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ORIGINAL ARTICLE

Evaluation of human milk fortification from the time of the first feeding: effects on infants of less than 31 weeks gestational age

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Objective: To determine whether human milk fortification from the time of the first feeding significantly improves weight gain and bone mineral status in infants of <31 weeks estimated gestational age as compared with delayed or standard human milk fortification.

Study Design: This was a retrospective pre–post design. In all, 95 infants born at <31 weeks estimated gestational age were compared. There were 53 infants in the early fortification group (EFG) and 42 infants in the delayed fortification group (DFG). They were compared with regard to weight gain at 34 weeks postmenstrual age (PMA), and their serum levels of calcium, phosphorus and alkaline phosphatase levels were compared as an indicator of bone mineral status. The practice change of fortifying all human milk given to preterm infants at <34 weeks PMA commenced in June 2009. The usual practice of fortification took place once an infant had reached a feeding volume of 50 to 100 ml kg^{-1} per day. The new practice fortified all human milk with a powdered human milk fortifier to 24 calories per ounce, starting with the first feeding, no matter how small the volume.

Result: There were no differences in weight gain between the EFG and the DFG. The group that received fortification from the time of the first feeding were significantly less likely to have alkaline phosphatase levels >500 U l^{-1} from 33 weeks PMA onward. There was no incidence of feeding intolerance with early fortification.

Conclusion: Fortification of human milk from the time of the first feeding does not affect weight gain at 34 weeks PMA, but is related to a lower incidence of elevated alkaline phosphate levels and does not cause feeding intolerance.

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Introduction

Survival rates for low birth weight infants continue to improve despite an increase in their total number over the past 10 years.[1] A major challenge in the care of these infants is ensuring adequate nutritional intake for growth, both in the hospital and after discharge, while balancing the potential complications associated with providing this nutrition. Nutritional requirements are higher for premature infants because most glucose, protein, mineral and fatty acid stores are accelerated in the third trimester of pregnancy.[2] Because of the late accretion of these important nutrients, the more premature the infant is at birth, the more deficient the nutrient stores.

Despite the infant growth targets based on intrauterine growth and fetal nutrient accretion rates established by the American Academy of Pediatrics, preterm infants born between 24 and 29 weeks gestation do not achieve the median birth weight of a fetus at the same postmenstrual age (PMA)[3] and 99% of extremely low birth weight infants are below the 10th percentile at 36 weeks PMA.[4] Early provision of adequate nutrition for preterm infants can help address their nutritional deficits, and improve growth and neurodevelopmental outcomes;[5–7] therefore, the importance of timeliness in meeting these nutritional needs cannot be ignored. One strategy to meet the preterm infant's nutritional needs is fortification of human milk. Human milk is considered to be the best source of nutrition for all infants[8] and has been shown to improve developmental outcomes.[9,10] However, in the case of preterm infants, differences in the constituents of human milk vary greatly depending on maternal age, nutrition and period of lactation.[11] Therefore, human milk does not always meet the preterm infant's increased nutrient and protein demand.[12] Standard fortification of human milk has shown to increase growth in preterm infants,[13] and newer methods of fortification such as individualized (targeted to actual human milk content) and adjustable (human milk content changed on the basis of infant serum lab values) fortification are proving to be beneficial.[14] In spite of these advances, there is still no consensus on the optimum method of fortification.

The use of commercially available human milk fortifiers is a common practice in the care of preterm infants. However, there are no data on the effects of using these commercial fortifiers from the time of the first feeding. Research on human milk fortifiers suggest that it is safe and beneficial for preterm infants[15–17] when fortification is initiated after the infant reaches between 100 and 150 ml kg^{-1} of feeding volume. Fortification with a human-based fortifier has been shown to be safe when given as early as at 40 ml kg^{-1} of feeding volume.[18] Protein fortification that is adjusted to each infant's metabolic response is also beneficial when initiation of fortification is begun only when the infant reaches a feeding volume of 90 ml kg^{-1}.[19] Studies on human milk fortification and its effects on growth outcomes have failed to address the use of fortified human milk, excluding any infants that were fed the preterm formula, and have not employed early fortification. Therefore, the purpose of this study was to evaluate the impact of human milk fortification from the time of the first feeding on growth and bone mineral status in infants born at <31 weeks gestation.

Methods

Study design

A retrospective pre–post design was used to compare the effects of early human milk fortification (with the first feeding) with delayed fortification (once the infant reached 50 to 80 ml kg^{-1} per day of enteral feeds). Fortification was done with a commercially available (Enfamil, Mead Johnson, LLC Evansville, IN, USA), powdered human milk fortifier, which was added to each mother's own milk or banked donor milk if maternal milk was not available. Data were retrieved from the neonatal database (Neodata) that is used daily in this neonatal intensive care unit and by an individual chart review following approval of the study by the Institutional Review Board.

