Effect of increasing protein content of human milk fortifier on growth in preterm infants born at <31 wk gestation: a randomized controlled trial

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ABSTRACT

Background: Preterm human milk–fed infants often experience suboptimal growth despite the use of human milk fortifier (HMF). The extra protein supplied in fortifiers may be inadequate to meet dietary protein requirements for preterm infants.

Objective: We assessed the effect of human milk fortified with a higher-protein HMF on growth in preterm infants.

Design: This is a randomized controlled trial in 92 preterm infants born at <31 wk gestation who received maternal breast milk that was fortified with HMF containing 1.4 g protein/100 mL (higher-protein group) or 1.0 g protein/100 mL (current practice) until discharge or estimated due date, whichever came first. The HMFs used were isocaloric and differed only in the amount of protein or carbohydrate. Length, weight, and head-circumference gains were assessed over the study duration.

Results: Length gains did not differ between the higher- and standard-protein groups (mean difference: 0.06 cm/wk; 95% CI: −0.01, 0.12 cm/wk; P = 0.08). Infants in the higher-protein group achieved a greater weight at study end (mean difference: 220 g; 95% CI: 23, 419 g; P = 0.03). Secondary analyses showed a significant reduction in the proportion of infants who were less than the 10th percentile for length at the study end in the higher-protein group (risk difference: 0.186; 95% CI: 0.370, 0.003; P = 0.047).

Conclusions: A higher protein intake results in less growth faltering in human milk–fed preterm infants. It is possible that a higher-protein fortifier than used in this study is needed. This trial was registered with the Australian New Zealand Clinical Trials Registry (http://www.anzctr.org.au/) as ACTRN12606000525583.


INTRODUCTION

Adequately nourishing infants born at <31 wk gestation is challenging for clinicians. These infants have high nutrient requirements for rapid tissue accretion and a limited capacity for nutrient transfer because of the immaturity of the gastrointestinal tract. Nutrient intakes, particularly protein, are low in comparison to requirements (1–7). Consequently, growth in the neonatal period is frequently suboptimal compared with intrauterine growth rates (2, 5-11). Human milk is the preferred feed for preterm infants (12) and confers developmental advantages for the infant (13), but it is generally acknowledged that human milk cannot adequately support growth and supplementation is required (14). Although fortification improves growth compared with unfortified human milk (15), there is evidence that infants fed preterm formula have higher rates of growth than infants fed isocaloric volumes of fortified human milk (16, 17), which appears to be due to inadequate protein intakes and arises because of incorrect assumptions about the protein concentration of preterm milk (18). Fortifier manufacturers assume an average protein concentration of preterm milk between 1.4 and 1.6 g/100 mL on the basis of samples taken early in lactation (19) and do not account for the decrease in protein over time (20–24). Consequently the current concentration of extra protein provided by HMF (20) (between 0.7 and 1.1 g/100 mL, depending on brand) is thought to be inadequate (25). Recent research has focused on an individualized approach to fortification...
on the basis of the metabolic response of the infant (26, 27). In these studies, most infants required extra fortification for most of the time, which provided additional evidence that the protein concentration of fortifiers is inadequate. In this double-blind randomized controlled trial, we aimed to determine the effect of increasing the protein content of HMF on growth in preterm infants.

SUBJECTS AND METHODS

Subjects

Infants admitted to the Women’s and Children’s Hospital and Flinders Medical Centre (Adelaide, Australia) from October 2006 to June 2008 were eligible to participate. Infants were included if they were born at <31 wk gestation and their mothers intended to supply breast milk. Infants were excluded if a major congenital or chromosomal abnormality known to affect growth was present, extra dietary protein was contraindicated, maternal milk supply was low, together with uncertainty about continuing to express milk, or they were likely to be transferred to an area where follow-up to discharge would be difficult. Infants were required to be randomly assigned before or within 3 d of commencing fortifier.

The trial was approved by the human research ethics committee of the Children, Youth and Women’s Health Service and Flinders Medical Centre. The infants’ parents provided written informed consent.

