was used as the basis for the sample size calculation, using an estimate of the variability in the weight gain of human milk-fed infants from 4 published articles.<sup>6,15,17,18</sup> The unweighted average of standard deviations (SDs) in the 4 studies for weight gain (g/kg/day) was 2.8 g/kg/day. A sample size of 98 subjects was determined to be sufficient to detect a difference of .57 SD between the fortifier groups. Lucas et al<sup>17</sup> reported that an increase in the rate of weight gain of 1.6 g/kg/day would allow for catch-up growth. Weight gain was analyzed by fitting a linear regression to logarithms of daily weights for each infant separately, exponentiating the slopes of regression, and using absolute weight at SDAY 1 as a covariate to mitigate the impact of variability in initial weights. The slopes were compared between groups using a 1-way ANOVA adjusting for site. Absolute weights at SDAYS 1, 8, 15, 22, and 29 were fitted with a repeated-measures model using SAS PROC MIXED (SAS Institute, Inc, Cary, NC). Differences in length and head circumference gains from SDAYS 1 to 29 or hospital discharge (whichever came first) were analyzed by ANOVA adjusted for site.

Serum biochemical indices of nutritional status were evaluated using repeated-measures ANOVA. When a significant feeding by time interaction was detected, means were compared at each time point. Intake and feeding tolerance were evaluated using ANOVA. Time-to-event data were analyzed by log-rank test. Count data were analyzed using Poisson regression. The percentage of infants having a certain feeding tolerance characteristic was compared between the 2 fortifier feeding groups using the Cochran Mantel-Haenszel test.

All statistical tests were 2-tailed and the level of significance was set at .05. Where applicable, site was used as a stratification/blocking variable. All statistical analyses were performed using SAS, *Release 6.09e* (SAS Institute, Inc). Unless indicated otherwise, the data are expressed as mean  $\pm$  SD.

## RESULTS

### Study Population

Seventy-four infants were randomized to the SF group, 64 of these infants (86%) reached SDAY 1, and 49 (66%) completed the feeding protocol. Similarly, 70 infants were randomized to the CF group, 55 of these infants (79%) reached SDAY 1, and 40 of these infants (57%) completed the feeding protocol. Of the infants who did not reach SDAY 1, 5 of 10 in the SF group and 8 of 15 in the CF group never received a fortified human milk feeding. Only 2 of 74 and 5 of 70 infants in the SF and CF groups, respectively,

exited because of intolerance as judged by the investigator at each site (primary reason for exit only). Among all possible reasons given for discontinuation of fortified milk feeding attributable to intolerance before SDAY 29, more infants fed CF exited because of gastric residuals and/or abdominal distention versus infants fed SF (0 of 74 vs 6 of 70;  $P = .012$ ). All other reasons cited for exiting the feeding protocol were not different between the groups. The most common reason cited for exiting the feeding protocol was insufficient milk supply and/or stopped pumping milk, followed by discharged from the hospital or transferred to another hospital and removed by investigator or parent.

The initial nutrition course seemed uncomplicated and similar for infants in both groups before study entry. Approximately 92% of infants in the SF group and 82% of infants in the CF group were supported with total parenteral nutrition for ~12 days. Infants in both groups regained birth weight within 12 days and initiated enteral feeding on approximately day 4 of life.

Characteristics of the infants and their families in the intent-to-treat analysis are summarized in Table 2. There were no statistically significant differences between groups with respect to birth weight, length, head circumference, gestational age, gender, multiple birth status, ethnicity, age at SDAY 1, and weight at SDAY 1. Likewise, these characteristics did not differ between groups when all infants enrolled in the study, regardless of their ability to reach SDAY 1, were included in the statistical analyses. Further, the average number of days to reach SDAY 1 from the time of first fortifier feeding did not differ among infants randomized to either the SF ( $2.2 \pm 2.2$  days; median: 1.5 days) or the CF group ( $3.3 \pm 5.3$  days; median: 2.0 days).