Practice change

Feeding practices in this unit for infants at <34 weeks PMA include early initiation of feeds and routine advancement of feedings every other day by no more than 20 ml kg^{-1} per day. In addition, infants receive 125 mg kg^{-1} of Lactinex (Becton, Dickinson, Sparks, MD, USA) every 12 h until they reach 34 weeks PMA. Lactinex is a probiotic that contains the organisms *Lactobacillus acidophilus* and *L. bulgaricus* as the active ingredients. Lactinex was chosen because of its bacterial count consistency, and it was used throughout the study period for both early and delayed fortification. The probiotic powder is mixed with the infant's feedings for administration. Before the practice change, once the infant had been tolerating feedings for 10 to 14 days without feeding intolerance, human milk was fortified to 24 calories per ounce using a powdered human milk fortifier (delayed fortification). The mean feeding volume at the time of late fortification was 85 ml kg^{-1} per day and the mean number of days of life on which this fortification occurred was 24. Feeding intolerance was defined as excessive residuals, emesis or an abnormal abdominal examination.

In June 2009, human milk given to all infants born at <34 weeks PMA was fortified at 24 calories per ounce from the first feeding (early fortification). No infant at <34 weeks PMA was given unfortified human milk. As donor milk in this unit is available for use by all infants until 34 weeks PMA, choosing infants <31 weeks gestation at birth for inclusion in this study gave each infant a minimum of 3 weeks to receive fortified human milk before evaluation. To accurately fortify small feeding volumes, a gram-scale was used to add powdered fortification to the milk when the 24-h volume needed was less than 25 ml. There were no other practice changes during this study period.

Setting and subjects

All infants admitted to a level III southeastern neonatal intensive care unit born at <31 weeks PMA between June 2008 and June 2010 were eligible for inclusion in this study. This time frame was chosen to capture infants 1 year before and after the early fortification practice change and to minimize variation in nutritional practices over the years that could affect the outcomes. Infants were included in the analysis if they exclusively received human milk until 34 weeks PMA, were free of major congenital anomalies, had enteral feedings for at least 48 h before death and, if transferred to another facility for care, were away for no longer than 7 days. Infant transfers included the need for surgical procedures such as ventricular access device placement, ventriculoperitoneal shunt placement, patent ductus arteriosus closure and surgery for retinopathy of prematurity.

One hundred and twenty-eight infants <31 weeks PMA were admitted between June 2008 and June 2010. Ninety-five of these infants were eligible for analysis. Most of the infants excluded from the analysis were fed the formula before 34 weeks PMA. Three infants born at 23 weeks PMA had a spontaneous gastrointestinal perforation before feeding in the first week of life and were transferred to another facility for surgical evaluation. Two infants were transferred out of the facility secondary to overcapacity and an additional four infants were excluded because the caloric content of their human milk exceeded 26 calories per ounce before 34 weeks PMA. The early fortification group (EFG) comprised 53 infants, whereas the delayed fortification group (DFG) included 42 infants. Characteristics of the infants are detailed in Table 1.

Outcome measures

The primary outcomes measured were growth at 34 weeks and bone mineral status throughout hospitalization. Growth was measured as weight gain in grams from birth until 34 weeks PMA. Thirty-four weeks was chosen, as it could not be guaranteed that an infant would get only human milk after this time; this was the age at which donor milk was no longer available. All weights were obtained using electronic scales. Registered nursing staff or nurse practitioners performed the measurements. Alkaline phosphatase, calcium and phosphorus levels were evaluated as an indicator of

Table 1 Demographic and clinical characteristics

	DFG (n = *42*)	*EFG* (n = *53*)
Gestational age at birth (weeks)	27.8 ± 1.8	27.8 ± 1.8
Birth weight (g)	1072 ± 300	1173 ± 330
Fetal growth status (SGA)	6 (14%)	3 (6%)
Male gender	17 (40%)	29 (55%)
Race		
White	10 (24%)	13 (25%)
African	18 (43%)	16 (30%)
Hispanic-non-white	9 (21%)	19 (36%)
Unknown/other	5 (12%)	5 (9%)
Maternal age (years)	28.9 ± 6.4	31.5 ± 7.3
Apgar at 1 min	5.3 ± 2.6	5.9 ± 2.3
Apgar at 5 min	7.8 ± 1.8	8.5 ± 0.9**
Day of life for first feeding (days)	2.5 ± 1.8	1.6 ± 0.8*
Sepsis	31 (74%)	24 (45%)*
Patent ductus arteriosus	11 (26%)	12 (23%)
NEC	0	1 (2%)
Days on ventilator	16 ± 23.1	18 ± 29.3
Transferred out <7 days during stay (*n*)	3 (7%)	3 (6%)
Discharge weight	2679 ± 684	2540 ± 672