Randomization

Infants were randomly assigned to either the higher-protein or control fortifier. Stratification was by gestational age (<28 and 28–32 wk) and infant sex. Multiple births were randomly assigned to the same group according to the sex and gestational age of the first born infant. The randomization schedule was developed by an independent researcher by using a computer random-number generator to select random permuted blocks of 4. The tins of fortifier were sequentially numbered by an independent staff member according to the allocation sequence. Because more than one tin was usually required for each infant, 4 tins were allotted to the same sequential number. If more fortifier was required, it was dispensed by the independent staff member who originally sequentially numbered the tins. This staff member had no other involvement in the study. Tins were placed in 4 boxes according to the randomization strata, and once consent was obtained, the researcher (JM) or a research assistant drew the next sequentially numbered tin of fortifier from the appropriate box. The study fortifiers were developed by an independent researcher by using a computer random-number generator to select random permuted blocks of 4. The tins of fortifier were sequentially numbered by an independent staff member according to the allocation sequence. Because more than one tin was usually required for each infant, 4 tins were allotted to the same sequential number. If more fortifier was required, it was dispensed by the independent staff member who originally sequentially numbered the tins. This staff member had no other involvement in the study. Tins were placed in 4 boxes according to the randomization strata, and once consent was obtained, the researcher (JM) or a research assistant drew the next sequentially numbered tin of fortifier from the appropriate box. The study fortifiers were supplied as a powder in 100-g tins and were identical in appearance, packaging, and rate of mixing. All infants in the study, their families, caregivers, research personnel, outcome assessors, and analysts were unaware of the group allocation.

Interventions

The trial HMF (Nestlé Product Technology Centre) was based on the commercial FM85 Human Milk Supplement (Nestlé). The fortifiers consisted of extensively hydrolyzed whey protein, maltodextrin, vitamins, and minerals and were made isocaloric by adjusting the carbohydrate amount. The higher-protein, standard-carbohydrate fortifier contained 1.4 g protein added to 100 mL EBM, and the control fortifier (standard protein, higher carbohydrate) contained 1 g protein/100 mL EBM, which was equivalent to standard clinical care. The composition of the HMF is detailed in Table 1. The feeding regimen was standardized across sites and was under the direction of the attending neonatologist. The fortifier was to be commenced when the infant’s enteral intake reached ~80 mL · kg⁻¹ · d⁻¹ and continued while the infant received EBM via a bottle or feeding tube. When there was an insufficient supply of EBM, the infant was fed a standard preterm-infant formula that contained 2.4 g protein/100 mL. The study ended at discharge or the expected date of delivery, whichever came first.

Outcome assessments

Length gain was the primary outcome because this measurement more closely reflects the lean body mass gain and is not affected by hydration status (28) or fat mass gain compared with weight gain. Length was measured weekly by 2 measurers to the nearest 1 mm by using a recumbent length board (O’Leary; Ellard Instruments) according to the WHO Multicentre Growth Reference Study (29). Measurements were repeated if there was a discrepancy >7 mm (30). The average of the 2 independent measurements was taken. Infants were weighed naked at approximately the same time daily when in Level I care and twice weekly in Level II care by using electronic balance scales that are accurate to 5 g. The scales are calibrated annually by using standard weights. Head circumference was measured weekly by using a paper tape placed across the frontal bones across the eyebrows and over the occipital prominence at the back of the head. Measurement differences >5 mm were discarded and remeasured. The average of the 2 independent measurements was taken.

All anthropometric measurements were taken by a researcher (JM) and one of 3 neonatal research nurses. On occasions when these individuals were unavailable, at least one trained researcher was present and measurements were done in conjunction with the infant’s primary nurse. Interobserver variability was assessed during the trial as the technical error of measurement between the researcher (JM; the common observer between both sites) and research nurses. The technical error of measurement recorded for length for each of the 3 research nurses was 0.188, 0.243, and 0.202 cm. This compared favorably with the technical error of measurement reports for length that varied between 0.22 and 0.58 cm (31).

Biochemical analyses

Blood samples were taken weekly initially and then once every 2 wk when the infants were in Level II care. SUN, creatinine,
albumin, and pH analyses were conducted at the respective hospital laboratories accredited by the National Association of Testing Authorities, Australia.