### Feeding Tolerance

There were no statistically significant differences between feeding groups with respect to the percent-

age of infants that had feedings withheld for  $\geq 1$  day (SF, 5 of 69; CF, 8 of 62), feedings withheld because of gastric residuals  $>5$  mL (SF, 5 of 69; CF, 7 of 62), abdominal distention attributable to gastric residuals (SF, 7 of 69; CF, 3 of 62), or at least 1 episode of emesis (SF, 46 of 69; CF, 40 of 62). Among those infants who had at least 1 episode of emesis, there were statistically significant differences in the percent days with emesis between the SF (3 days, 11%) and CF (5 days, 18%) groups ( $P = .007$ ).

### Anthropometrics

Differences between groups were noted for weight, length, and head circumference gains in the intent-to-treat (all infants as randomized and reaching SDAY 1) and the subgroup analyses among infants that strictly adhered to the feeding protocol (Fig 1 and Table 3). The mean weight gains for the SF and CF groups were  $17.6 \pm 4.1$  g/kg/day and  $14.9 \pm 3.2$  g/kg/day, respectively, in the intent-to-treat analysis ( $P = .0004$ ). Infants fed the SF weighed, on average, 219 g more than infants fed the CF at hospital discharge after SDAY 29 ( $P = .028$ ). The time to reach 1800 g were SDAys 18 and 25 for infants receiving SF and CF, respectively ( $P = .001$ ). The absolute difference in weight gain between feeding groups was greater in the subgroup analysis (fed SF,  $18.4 \pm 3.0$  g/kg/day and fed CF,  $14.3 \pm 2.9$  g/kg/day). Infants randomized to the SF group also had significantly greater length gains than did infants randomized to the CF group both in the intent-to-treat ( $P = .034$ ) and subgroup analyses ( $P = .030$ ). Using least square (LS) mean values adjusted for a possible impact of study site, the length-gain differences were .14 and .18 cm/week, for the intent-to-treat and subgroup analyses, respectively. Head circumference gain did not reach statistical significance in the intent-to-treat analysis but was significant in the subgroup analysis ( $1.06 \pm .19$  cm/week vs  $.84 \pm .22$  cm/week;  $P = .0007$ ).

**TABLE 2.** Intent-to-Treat Analysis: Baseline Characteristics of Preterm Infants Reaching SDAY One

	SF	CF
Birth weight, g	1247 $\pm$ 190 (64)	1274 $\pm$ 196 (55)
Gestational age at birth, wk	29.4 $\pm$ 1.5 (64)	29.7 $\pm$ 1.6 (55)
Birth length, cm	38.9 $\pm$ 2.2 (59)	39.2 $\pm$ 2.6 (49)
Birth head circumference, cm	27.0 $\pm$ 1.6 (63)	27.2 $\pm$ 1.7 (54)
Gender, <i>n</i>		
Male	34	27
Female	30	28
Multiple birth status, <i>n</i>		
Singleton	46	41
Twin	12	11
Triplet	6	3
Ethnicity, <i>n</i>		
White	44	35
Black	4	7
Hispanic	15	12
Other	1	1
Postnatal age at SDAY 1, d	17.9 $\pm$ 7.9 (64)	15.7 $\pm$ 8.3 (55)
Weight at SDAY 1, g	1342 $\pm$ 195 (63)	1332 $\pm$ 259 (55)
Days to reach SDAY 1 from day of 1st fortifier feeding	2.2 $\pm$ 2.2 (64)	3.3 $\pm$ 5.3 (55)

\* Values are mean  $\pm$  SD (number of subjects) unless otherwise noted. No statistically significant differences between fortifier groups were found.