Abbreviations: DFG, delayed fortification group; EFG, early fortification group; NEC, necrotizing enterocolitis; SGA, small for gestational age.
Mean ± s.d. or *n* (%).
Race: Asian, other/unknown combined for the χ^2 analysis; sepsis: positive blood/urine culture and/or elevated C-reactive protein level.
Days on ventilator transformed to a natural log for analysis to normalize the data. Unadjusted value of days on ventilator presented in table.
*$P \leqslant 0.05$ and **$P \leqslant 0.01$ for group differences.

bone mineral status. The alkaline phosphatase levels were not fractionated. Starting at 2 weeks of age, infants had comprehensive nutritional profiles drawn weekly until discharge. Days to full feeds, episodes of feeding intolerance and incidence of necrotizing enterocolitis (NEC) were also evaluated to assess whether early fortification could potentially be an unsafe practice. Days to full feeds were measured as the number of days it took for an infant to first reach full feedings, defined as reaching a minimum of 125 ml kg^{-1} per day of enteral intake. The days were calculated from the first day of feeding and included any day on which feedings were held. Feeding intolerance was defined as an infant being made nothing by mouth for at least 24 h secondary to emesis or abdominal distention, before 34 weeks PMA. An infant either had feeding intolerance or did not, and the number of episodes was not taken into account. Incidence of NEC was also recorded and defined as clinical signs plus pneumatosis intestinalis or portal venous gas on abdominal radiographs.[20]

Data analysis

Non-directional hypotheses were tested and the significance level was set at 0.05. The significance level was not adjusted for multiple tests because of the exploratory nature of the study.

Group differences in demographic and clinical characteristics were examined using independent *t*-tests and χ^2-tests. Non-parametric Wilcoxon rank sum tests and Fisher's exact tests were applied when the assumptions of the above tests were not met. Birth weight and any clinical or demographic measures for which the groups differed at baseline ($P \leqslant 0.10$) were considered to be potential covariates in the growth and bone mineral status analyses.

Analysis of covariance methods were used to test for group differences in mean weight gain at 34 weeks PMA. Random coefficients regression model (a type of mixed-effects model for repeated measurements) was used to test for group differences in the trajectory of change in alkaline phosphatase, calcium and phosphorus levels across the period of 27 through 38 weeks PMA, controlling for the initial level of bone mineral measure, birthweight and other significant covariates. Group differences in the safety outcomes were conducted using independent *t*-tests. A random coefficients regression model was also conducted to examine the change in protein levels in the groups during the 27 to 38 weeks PMA period.

Results

Demographic and clinical characteristics

Table 1 presents the characteristics of the two fortification groups. The groups differed significantly in their Apgar score at 5 min ($P<0.01$), day of life for first feeding ($P<0.005$) and incidence of sepsis ($P<0.005$), with the EFG having a higher mean 5-min Apgar score, a higher proportion of infants who were fed earlier and a lower likelihood of sepsis when compared with the DFG. Apgar modes were the same in both the early and late fortification group at 1 (mode = 8) and 5 (mode = 9) min.

Growth outcomes

The initial analysis of covariance model that tested differences in mean weight gain at 34 weeks PMA in the groups included birthweight, SGA, maternal age, gender, sepsis, day of life for first feeding and Apgar at 5 min as covariates. Maternal age, gender, day of life for first feeding and Apgar at 5 min were omitted from the final model because of lack of significance ($P>0.10$). The groups did not differ significantly on mean weight gain at 34 weeks PMA ($F_{1,90} = 1.17$, $P<0.29$), after controlling for birthweight ($F_{1,90} = 29.17$, $P<0.001$), sepsis ($F_{1,90} = 6.3$, $P<0.02$) and SGA ($F_{1,90} = 6.65$, $P<0.02$). The mean weight gain in grams adjusted for covariates was slightly lower in the EFG (728 ± 249.3) when compared with the DFG (785 ± 250.1). As previous studies have shown fortification to benefit smaller preterm infants,[17] analysis of covariance was performed on weight gain in the subgroup of 60 infants born at ⩽28 weeks. The results indicated that the EFG (875 ± 251) and DFG (940 ± 251) did not differ significantly ($P>0.05$). The random coefficients regression model analysis

adjusting for covariates confirmed that serum protein levels remained stable from 27 to 38 weeks in both groups. No group, time or group-by-time interactions were observed ($P>0.05$). Similarly, the *t*-test on the average protein level for each infant did not show a group difference ($P>0.05$).

Bone mineral status

The trajectory analyses, after adjusting for initial level of the bone mineral serum measurement, birthweight and sepsis, did not indicate a significant fortification group effect with regard to alkaline phosphatase, calcium and phosphorus levels from 27 through 38 weeks PMA (all $P>0.05$). SGA, maternal age, gender, day of life for first feeding and Apgar at 5 min were dropped from the final model. Table 2 presents the unadjusted and adjusted (predicted) means for each measure over time.