**Dietary intake**

Dietary intake data from fluid-balance charts were collected daily and included the volume, caloric density, and supplements added for both EBM and formula. An aliquot of unfortified EBM was collected once per week from the infant’s daily volume and used to represent the weekly composition of EBM. Protein and fat content was determined by infrared spectroscopy with the use of a MilkoScan Minor (Foss) (32), which was calibrated for this trial. Protein was calculated as total nitrogen and determined by the Kjeldahl method, minus nonprotein nitrogen × 6.38, and with the assumption that 27% of the nonprotein nitrogen was bioavailable (33). Protein and fat intakes were calculated from the volume of milk ingested, the protein and fat concentration of EBM, and the manufacturer’s information on the study fortifiers and any formula consumed.

The energy content of the milk was calculated by using an assumed concentration of lactose of 6.8 g/100 mL (34) and by using the Atwater factors of 4, 4, and 9 kcal/g for protein, carbohydrate, and fat, respectively.

**Clinical data**

Feeding tolerance was recorded. Confirmed cases of necrotizing enterocolitis, sepsis, brain injury, retinopathy of prematurity, and oxygen requirement at 36 wk postmenstrual age were recorded. Clinical definitions were consistent with the Australian and New Zealand Neonatal Network (35).

**Sample size and statistical analyses**

With the use of repeated measurements, we estimated that a sample size of 80 (40 in each group) would detect a difference of 0.13 cm/wk with a power of 88% for a 2-sided significance of 5%. This was the order of difference shown in a study that used adjustable fortification (26). Because this pragmatic trial included infants fed a mixed diet of fortified milk and formula, we planned, a priori, to conduct a subgroup analysis of those infants who adhered more strictly to human milk feeding, which was defined as infants who received >70% of their enteral intake as fortified breast milk. All analyses were conducted according to the intention-to-treat principle, and the level of significance was $P < 0.05$. z scores were calculated by using mean and SD estimates in an Australian population of preterm infants (36). Differences between groups over time were assessed with a repeated-measures model by using generalized estimating equations to adjust variance estimates for clustering on siblings. Differences between groups for the outcome small-for-gestational-age and for clinical outcomes were performed by using generalized linear models. Again, generalized estimating equations were incorporated to adjust variance estimates for clustering on siblings. All models were adjusted for sex and gestational age. All statistical models were fit with SAS software (version 9.2; SAS Institute Inc). Statistical analyses of the primary outcome were conducted blinded to the study group.

![FIGURE 1. Participant flow through the trial.](image-url)
were randomly allocated to the control group (Figure 1). One set of twins (4 infants) were randomly allocated to the higher-protein group, and 10 sets of twin (20 infants) were allocated to the control group (Table 2).

### RESULTS

#### Study population

Ninety-two infants were enrolled in the trial; 43 infants were randomly allocated to the higher-protein group, and 49 infants were randomly allocated to the control group (Figure 1). One infant was withdrawn from each group by parental request because of perceived gastrointestinal intolerance; however, the parents agreed to the continued collection of anthropometric measures so that all infants were included in the primary analyses. Baseline demographic and clinical characteristics were comparable between groups except for multiple births because 2 sets of twins (4 infants) were randomly allocated to the higher-protein group, and 10 sets of twin (20 infants) were allocated to the control group (Table 2).

#### Nutritional management

Nutritional management of the infants did not differ between groups with the exception of the mean (±SD) amount of protein provided by the HMF (2.6 ± 1.2 and 1.8 ± 0.8 g/d; P < 0.0001) for the higher-protein and control groups, respectively. From study weeks 1 to 4, the protein intake was 0.6 g · kg⁻¹ · d⁻¹ higher for the intervention group at a median of 4.2 g · kg⁻¹ · d⁻¹.