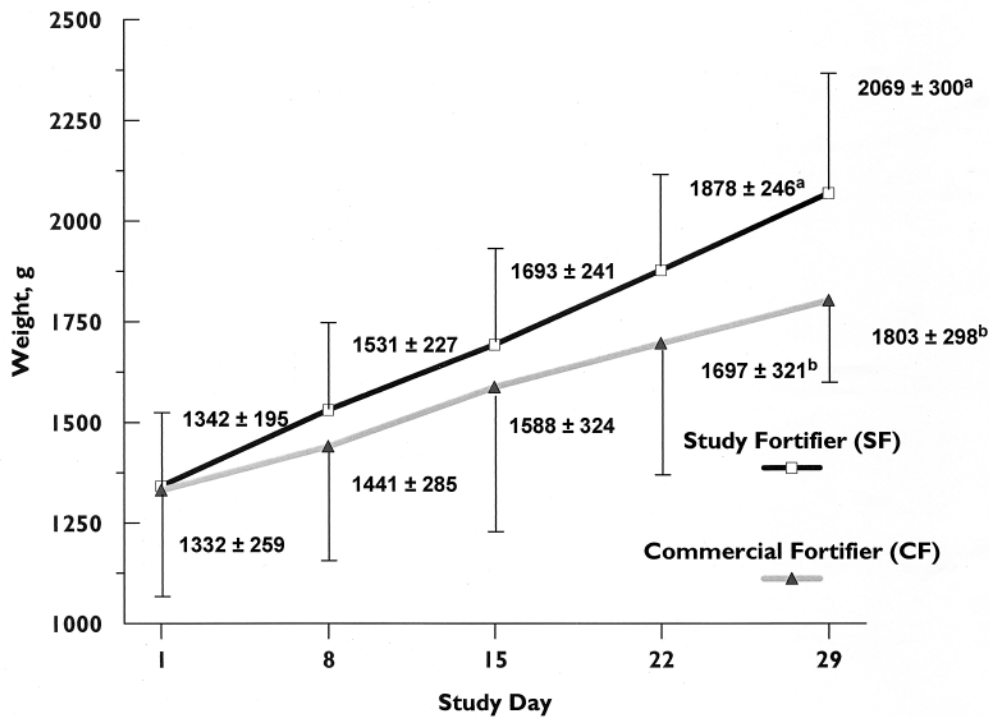


Fig 1. Intent-to-treat analysis: weight of preterm infants fed human milk fortified with either SF or CF. Values are mean  $\pm$  SD. Values with different superscript letters at each time point represent statistically significant differences.

TABLE 3. Intent-to-Treat and Subgroup Analyses: Weight, Length, and Head Circumference Outcome Variables\*

Variable	SF	CF	P Value
Weight gain, g/kg/d			
Intent-to-treat analysis	17.6 $\pm$ 4.1 (64)	14.9 $\pm$ 3.2 (55)	.0004
Subgroup analysis	18.4 $\pm$ 3.0 (37)	14.3 $\pm$ 2.9 (30)	.0001
Length gain, cm/wk			
Intent-to-treat analysis	1.09 $\pm$ .29 (60)	.95 $\pm$ .39 (52)	.0345
Subgroup analysis	1.11 $\pm$ .28 (35)	.95 $\pm$ .33 (30)	.0301
Head circumference gain, cm/wk			
Intent-to-treat analysis	1.04 $\pm$ .23 (60)	.94 $\pm$ .25 (51)	.0743
Subgroup analysis	1.06 $\pm$ .19 (35)	.84 $\pm$ .22 (29)	.0007

\* Values are mean  $\pm$  SD. Numbers in parentheses equal the number of subject.

### Biochemical Indicators of Nutritional Status

There were no statistically significant differences in the biochemical indicators of protein status, vitamin A, and vitamin E status between groups in either the intent-to-treat or subgroup analyses. In the intent-to-treat group there were significant differences in serum concentrations of sodium (LS means  $\pm$  standard error of the mean; 137  $\pm$  .05 mEq/L vs 135  $\pm$  .05 mEq/L;  $P = .029$ ), potassium (5.28  $\pm$  .07 mEq/L vs 4.92  $\pm$  .07 mEq/L;  $P = .0001$ ), and alkaline phosphatase (327  $\pm$  14 U/L vs 272  $\pm$  16 U/L;  $P = .007$ ) in the SF versus CF groups, respectively. There was also a statistically significant feeding group by time interaction in the intent-to-treat analysis observed for serum magnesium at SDAY 15 (SF > CF: 2.18  $\pm$  .04 mEq/L vs 2.01  $\pm$  .04 mEq/L;  $P = .002$ ). In the subgroup analysis, there were significant differences in serum concentrations of calcium (10.3  $\pm$  .24 mg/dL vs 11.2  $\pm$  .26 mg/dL;  $P = .016$ ), phosphorus (6.4  $\pm$  .17 mg/dL vs 7.0  $\pm$  .20 mg/dL;  $P = .021$ ), sodium (136  $\pm$  .6 mEq/L vs 135  $\pm$  .6 mEq/L;  $P = .047$ ) and potassium (5.3  $\pm$  .09 mEq/L vs 4.8  $\pm$  .09