Calcium change was best depicted as a significant quadratic function over time in both groups (time: $F_{1, 542}=3.96$, $P<0.05$; time^2: $F_{1, 566}=5.50$, $P<0.02$) and only initial calcium level was a significant covariate ($F_{1, 113}=25.69$, $P<0.001$). Phosphorus significantly increased in a linear fashion across the time interval in both groups (time: $F_{1, 53.4}=12.58$, $P<0.001$), but the pattern of change did not differ in the two groups (group: $F_{1, 48.5}=0.69$, $P=0.41$; group-by-time interaction: $F_{1, 52.2}=0.64$, $P=0.43$). In addition, initial phosphorus level, birthweight and sepsis significantly influenced phosphorus levels over time (all $F>4.5$, $P<0.03$). A significant quadratic relation of alkaline phosphatase in each group over time was observed (time: $F_{1, 473}=10.39$, $P<0.002$; time^2: $F_{1, 560}=17.21$, $P<0.001$), with the levels of alkaline phosphatase consistently but not significantly higher on average in DFG relative to the EFG (group: $F_{1, 132}=0.53$, $P>0.05$). EFG tended to have a more pronounced quadratic function relative to the DFG (group-by-time interaction: $F_{1, 414}=1.66$, $P<0.20$; group-by-time^2 interaction: $F_{1, 554}=2.71$, $P=0.10$) after controlling for birthweight ($F_{1, 128}=7.62$, $P<0.01$), sepsis ($F_{1, 114}=3.19$, $P<0.08$) and the initial levels of alkaline phosphatase ($F_{1, 111}=124.79$, $P<0.001$).

Alkaline phosphatase values were then dichotomized into low ($<500\,U\,l^{-1}$) and high ($>500\,U\,l^{-1}$) levels to compare the percentage of high scores at weeks 27 through 38. When an alkaline phosphatase value was missing, an estimated value derived from the above random coefficients regression model trajectory analysis of change over time in alkaline phosphatase was imputed so that between-group difference in the percentage of infants with alkaline phosphatase value was >500 at each PMA week (Figure 1d). The DFG had a significantly higher proportion of infants with alkaline phosphatase ($>500\,U\,l^{-1}$) than did the EFG group at week 27 (DFG = 31.0%, EFG = 11.3%, $\chi^2=5.64$, d.f. = 1, $P<0.02$), week 28 (DFG = 35.7%, EFG = 15.1%, $\chi^2=5.42$, d.f. = 1, $P<0.02$) and week 29 (DFG = 38.1%, EFG = 18.9%, $\chi^2=4.36$, d.f. = 1, $P<0.04$). The groups did not differ significantly after week 29. Figures 1a–d provide a graphical representation of the bone mineral status indicators from PMA weeks 27 through 38.

Safety

The EFG and DFG did not differ significantly in terms of days to full feeds and measures of feeding tolerance ($P>0.05$). Between-group differences were not observed on any of the other safety measures. Interestingly, five infants in the EFG had documented bloody stools without NEC and an unremarkable abdominal

Table 2 Bone mineral status: unadjusted and adjusted means and s.d. for each group