### TABLE 2

Baseline infant and maternal demographic and clinical characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Higher protein (n = 43)</th>
<th>Standard protein (n = 49)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recruitment hospital [n (%)]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women’s and Children’s Hospital</td>
<td>37 (86)</td>
<td>43 (88)</td>
</tr>
<tr>
<td>Flinders Medical Centre</td>
<td>6 (14)</td>
<td>6 (12)</td>
</tr>
<tr>
<td>Maternal age (y)</td>
<td>29 ± 5</td>
<td>30 ± 5</td>
</tr>
<tr>
<td>Mother smoked during pregnancy [n (%)]</td>
<td>9 (22)</td>
<td>6 (15)</td>
</tr>
<tr>
<td>Previous preterm birth [n (%)]</td>
<td>6 (15)</td>
<td>5 (13)</td>
</tr>
<tr>
<td>Received antenatal steroids [n (%)]</td>
<td>36 (87)</td>
<td>35 (90)</td>
</tr>
<tr>
<td>Cesarean section [n (%)]</td>
<td>27 (63)</td>
<td>32 (65)</td>
</tr>
<tr>
<td>Multiple births [no. of infants (%)]</td>
<td>4 (9)</td>
<td>20 (41)</td>
</tr>
<tr>
<td>Gestational age (wk)</td>
<td>27.5 ± 2.2</td>
<td>28 ± 1.5</td>
</tr>
<tr>
<td>Male infants [n (%)]</td>
<td>19 (44)</td>
<td>21 (43)</td>
</tr>
<tr>
<td>Birth anthropometric measures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (g)</td>
<td>1012 ± 315</td>
<td>1056 ± 289</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>35.7 ± 3.5</td>
<td>35.6 ± 3.4</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>25.3 ± 2.5</td>
<td>25.6 ± 2</td>
</tr>
<tr>
<td>Infants born at &lt;28 wk gestation [n (%)]</td>
<td>23 (54)</td>
<td>20 (41)</td>
</tr>
<tr>
<td>Postnatal age at study entry (d)</td>
<td>12.9 ± 6.1</td>
<td>13.1 ± 5.5</td>
</tr>
<tr>
<td>Age feedings commenced (d)</td>
<td>3 (2–4)</td>
<td>3 (2–4)</td>
</tr>
<tr>
<td>Age human milk fortifier introduced (d)</td>
<td>13 (10–16)</td>
<td>13 (10–18)</td>
</tr>
<tr>
<td>Volume of enteral intake when human milk fortifier was introduced (mL · kg⁻¹ · d⁻¹)</td>
<td>120 (94–140)</td>
<td>120 (96–156)</td>
</tr>
</tbody>
</table>

1 With the use of t tests and Fisher’s exact tests (for continuous and categorical variables, respectively), the only significant difference in baseline variables between groups was for multiple birth (P = 0.0007).
2 Of n = 80 women (41 in the higher-protein group, and 39 in the standard-protein group).
3 Mean ± SD (all such values).
4 n = 43 infants in the higher-protein group, and n = 48 in the standard-protein group.
5 Median; 25th–75th percentile in parentheses (all such values).

### TABLE 3

Growth characteristics of participants

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Higher protein (n = 43)</th>
<th>Standard protein (n = 49)</th>
<th>Mean difference (95% CI)</th>
<th>P²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length gain (cm/wk)</td>
<td>1.15 (1.10–1.19)</td>
<td>1.09 (1.05–1.13)</td>
<td>0.06 (−0.01, 0.12)</td>
<td>0.08</td>
</tr>
<tr>
<td>Weight gain (g/d)</td>
<td>24 (20–28)</td>
<td>26 (24–28)</td>
<td>−2 (−7, 2)</td>
<td>0.33</td>
</tr>
<tr>
<td>Head circumference gain (cm/wk)</td>
<td>0.94 (0.90–0.98)</td>
<td>0.95 (0.92–0.99)</td>
<td>−0.02 (−0.07, 0.04)</td>
<td>0.56</td>
</tr>
<tr>
<td>Length at study end (cm)</td>
<td>46.3 ± 2.1</td>
<td>45.5 ± 3.0</td>
<td>0.8 (−0.3, 1.9)</td>
<td>0.14</td>
</tr>
<tr>
<td>Weight at study end (g)</td>
<td>2760 ± 498</td>
<td>2539 ± 494</td>
<td>221 (23, 419)</td>
<td>0.03</td>
</tr>
<tr>
<td>Head circumference at study end (cm)</td>
<td>33.5 ± 1.8</td>
<td>33.8 ± 1.8</td>
<td>−0.3 (−1.0, 0.3)</td>
<td>0.33</td>
</tr>
</tbody>
</table>