mEq/L;  $P = .0002$ ) in the SF versus CF groups, respectively. There were also statistically significant feeding group by time interactions in the subgroup analysis observed for serum concentrations of magnesium ( $P = .037$ ) and alkaline phosphatase ( $P = .042$ ). On SDAY 15 the mean concentration of serum magnesium was greater among infants fed SF (2.2  $\pm$  .04 mg/dL) than among infants fed CF (2.0  $\pm$  .05 mg/dL;  $P = .0004$ ), and alkaline phosphatase was greater among infants fed SF (356  $\pm$  24 U/L) than among those fed CF (262  $\pm$  26 U/L;  $P = .007$ ).

### Enteral Intake

In both the intent-to-treat and subgroup analyses, the total number of days that study subjects were on the feeding protocol did not differ between groups. In the intent-to-treat analysis, both groups reached full enteral feeds (150 mL/kg/day)  $\sim$ 3 days after SDAY 1. They were fed the study fortifier for an average of 26 days in the SF group and 25 days in the CF group. There were no differences between the groups with respect to mean total energy intake at

SDAY 1 or during the study (Table 4). Furthermore, the percent of total energy intake from full-strength fortified human milk was >80% in each group. The mean total protein intake was greater in the SF than in the CF group (3.5 g vs 3.1 g/kg/day, respectively).

### Morbidity

In both the intent-to-treat and subgroup analyses, the number of subjects with suspected or confirmed NEC or systemic infection did not statistically differ between groups, either before the first fortifier feeding or during the period in which the infants were receiving the study fortifier. There were 3 cases of suspected NEC in the SF group (4%) and 8 cases of suspected NEC in the CF group (13%) reported during the period in which the infants were receiving the study fortifier. The 1 case of confirmed NEC reported during the same period was in the CF group. The groups were similar for the number of infants requiring supplemental oxygen, mechanical ventilation, steroid use, or having apnea and bradycardia.

### DISCUSSION

The primary objectives of this study were to assess whether a newly formulated powdered HMF promoted improved weight gain, was well-tolerated, and was not associated with morbidity among preterm infants. Infants randomized to the SF had significantly greater weight and length gains during the study than did infants randomized to the CF group. These results were observed regardless of whether the statistical analyses were performed on all subjects who were randomized into the study and reached SDAY 1 (intent-to-treat) or were limited to those able to strictly adhere to the feeding protocol of the study (subgroup). Although both groups met intrauterine accretion rates for growth, (15 g/kg/day), only the SF group exhibited weight gains that approached that of the 20 g/kg/day necessary for catch-up growth.<sup>19</sup> In addition, infants in the SF group reached a weight of 1800 g at SDAY 18, compared with those who received CF who did not reach this weight until SDAY 25. Infants in the SF group also had improved length gain compared with the CF group, suggesting that the former group of infants had significantly improved accretion rates of lean body mass. Likewise in the subgroup analysis,

infants in the SF group had greater head circumference gains, compared with infants consuming the CF.