PMA week	Alkaline phosphatase ($U\,l^{-1}$)				Phosphorus ($mg\,dl^{-1}$)				Calcium ($mg\,dl^{-1}$)			
	DFG		EFG		DFG		EFG		DFG		EFG	
	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted
27	471 ± 145	415 ± 170	342 ± 91	306 ± 145	4.1 ± 2.0	6.0 ± 0.8	5.7 ± 1.3	6.0 ± 0.7	10.0 ± 0.7	9.9 ± 0.2	9.8 ± 0.2	10.3 ± 0.2
28	553 ± 131	432 ± 165	424 ± 185	345 ± 146	4.4 ± .8	6.1 ± 0.7	5.6 ± 1.7	6.1 ± 0.7	9.9 ± 0.8	9.8 ± 0.2	10.3 ± 0.6	10.2 ± 0.2
29	447 ± 198	445 ± 162	521 ± 229	374 ± 147	6.2 ± 1.8	6.1 ± 0.7	5.9 ± 1.5	6.2 ± 0.6	9.5 ± 0.5	9.8 ± 0.2	9.9 ± 0.6	10.1 ± 0.2
30	459 ± 213	454 ± 160	414 ± 142	395 ± 149	6.3 ± 1.4	6.2 ± 0.7	6.4 ± 1.8	6.3 ± 0.6	9.8 ± 0.5	9.8 ± 0.2	9.9 ± 0.4	10.0 ± 0.2
31	452 ± 181	460 ± 168	470 ± 278	408 ± 152	6.7 ± 1.9	6.3 ± 0.6	5.9 ± 1.1	6.4 ± 0.5	9.9 ± 0.5	9.8 ± 0.2	10.2 ± 0.6	10.0 ± 0.2
32	476 ± 262	462 ± 164	447 ± 247	411 ± 155	6.2 ± 1.2	6.3 ± 0.6	6.3 ± 0.9	6.5 ± 0.5	9.7 ± 0.6	9.8 ± 0.2	10.0 ± 0.5	10.0 ± 0.2
33	447 ± 218	460 ± 168	399 ± 193	406 ± 158	6.3 ± 1.3	6.4 ± 0.5	6.7 ± 1.1	6.6 ± 0.5	9.8 ± 0.6	9.8 ± 0.2	9.8 ± 1.3	9.9 ± 0.3
34	479 ± 261	453 ± 174	368 ± 166	392 ± 163	6.1 ± 1.0	6.5 ± 0.5	6.9 ± 1.1	6.8 ± 0.5	9.9 ± 0.4	9.9 ± 0.2	10.0 ± 0.5	10.0 ± 0.3
35	452 ± 277	443 ± 181	379 ± 187	369 ± 167	6.4 ± 0.8	6.5 ± 0.5	6.9 ± 0.7	6.9 ± 0.5	9.9 ± 0.4	9.9 ± 0.3	9.8 ± 0.5	10.0 ± 0.3
36	449 ± 229	429 ± 190	430 ± 249	338 ± 172	6.5 ± 1.1	6.6 ± 0.5	6.4 ± 0.8	7.0 ± 0.5	9.9 ± 0.4	10.0 ± 0.3	9.9 ± 0.5	10.0 ± 0.3
37	498 ± 220	412 ± 200	424 ± 211	297 ± 178	6.5 ± 0.9	6.7 ± 0.5	7.1 ± 0.5	7.1 ± 0.5	9.8 ± 0.6	10.0 ± 0.3	10.0 ± 0.4	10.1 ± 0.3
38	496 ± 282	390 ± 210	430 ± 130	248 ± 184	6.4 ± 0.8	6.7 ± 0.5	7.2 ± 0.5	7.2 ± 0.5	9.9 ± 0.4	10.1 ± 0.3	10.0 ± 0.6	10.1 ± 0.3

Abbreviations: DFG, delayed fortification group; EFG, early fortification group; PMA, postmenstrual age.
Adjusted scores are estimated (predicted) scores that take into account the fixed and random effects specified in random coefficients regression model for repeated measurements.

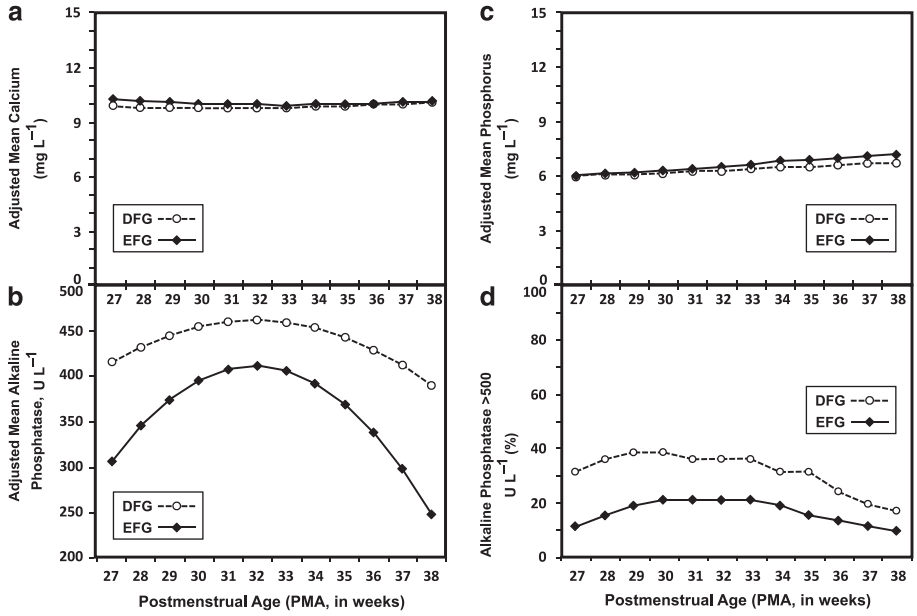

Figure 1 (**a**–**c**) Change in calcium, phosphorus and alkaline phosphate between 27 and 38 weeks PMA, and (**d**) cumulative percent rate between 27 and 38 weeks during which alkaline phosphate level is $>500\,U\,l^{-1}$.