1 Calculated by using generalized estimating equations.
2 Adjusted for sex, gestational age, and sibling clustering.
3 From enrollment to study end; study end occurred when the infant was discharged from home or reached expected date of delivery, whichever occurred first.
4 Median; 25th–75th percentile in parentheses (all such values).
5 Mean ± SD (all such values).
There was a trend to an improved mean rate of length gain in the higher-protein group, but this trend was not significant [1.15 cm/wk (95% CI: 1.10, 1.19 cm/wk) and 1.09 cm/wk (95% CI: 1.05, 1.13 cm/wk) in the higher and standard-protein groups, respectively; mean difference: 0.06 cm/wk (95% CI: −0.01, 0.12 cm/wk); \( P = 0.08 \) (Table 3). Length \( z \) scores decreased over the course of the study, independent of the dietary group (Figure 2). Length \( z \) scores of infants in the control group showed a trend to decrease more rapidly than those in the higher-protein group, but this was not significant (0.0032 per study day; 95% CI: −0.0005, 0.0068; \( P = 0.87 \)). In the preplanned per-protocol analyses of infants who received >70% of their enteral intake as fortified breast milk \([n = 59 (64%); n = 29 in the higher- and n = 30 in the standard-protein groups] \), there was a trend toward greater length gain in the higher-protein group. The effect size was similar to that seen in the whole cohort of infants but was not significant [mean weekly length gain of 1.15 cm/wk (95% CI: 1.09, 1.21 cm/wk) and 1.08 cm/wk (95% CI: 1.02, 1.13 cm/wk) in the higher- and standard-protein groups, respectively; mean difference: 0.07 cm/wk (95% CI: −0.01, 0.15 cm/wk); \( P = 0.09 \)].

Infants randomly assigned to receive higher-protein HMF had greater weights at study end than did infants randomly assigned to receive standard protein (mean difference: 221 g; 95% CI: 23, 419 g; \( P = 0.03 \) (Table 3). There were no significant differences in lengths or head circumferences at study end.

Secondary analyses indicated that significantly fewer infants had lengths that were less than the 10th percentile at study end in the higher-protein group than in the control group (risk difference: −0.187; 95% CI: −0.370, −0.003; \( P = 0.047 \); adjusted for gestational age and sibling clustering) (Table 4). Seven infants per group were born with lengths less than 10th percentile, which remained less than the 10th percentile at study end. Fewer infants in the higher-protein group who were born with lengths appropriate to their gestational age fell below the 10th percentile at study end \([n = 14 (33%)] \) compared with \( n = 23 (47%) \); missing data: \( n = 2 \). The number needed to treat to prevent one infant from becoming small-for-gestational-age for length,

![FIGURE 2. Length z scores from enrollment to study end. The z scores of infants in the standard-protein group showed a trend to decrease more rapidly than did z scores of infants in the higher-protein group, but this was not significant by using generalized estimating equations adjusted for gestational age and sibling clustering. The z scores were calculated by using mean (±SD) estimates in an Australian population of preterm infants (36). \( n = 49 \) and 43 in standard- and higher-protein groups, respectively.

**TABLE 4**

<table>
<thead>
<tr>
<th></th>
<th>Higher protein ( (n = 43) )</th>
<th>Standard protein ( (n = 49) )</th>
<th>Risk difference ( (95% \text{ CI}) )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Length [( n (%) )]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth</td>
<td>7 (16)</td>
<td>7 (14)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Study end</td>
<td>21 (49)</td>
<td>31 (63)</td>
<td>(-0.187 (-0.370, -0.003)) ( ^4 )</td>
<td>0.047</td>
</tr>
<tr>
<td><strong>Weight [( n (%) )]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth</td>
<td>7 (16)</td>
<td>6 (12)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Study end</td>
<td>15 (35)</td>
<td>17 (35)</td>
<td>(-0.02 (-0.22, 0.18)) ( ^4 )</td>
<td>0.84</td>
</tr>
<tr>
<td><strong>Head circumference [( n (%) )]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth</td>
<td>3 (7)</td>
<td>5 (10)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Study end</td>
<td>8 (19)</td>
<td>11 (22)</td>
<td>(-0.04 (-0.20, 0.13)) ( ^5 )</td>
<td>0.66</td>
</tr>
</tbody>
</table>

\(^1\) Study end occurred when infant was discharged home or reached expected date of delivery, whichever occurred first.