The total caloric intake between groups was not significantly different in either the intent-to-treat or subgroup analyses; therefore, energy intake cannot explain the growth differences observed. A number of specific nutrient and ingredient compositional differences do exist between the 2 fortifiers that may account, at least in part, for the observed differences in growth. The protein intake was significantly greater in the SF group versus the CF group. Kashyap et al<sup>5</sup> reported a linear relationship among total protein intake, nitrogen retention, and total weight gain in low birth weight infants fed either unsupplemented or fortified human milk, suggesting that growth, specifically lean body accretion, in preterm infants is highly influenced by protein intake.<sup>5</sup> Protein intakes in this study were in the range of those reported herein. More recently, Carlson et al<sup>10</sup> reported that small preterm infants fed fortified human milk (available commercially) and supplemented with an additional .8 g of protein/100 mL of human milk had greater weight gains (17.3 g/kg/day) than did infants fed fortified human milk without the additional protein supplement (14.9 g/kg/day).<sup>10</sup> However, despite differences in protein intake in this study, we found that no differences existed between groups with respect to biochemical indicators of protein status (ie, serum urea nitrogen, albumin, and transthyretin), possibly because all values were within the normal range.

Differences in the level and ingredient sources of various minerals and vitamins in SF versus CF may also contribute to improved growth. For example, in addition to the calcium inherent to the whey protein concentrate and nonfat dry milk ingredients, calcium was added to the SF in the form of relatively insoluble calcium salts, calcium phosphate tribasic and calcium carbonate. The CF contains the soluble calcium salts, calcium gluconate and calcium glycerophosphate. It is speculated that these soluble calcium salts may bind fatty acids in the fortified human milk mixture, thereby impairing fat absorption and lowering energy intake.<sup>12</sup> Indeed, it has been reported that infants fed the CF containing these soluble calcium salts have lower skinfold thicknesses than do

**TABLE 4.** Intent-to-Treat Analysis: Mean Reported Energy and Protein Intakes During the Study Period (SDAY 1–29)\*

Variable	SF		CF	
	kcal/kg/Day	Grams of Protein/kg/Day	kcal/kg/Day	Grams of Protein/kg/Day
Fortified human milk (full-strength)	104.7 ± 23.9	3.1 ± .7 <sup>a</sup>	98.7 ± 32.9	2.6 ± .9 <sup>b</sup>
Human milk only	1.4 ± 2.4	.03 ± .05	5.7 ± 15.6	.12 ± .3
Other intake†	11.5 ± 22.2	.38 ± .7	11.3 ± 22.9	.37 ± .8
Total intake	117.99 ± 13.1	3.50 ± .4 <sup>a</sup>	117.5 ± 16.3	3.07 ± .5 <sup>b</sup>

\* Values are mean ± SD. Fortified human milk at half-strength is not shown in this table but is included in the total kcal/kg/day and total protein/kg/day. The intake of half-strength human milk was minimal in both groups (<2 kcal/kg/day). Human milk values used in the calculations are from Ross Products Division, 1998.<sup>27</sup> <sup>a,b</sup>Values with different superscript letters within each row denote statistically significant differences [ $P = .001$ ].

† Other intake includes any enteral intake other than fortified human milk or human milk alone.

infants fed CF containing relatively insoluble calcium salts.<sup>11</sup> Recently, Schanler et al<sup>2</sup> observed that weight gain correlated directly with fat absorption in premature infants fed fortified human milk.

Improved mixability of SF with human milk and enhanced homogeneity of milk fat in the resultant mixture may improve energy and nutrient delivery to the preterm infant and may also account for their improved growth. Processing procedures are known to impact on the mixability of powdered food products with liquids, and problems with clumping of CF and adherence to the bottle walls have been cited.<sup>15</sup> Addition of phosphatidylcholine to the SF may have served to keep the milk fat emulsified in the fortified milk mixture during feeding.