Table 3 Outcome measures

	DFG (n = *42*)	*EFG* (n = *53*)	*DFG to EFG (ES)*
Day of life to full feeds (days)	28.4 ± 11.7	27 ± 11.8	−0.12
Feeding intolerance without NEC	6 (14%)	6 (11%)	+0.15
Bloody stools without NEC	1 (2%)	4 (8%)	−0.66
Serum protein levels ($g\,dl^{-1}$)	4.6 ± 0.3	4.7 ± 0.5	−0.24
Weight at 34 weeks (g)	1895 ± 310	1867 ± 303	+0.09
Length of stay (days)	72.4 ± 30.5	63 ± 30.1	−0.31

Abbreviation: DFG, delayed fortification group; EFG, early fortification group; ES, effect size; NEC, necrotizing enterocolitis.
Covariates that differed ($P \leqslant 0.01$) were used in this analysis.
Values are shown as mean ± s.d. or *n* (%).
Mean serum protein level for each infant was calculated and included in the analysis; all $P > 0.05$ for group differences.

examination; only one infant in the DFG had bloody stools. One infant in the DFG had the diagnosis of microcolon at 36 weeks PMA. Table 3 summarizes the safety outcomes.

The two groups were not statistically significant in terms of safety outcome measures. Despite a small sample size and insufficient statistical power, the results indicated that the EFG infants had a relatively lower risk of feeding intolerance without NEC and a shorter length of stay, on average, relative to the DFG. The DFG was 1.31 times more likely to have feeding intolerance compared with the EFG (odds ratio = 1.31, 95% confidence interval (CI) = 0.39 to 4.39), and the risk of this event in the DFG was estimated to be 126% of that reported in the EFG (relative risk = 1.26, 95% CI = 0.44 to 3.63). The 95% CIs for the odds ratio and relative risk findings, however, were wide and the effect size (ES) was small (ES = 0.15). The mean length of stay was also less in the EFG and the magnitude of the effect was in the moderate range (ES = −0.31). The proportion of cases with bloody stools without NEC was lower in the DFG compared with the EFG (ES = −0.66). The estimated odds of having this event was less than one-third (odds ratio = 0.30, 95% CI = 0.03 to 2.78) as high among those in the DFG as among those in the EFG. Similarly, the risk of having this adverse event was lower in the DFG (relative risk = 0.32, 95% CI = 0.04 to 2.72) than in the EFG. However, the 95% CIs for the odds ratio and relative risk estimates were large. The groups had similar outcomes and resulting small ESs ($\leqslant 0.25$) on the remaining safety measure.

Discussion

In this retrospective analysis, infants who received early fortification of human milk did not differ in weight gain at 34 weeks PMA when compared with infants whose fortification was delayed. However, the average length of time for infants to receive the fortified human milk was only 5 weeks, which may have been too short a time for a cumulative effect on weight gain to be appreciated, especially for the extremely preterm infant. Despite a small sample size and low statistical power, the study did provide valuable information regarding the direction and magnitude of effect (ES = 0.23) with regard to weight gain. As commercial fortifiers are based on assuming human milk content, under or over-nutrition could be a factor as exact composition of the human milk before fortification was unknown. Human milk analyzers may soon be a common finding in neonatal intensive care units as this

will be the first step to being able to customize feedings to the very preterm infants based on their individual needs. It would have been beneficial to see if there was a difference between those infants who received maternal milk versus those who had only donor milk. Donor milk is most often from mothers who delivered at term, and has been found to be lower in protein and other nutrients crucial to the preterm infant.[21] Although there were no differences in weight gain at 34 weeks PMA, the study was underpowered to detect a significant difference in this area. In addition, weight gain may be related to acid–base balance and metabolic acidosis may, in fact, affect weight gain.[22] The incidence of metabolic acidosis was not evaluated. Recent data suggest that early fortification is well tolerated, but the use of bovine-based human milk fortifier may contribute to an increased rate of NEC when compared with human-based human milk fortification.[18] Further study is needed to determine the best initiation time and fortification product for human milk fortification.

There are multiple risk factors for development of osteopenia of prematurity, and an inadequate supply of calcium and phosphorus after delivery remains a prominent cause. In this study, infants receiving early fortification had a significantly lower incidence of having an alkaline phosphatase level >500 starting at 33 weeks PMA. Elevated alkaline phosphatase levels and low serum phosphorus levels have been shown to be an early predictor of metabolic bone disease in premature infants[23,24] as well as a good predictor of length at 18 months and height at adolescence.[25,26] More recent data suggest that calcium and phosphorus supplementation does not need to necessarily match the high intrauterine accretion rates, suggesting that adaptation and stimulation, by the preterm infant itself, may provide part of the mineral requirements for postnatal bone formation.[27] Without dual-emission X-ray absorptiometry to analyze actual bone mineral status, we do not know the direct impact that early fortification may have with regard to bone mineral density. Long-term follow-up of growth to include bone mineral status and peak bone mass would be beneficial in these groups.