\(^2\) Calculated by using generalized linear models.

\(^3\) One infant (standard-protein group) did not have birth length measured, and one infant (standard-protein group) did not have length and head circumference measured at study end.

\(^4\) Adjusted for sex, gestational age, and sibling clustering.

\(^5\) Adjusted for sex and sibling clustering; not adjusted for gestational age because of modeling limitations.
which was calculated as the inverse of the risk difference, was 5 (95% CI: 3, 117). There were no significant differences between groups in weight or head-circumference gains in the intention-to-treat analyses or in the subgroup of infants who were predominantly human milk fed or in the proportion of infants less than the 10th percentile for weight or head circumference.

**Biochemistry**

SUN concentrations reduced over the course of the study, independent of the dietary group ($P < 0.0001$) (Figure 3). Infants in the higher-protein group had significantly higher urea concentrations ($P < 0.0001$) than did infants in the control group over the duration of the study. Seven infants, all of whom were in the higher-protein group, developed transient uremia (SUN concentration $\geq 8$ mmol/L), which resolved spontaneously without intervention. Low SUN concentrations ($\leq 1$ mmol/L) were recorded in 15 infants (4 and 11 infants in the higher-protein and control groups, respectively). Plasma creatinine concentrations reduced significantly over time, independent of the dietary group ($P = 0.8$). Plasma albumin concentrations significantly increased over time, independent of the group ($P < 0.0001$), with no significant difference between groups ($P = 0.4$). pH also increased over time ($P = 0.002$) but did not differ between groups ($P = 0.8$) (Figure 4).

**Clinical outcomes**

Feeding tolerance did not differ between groups. The mean ± SD days taken to reach full enteral feedings ($\geq 150$ mL $\cdot$ kg$^{-1} \cdot$ d$^{-1}$) was $18 \pm 10.2$ and $17 \pm 9.1$ d ($P = 0.88$) in the higher-protein and control groups, respectively. Feedings were interrupted on a median (minimum, maximum) of $2$ d (0, 6 d) and 1 d (0, 7 d) ($P = 0.16$) in the higher-protein and control groups, respectively. There were no significant differences between groups for any clinical outcomes (Table 5).

**DISCUSSION**

The aim of this study was to assess the effect of a higher-protein HMF on preterm-infant growth. Current practices with human milk fortification have shown only modest improvements in growth. This study was designed to provide a protein concentration to more adequately meet protein requirements. To our knowledge, our trial is unique in that we used HMF with the highest protein concentration (1.4 g/100 mL) while maintaining constant energy through adjustment of the carbohydrate concentration. Most recent trials that attempted to increase protein intake in preterm infants fed human milk have used an individualized or adjustable regimen that increased protein, energy, and other micronutrients that may all have independent effects on growth (26, 27). In our trial, infants randomly allocated to receive higher protein had greater length gains, which was of borderline significance. It may be that the effect size of energy-adjusted protein supplementation on length gain is smaller than previously suggested from studies in which other nutrients are not controlled (26). It is also possible that the statistical adjustments needed to account for the unanticipated imbalance of multiple births limited the power to detect a significant difference.

Weight at study end was significantly greater in the higher-protein group. The importance of this finding is not clear given that there were no significant differences between groups in the rate of weight gain or small-for-gestational-age weight classification. This result may have been confounded by the longer length of stay in the higher-protein group.