Overall, both powdered HMFs were very well-tolerated, as suggested by the fact that few infants in the study had feedings discontinued primarily attributable to feeding intolerance. The addition of fat to the SF reduced the osmolality of the fortified milk mixture to a range of 375 to 385 mOsm/kg versus the CF ranging from 400 to 410 mOsm/kg. A reduction in osmolality may improve the feeding tolerance of an enteral product.<sup>13</sup>

The 2 groups were comparable with respect to serum concentrations of retinol and  $\alpha$ -tocopherol. Mean retinol levels in both groups were slightly below the levels considered deficient in adults ( $<7 \mu\text{mol/L}$ ); however, these levels are expected in preterm infants.<sup>20</sup> The mean serum  $\alpha$ -tocopherol values for both groups were within the normal reference ranges.<sup>21</sup>

The most pronounced differences in biochemical indices of nutritional status between infants fed the 2 powdered HMFs relate to serum concentrations of calcium, phosphorus, alkaline phosphatase, and magnesium. The calcium concentrations among infants fed CF tended to be higher than those of infants fed SF. This difference likely reflects the fact that CF contains highly soluble calcium salts. The mean serum calcium concentration in infants included in the subgroup analysis fed CF was generally higher than in those infants in the SF study group (LS means: 11.2 mg/dL vs 10.3 mg/dL). The tendency of serum calcium concentrations to be higher among infants fed CF versus infants fed other human milk fortifiers has been reported previously.<sup>15,22</sup>

In general the alkaline phosphatase values of infants fed SF were higher than those of infants fed CF, but both groups were within the normal range for preterm infants.<sup>20</sup> These higher values likely reflect the improved growth rate of infants fed SF. Alkaline phosphatase values have been shown to increase with osteoblastic activity and have been correlated with the rate of growth.<sup>23-25</sup> In situations in which mineral intake is deficient, there may be enhanced synthesis or activity of alkaline phosphatase with reduced bone mineralization. Marked elevations of alkaline phosphatase to values 4 to 5 times the adult reference range may occur and are associated with decreased levels of serum phosphorus, as observed in rickets.<sup>26</sup> However, serum phosphorus values of infants in this study were well within normal range, indicating that the higher serum alkaline phosphatase

values observed in the SF group were related to the enhanced linear growth rate.

## CONCLUSION

A new powdered HMF has been formulated and shown to enhance the growth of preterm infants, compared with those fed a commercially available powdered HMF in the United States. Although it is difficult to assess which nutrient(s) or ingredient(s) might be responsible for enhanced growth, the higher protein content, removal of the highly soluble calcium salts, and inclusion of fat and phosphatidylcholine in the new fortifier may contribute, at least in part, to these observations. In addition, the higher alkaline phosphatase in the SF group is consistent with a greater rate of linear growth in that group.

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## REFERENCES

1. American Academy of Pediatrics, Committee on Nutrition. *Pediatric Nutrition Handbook*. 4th ed. Elk Grove Village, IL: American Academy of Pediatrics; 1998
2. Schanler RJ, Shulman RJ, Lau C. Feeding strategies for premature infants: beneficial outcomes of feeding fortified human milk versus preterm formula. *Pediatrics*. 1999;103:1150-1157
3. Schanler RJ. Suitability of human milk for the low birthweight infant. *Clin Perinatol*. 1995;22:207-222
4. Canadian Paediatric Society, Nutrition Committee. Nutrient needs and feeding of preterm infants. *Can Med Assoc J*. 1995;152:1765
5. Kashyap S, Schulze KF, Forsyth M, et al. Growth, nutrient retention, and metabolic response in low-birth-weight infants fed supplemented and unsupplemented preterm human milk. *Am J Clin Nutr*. 1990;52:254-262
6. Polberger SKT, Axelsson IA, Raiha NC. Growth of very low birth weight infants on varying amounts of human milk protein. *Pediatr Res*. 1989;25:414-419
7. Atkinson SA, Raddle IC, Anderson GH. Macromineral balances in premature infants fed their own mother's milk or formula. *J Pediatr*. 1983;102:99
8. Pettifor JM, Rajah R, Venter A, et al. Bone mineralization and mineral homeostasis in very low-birth-weight infants fed either human milk or fortified human milk. *J Pediatr Gastroenterol Nutr*. 1989;2:217-224
9. Ziegler EE, O'Donnell AM, Nelson SE, et al. Body composition of the reference fetus. *Growth*. 1976;40:329-341
10. Carlson SJ, Johnson KJ, Cress GA, Connolly NW, Ziegler EK. Higher protein intake improves growth of VLBW infants fed fortified breast milk. *Pediatr Res*. 1999;45:278A
11. Schanler RJ, Abrams SA. Postnatal attainment of intrauterine macromineral accretion rates in low birth weight infants fed fortified human milk. *J Pediatr*. 1995;126:441-447
12. Schanler RJ, Henderson TR, Hamosh M. Fatty acid soaps may be responsible for poor fat absorption in premature infants fed fortified human milk. *Pediatr Res*. 1999;45:290A
13. Jew RK, Owen D, Kaufman D, Balmer D. Osmolality of commonly used medications and formulas in the neonatal intensive care unit. *Nutr Clin Pract*. 1997;12:158-163
14. De Curtis MD, Canduss OM, Pieltain C, Rigo J. Effect of fortification on the osmolality of human milk. *Arch Dis Child Fetal Neonatal Ed*. 1999;81:141-143
15. Sankaran K, Papageorgiou A, Ninan A, Sankaran R. A randomized, controlled evaluation of two commercially available human breast milk fortifiers in healthy preterm neonates. *J Am Diet Assoc*. 1996;96:1145-1149