Literature shows that the fortification of human milk increases the osmolality of the milk[28] and also may decrease gastric motility as evidenced by increased residuals.[29] Fortification also slows gastric emptying, but in a preliminary study this has not been shown to be significant.[30] Residuals are checked on our infants, but rarely affect routine feedings without evidence of emesis or an abnormal abdominal exam. In this comparison, there was neither an increase in time to full feeds nor an increase in feeding intolerance with the EFG, suggesting the early fortification was well tolerated. The incidence of bloody stools with no other gastrointestinal symptoms was of interest, but not significant. This finding could be the result of a cow's milk protein sensitization from the powdered Human milk fortification[31] and it may prove beneficial to follow in future studies with larger samples. Increased protein administration, by way of early fortification, to preterm infants at an early age brings into question whether or not they can tolerate the renal solute load, but evidence clearly shows that even in extremely preterm human milk (<28 weeks) the protein content averages 2.3 g dl^{-1}[32] and the addition of human milk fortifier adds only 0.28 g per 25 ml, which is still a minimal amount of protein when compared with the recommended daily intake as mentioned previously. Protein intakes are clearly not what we assume after analysis of human milk.[19] New findings suggest that the optimal fortification will be tailored to each preterm infant[14] and this may require that human milk analyzers become a necessary tool in neonatal intensive care units.

Additional research investigating the incidence of metabolic acidosis with the use of early fortification may be helpful. Also, the use of early fortification, combined with preterm human milk only, and its effect on growth and tolerance in infants <1000 g need to be studied. The impact that early fortification might have had on the infants of mothers <20 years of age is of interest. Maternal nutrition may account for more than 50% of the cause of low birth weight.[33] This study sample only had seven mothers under the age of 20 and therefore we were not powered to study these effects. As infants in the EFG had a decreased occurrence of alkaline phosphatase levels >500 U l^{-1}, it would be of interest to see whether there is a difference in their peak bone mass as adults.

Fortification of human milk for feeding premature infants is a necessary practice[34] as human milk is not adequate for the preterm infant without the added nutrients.[32] Because of this, fortification with powdered, and now liquid, human milk fortifiers is common practice. In this preliminary study, fortification of human milk from the time of the first feeding was related to a lower incidence of elevated alkaline phosphate levels and was not related to any feeding intolerance. Until the optimum fortification method is determined, we should consider using standard fortification at the earliest opportunity.

Conflict of interest

The authors declare no conflict of interest.

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References

1 Behrman R, Butler A., Institute of Medicine (U.S.). Committee on Understanding Premature Birth and Assuring Healthy Outcomes. *Preterm Birth: Causes, Consequences and Prevention*. National Academics Press: Washington, DC, 2007.