In secondary analyses, we showed a significant reduction in the proportion of infants less than the 10th percentile for length at
Oxygen at 36 wk postmenstrual age 

Days receiving intravenous lipid 13

Days receiving parenteral nutrition 6

Length of stay (d) 77

Postnatal steroids [n (%)] 14 (33)

Sepsis [n (%)] 6 (14)

Time to reach full enteral feedings 18

confirmed necrotizing enterocolitis [n (%)] 3 (7)

Bowel surgery [n (%)] 1 (2)

Oxygen at 36 wk postmenstrual age [n (%)] 20 (47)

Any retinopathy of prematurity [n (%)] 13 (30)

Sepsis [n (%)] 6 (14)

Postnatal steroids [n (%)] 14 (33)

Length of stay (d) 77 ± 21

Postmenstrual age at discharge (wk) 38.2 ± 1.5

Time to reach full enteral feedings of ≥150 mL · kg⁻¹ · d⁻¹ (d) 18 ± 10.2

Days receiving parenteral nutrition 18 ± 12.1

Days receiving intravenous lipid 13 ± 11.0

Feeding interrupted (d)³

Higher protein

Standard protein

Effect

(n = 43)

(n = 49)

(95% CI) P

confirmed necrotizing enterocolitis [n (%)]

3 (7)

5 (10)

0.58 (0.16, 2.12)¹

0.41

Bowel surgery [n (%)]

1 (2)

2 (4)

Postnatal steroids [n (%)]

20 (47)

17 (35)

1.34 (0.86, 2.10)²

0.19

Any retinopathy of prematurity [n (%)]

13 (30)

15 (31)

1.01 (0.59, 1.76)⁰

0.96

Sepsis [n (%)]

6 (14)

7 (14)

0.97 (0.37, 2.56)⁰

0.95

Postmenstrual age at discharge (wk)

38.2 ± 1.5

38.4 ± 1.4

-0.33 (-0.91, 0.25)⁰

0.26

Time to reach full enteral feedings of ≥150 mL · kg⁻¹ · d⁻¹ (d)

18 ± 10.2

17 ± 9.1

1.02 (0.80, 1.29)⁰

0.88

Length of stay (d)

77 ± 21

73 ± 16

0.46 (-0.64, 5.56)⁰

0.06

Days receiving parenteral nutrition

18 ± 12.1

20 ± 13.2

0.91 (0.72, 1.16)⁰

0.45

Days receiving intravenous lipid

13 ± 11.0

14 ± 10.9

0.92 (0.68, 1.24)⁰

0.57

Feeding interrupted (d)

2 (0–6)⁰

1 (0–7)

0.62 (0.32, 1.20)⁰

0.16

¹ RR adjusted for gestational age and sibling clustering.

² Mean ± SD (all such values).

³ Mean difference adjusted for gestational age and sibling clustering.

⁴ Mean ratio adjusted for gestational age and sibling clustering.

⁵ One or more prescribed feedings in a 24-h period were not given.

⁶ Median; minimum–maximum in parentheses (all such values).

The pragmatic nature of this trial may have influenced the results. For example, standard preterm formula was used if the supply of EBM was insufficient, which reflected current practice in neonatal units in which human milk banking is unavailable. Although it was possible that the use of preterm formula diluted our intervention and made it harder to detect a difference, it was reassuring that the direction and size of the effect of the per-protocol analysis (receiving >70% human milk) was consistent with the intention-to-treat analysis. In addition, because of considerations of maternal milk supply and resources needed for the study, weekly breast milk samples were collected and analyzed for macronutrients, and these results were extrapolated for the whole week. A more accurate approach would be to analyze macronutrient content daily from a 24-h pooled sample.

The findings of our study suggest that feeding HMF with a protein concentration of 1.4 g/100 mL results in less growth failure in preterm infants born <31 wk gestation. Our strategy of increasing the protein concentration of fortifier has no detectable short-term side effects and is less time consuming than individualized regimes. It is possible that a higher-protein fortifier than used in this study is required to achieve optimal growth. To determine whether this is so, sufficiently large randomized controlled trials that are powered for important clinical outcomes, including neurodevelopment, will be needed.

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to their institutions to support conference travel and continuing education for postgraduate students and early career researchers. None of the authors had a personal or financial conflict of interest related to the research.

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