16. Nomura AMY, Stemmermann GN, Lee J, Craft NE. Serum micronutrients and prostate cancer in Japanese Americans in Hawaii. *Cancer Epidemiol Biomarkers Prev.* 1997;6:487-491
17. Lucas A, Fewtrell MS, Morley R, et al. Randomized outcome trial of human milk fortification and developmental outcome of preterm infants. *Am J Clin Nutr.* 1996;64:142-151
18. Wauben IP, Atkinson SA, Grad TG, et al. Moderate nutrient supplementation of mother's milk for preterm infants supports adequate bone mass and short-term growth: a randomized, controlled trial. *Am J Clin Nutr.* 1988;67:465-472
19. Lucas A. Enteral nutrition. In: Tsang RC, Lucas A, Vaug R, Zlotkin S, eds. *Nutritional Needs of the Preterm Infant.* Baltimore, MD: William & Wilkins; 1993:209-21
20. Avery GB, Fletcher MA, MacDonald MS. *Neonatology—Pathophysiology and Management of the Newborn.* 4th ed. Philadelphia, PA: JB Lippincott Co; 1994
21. Bell CF. Upper limit of vitamin E in infant formulas. *J Nutr.* 1989;119:1829-1831
22. Fenton TR, Tough SC. Comparison of tolerance, parental attitudes and duration of breast feeding with powdered versus liquid breast milk enrichment products for very low birth weight infants *Pediatr Res.* 1999;45:282A
23. Clark LC, Beck E. Plasma "alkaline" phosphatase activity. I. Normative data for growing children. *J Pediatr.* 1950;36:335-341
24. Kattwinkel J, Taussig LM, Statland BE, Verter JI. The effects of age on alkaline phosphatase and other serologic liver function tests in normal subjects and patients with cystic fibrosis. *J Pediatr.* 1973;82:234-242
25. Posen S, Grunstein HS. Turnover rate of skeletal alkaline phosphatase in humans. *Clin Chem.* 1982;28:153-144
26. Glass EJ, Hume R, Hendry GMA, et al. Plasma alkaline phosphatase activity in rickets of prematurity. *Arch Dis Child.* 1982;57:373-376
27. Ross Products Division, Abbott Laboratories, Inc. *Meeting the Special Nutrient Needs of Low-Birth-Weight and Premature Infants in the Hospital.* Columbus, Ohio: Ross Products Division, Abbott Laboratories, Inc; 1998:56

## UNBORN CRIMINALS!?

Two highly regarded economists contend that a large share of the drop in crime in the 1990s—perhaps as much as half—can be attributed to the sharp increase in abortions after the Supreme Court ruling in *Roe v. Wade* in 1973.

Goode E. Linking drop in crime to rise in abortion. *New York Times.* August 20, 1999

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