2 Shah M, Shah S. Nutrient deficiencies in the premature infant. *Pediatr Clin N Am* 2009; **56**: 1069–1083.
3 Ehrenkranz M, Younes N, Lemons J, Fanaroff A, Donovan E, Wright L *et al.* Longitudinal growth of hospitalized very low birth weight infants. *Pediatrics* 1999; **104**: 280–289.
4 Lemons J, Bauer C, Oh W, Korones S, Papille, Stoll B *et al.* Very low birth weight outcomes of the National Institute of Child Health and Development Neonatal Research Network. *Pediatrics* 2001; **107**: E1.
5 Stephens B, Walden R, Gargus R, Tucker R, McKinley L, Mance M *et al.* First-week protein and energy intakes are associated with 18-month developmental outcomes in extremely low birth weight infants. *Pediatrics* 2009; **123**: 1337–1343.
6 Isaacs E, Morley R, Lucas A. Early diet and general cognitive outcome at adolescence in children born at or below 30 weeks gestation. *J Pediatr* 2009; **155**: 229–234.
7 Martin C, Brown Y, Ehrenkranz R, O'Shea TM, Allred E, Belfort M *et al.* Extremely low gestational age newborns study investigators. Nutritional practices and growth velocity in the first month of life in extremely premature infants. *Pediatrics* 2009; **124**: 647–657.
8 Gartner LM, Morton J, Lawrence RA, Naylor AJ, O'Hare D, Schanler RJ *et al.* Breastfeeding and the use of human milk. *Pediatrics* 2005; **115**: 496–506.
9 Ehrenkranz RA, Dusick AM, Vohr BR, Wright LL, Wrage LA, Poole WK *et al.* Growth in the neonatal intensive care unit influences neurodevelopmental and growth outcomes of extremely low birth weight infants. *Pediatrics* 2006; **117**: 1253–1261.
10 Vohr BR, Poindexter BB, Dusick AM, McKinley LT, Wright LL, Langer JC *et al.* Beneficial effects of breast milk in the neonatal intensive care unit on the developmental outcome of extremely low birth weight infants at 18 months of age. *Pediatrics* 2006; **118**: e115–e123.
11 Sauer CW, Kim JH. Human milk macronutrient analysis using point-of-care near-infrared spectrophotometry. *J Perinatol* 2010; **31**(5): 339–343.
12 Kuschel CA, Harding JE. Protein supplementation of human milk for promoting growth in preterm infants. *Cochrane Database Syst Rev* 2000; (3): CD000433.
13 Kuschel C, Harding J. Multicomponent fortified human milk for promoting growth in preterm infants. *Cochrane Database Syst Rev* 2000; (3): CD000343 update in 2004; (1): CDC000343.
14 Arslanoglu S, Moro G, Ziegler E, WAPM Working Group on Nutrition. Optimization of human milk fortification in preterm infants: new concepts and recommendations. *J Perinat Med* 2010; **38**: 233–238.
15 Reis BB, Hall TR, Schanler RJ, Berseth CL, Chan G, Ernst JA *et al.* Enhanced growth of preterm infants fed a new powdered human milk fortifier: A randomized, controlled trial. *Pediatrics* 2000; **106**: 581–588.
16 Berseth CL, Van Aerde JE, Gross S, Stolz SI. Growth, efficacy, and safety of feeding an iron-fortified human milk fortifier. *Pediatrics* 2004; **114**: e699–e706.
17 Mukhopadhyay K, Narang A, Mahajan R. Effect of human milk fortification in appropriate for gestation and small for gestation preterm babies: A randomized, controlled trial. *Indian Pediatr* 2007; **44**: 286–290.
18 Sullivan S, Schanler R, Kim J, Patel A, Trawoger R, Kiechl-Kohlendorfer U *et al.* An exclusively human milk-based diet is associated with a lower rate of necrotizing enterocolitis than a diet of human milk and bovine milk-based products. *J Pediatr* 2010; **156**: 562–567.
19 Arslanoglu S, Moro GE, Ziegler EE. Preterm infants fed fortified human milk receive less protein than they need. *J Perinatol* 2009; **29**: 489–492.
20 Martin R, Fanaroff A, Walsh M. *Fanarof and Martin's Neonatal-Perinatal Medicine: Diseases of the Fetus and Infant*, 9th edn. Mosby, Inc: St Louis, MO, 2011.
21 Heiman H, Schanler RJ. Benefits of maternal and donor milk for premature infants. *Early Hum Dev* 2006; **82**: 781–787.
22 Rochow N, Jochum F, Redlich A, Korinekova Z, Linnermann K, Weitmann K *et al.* Fortification of breast milk in VLBW infants: Metabolic acidosis is linked to the composition of fortifiers and alters weight gain and bone mineralization. *Clin Nutr* 2011; **30**: 99–105.
23 Hung Y, Chen P, Jeng S, Hsieh C, Peng S, Yen R *et al.* Serial measurements of serum alkaline phosphatase for early prediction of osteopaenia in preterm infants. *J Paediatr Child Health* 2011; **47**: 134–139.
24 Kulkarni PB, Hall RT, Rhodes PG, Sheehan MB, Callenbach JC, Germann DR *et al.* Rickets in very low-birth-weight infants. *J Pediatr* 1980; **96**: 275–282.
25 Fewtrell MS, Cole TJ, Bishop NJ, Lucas A. Neonatal factors predicting childhood height in preterm infants: Evidence for a persisting effect of early metabolic bone disease? *J Pediatr* 2000; **137**: 668–673.
26 Lucas A, Brooke OG, Baker BA, Bishop N, Morley R. High alkaline phosphatase activity and growth in preterm neonates. *Arch Dis Child* 1989; **64**: 902–909.
27 Aly H, Moustafa MF, Hassanein SM, Massaro AN, Amer HA, Patel K. Physical activity combined with massage improves bone mineralization in premature infants: a randomized trial. *J Perinatol* 2004; **24**: 305–309.
28 DeCurtis M, Candusso M, Pieltain C, Rigo J. Effect of fortification on the osmolality of human milk. *Arch Dis Child* 1999; **81**: F141–F143.
29 Moody GJ, Schanler RJ, Lau C, Shulman RJ. Feeding tolerance in premature infants fed human milk. *J Pediatric Gastroenterol Nutr* 2000; **30**: 408–412.
30 Yigit S, Akgoz A, Memisoglus A, Akata D, Ziegler E. Breastmilk fortification: Effect on gastric emptying. *J Maternal Fetal Neonatal Med* 2008; **21**: 843–846.
31 Vlieghe V, Roches AD, Payot A, Lachance C, Nuyt AM. Human milk fortifier in preterm babies: source of cow's milk protein sensitization? *Allergy* 2009; **64**: 1690–1691.
32 Bauer J, Gerss J. Longitudinal analysis of macronutrients and minerals in human milk produced by mothers of preterm infants. *Clin Nutr* 2010; **30**: 215–220.
33 Kramer MS. Determinants of low birth weight: methodological assessment and meta-analysis. *Bull World Health Organ* 1987; **65**: 663–737.
34 Heiman H, Schanler R. Enteral nutrition for premature infants: the role of human milk. *Seminars in Fetal & Neonatal Medicine* 2007; **12**: 26–34.