Effect of Increased Enteral Protein Intake on Growth in Human Milk–Fed Preterm Infants
A Randomized Clinical Trial

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➕ **Supplemental content**

IMPORTANCE Protein, supplied in currently available commercial fortifiers, may be inadequate to meet the requirements of very preterm infants; in addition, intraindividual and interindividual variability of human milk protein and energy content potentially contribute to unsatisfactory early postnatal growth.

OBJECTIVE To determine effects on growth of different levels of enteral protein supplementation in predominantly human milk–fed preterm infants.

DESIGN, SETTING, AND PARTICIPANTS This randomized clinical and partially blinded single-center trial was conducted in a neonatal tertiary referral center in Germany. Sixty preterm infants (gestation <32 weeks and weight <1500 g at birth) were recruited from October 2012 to October 2014 and included 35% of 173 eligible infants. Median (interquartile range [IQR]) gestational age at birth was 29.9 (28.7-31.2) weeks. All analyses were conducted in an intention-to-treat population.

INTERVENTIONS Infants were randomly assigned to either a lower-protein (adding 1 g of bovine protein/100 mL of breast milk through a commercial human milk fortifier; n = 30) or a higher-protein group at a median (IQR) postnatal age of 7 (6-8) days. The higher-protein group (n = 30) received either standardized higher-protein supplementation (study fortifier adding 1.8 g of bovine protein/100 mL of breast milk [n = 15]) or individualized high-protein supplementation based on protein and fat content of administered breast milk (n = 15). Study interventions were continued for a median (IQR) of 41 (30-57) days and until definite discharge planning.

MAIN OUTCOMES AND MEASURES Primary outcome was weight gain (g/kg/d) from birth to the end of intervention.

RESULTS Sixty preterm infants (gestation <32 weeks and weight <1500 g at birth), 33 girls, were recruited from October 2012 to October 2014 and included 35% of 173 eligible infants. Median (IQR) gestational age at birth was 29.9 (28.7-31.2) weeks. Demographic characteristics and hospital courses were similar in both groups, and birth weights ranged from 580 to 1495 g in the lower-protein group and 490 to 1470 g in the higher-protein group. Weight gain was similar in the lower- and higher-protein groups: mean (95% CI), 16.3 g/kg/d (15.4-17.1 g/kg/d) in the lower-protein group vs 16.0 g/kg/d (15.1-16.9 g/kg/d) in the higher-protein group) (P = .70), despite an increase in actual protein intake by 0.6 g/kg/d (0.4-0.7 g/kg/d) (P < .001). Head circumference and lower leg longitudinal growth were also similar, as was the proportion of cumulative total enteral feeding volume provided as breast milk: median (IQR) proportion of breast milk, 92% (79%-98%) in the lower-protein group vs 94% (62%-99%) in the higher-protein group (P = .89).

CONCLUSIONS AND RELEVANCE An increase in protein intake by 0.6 g/kg/d to a mean intake of 4.3 g/kg/d did not further enhance growth of very preterm infants with a median birth weight of 1200 g, who achieved near-fetal growth rates. This might point to a ceiling effect for enteral protein intake with respect to its influence on growth.

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The optimal dose of enteral protein for very preterm infants has not yet been established. Breast milk fortification assuming an average composition of breast milk and adding fortifiers at a fixed dose is widely practiced.[1] Several reports have shown an association of fortified maternal milk feeding with early postnatal growth restriction compared with preterm formula feeding.[2-4] Owing to intraindividual and interindividual variability of human milk protein content[5,6] and the importance of protein supply for growth and neurodevelopment of very preterm infants,[7,8] individualized fortification may optimize protein supply in predominantly human milk–fed preterm infants.

Beyond research defining the optimal dose of enteral protein supply, studies are required to evaluate whether a potential protein deficit is best prevented by standardized supplementation with more protein or by targeted protein fortification of human milk adjusted for individual milk protein content.

This study aimed to evaluate the effects on growth of different levels of enteral protein supplementation in predominantly human milk–fed very preterm infants based on recent recommendations.[9] Using a priori ordered hypotheses, we also evaluated the effects of a standardized higher-protein fortification vs individualized fortification adjusted for individual breast milk protein and fat content.

Methods

This randomized and partially blinded clinical trial was conducted at the Department of Neonatology at Tuebingen University Children's Hospital, Germany. Enrollment began in October 2012 and ended in October 2014. The institutional review board of Tuebingen University Hospital approved the protocol (Supplement 1), and written informed parental consent was obtained. The trial was registered with clinicaltrials.gov (NCT01773902).

A Priori Ordered Research Hypotheses

We worked from 2 ordered research hypotheses: (1) superordinate hypothesis: higher vs lower enteral protein intake will improve postnatal weight gain (and growth); (2) subordinate hypothesis: individualized higher protein intake will improve postnatal weight gain over standardized higher protein intake.

Participants

Inborn infants with gestational age less than 32 weeks at birth and a birth weight lower than 1500 g were eligible to participate if their mothers intended to supply breast milk and they had reached an enteral feeding volume of at least 100 mL/kg/d until postnatal day 7. Infants were excluded if a major congenital or chromosomal abnormality was present.

Randomization, Allocation Concealment, and Blinding

A computer-generated randomization scheme was produced by an independent statistician to assign the infants to intervention groups in a 2:1:1 ratio. Randomization was performed using sequentially numbered, sealed, opaque envelopes. Siblings of multiple births were randomized individually.

Key Points

Question Does additional enteral protein intake enhance growth of predominantly human milk–fed preterm infants?

Findings In this randomized clinical trial of 60 very premature infants, increasing enteral protein intake from 3.7 to 4.3 g/kg/d had no significant effect on weight gain to discharge (16.3 in lower vs 16.0 g/kg/d in higher protein group), but near-fetal growth rates were achieved in both groups during postnatal hospitalization.

Meaning There may be a ceiling effect for enteral protein intake with respect to growth in preterm infants with a median birth weight of 1200 g.

Caregivers and parents were blinded to the type of study fortifier in all groups (individual patient-dedicated fortifiers were provided in identical containers), but owing to local fortification practices, individualized fortification became evident as soon as additional protein supplements were administered.

Interventions

Infants were randomly assigned in proportions of 2:1:1 to 1 of 3 parallel treatment groups: (1) a lower-protein group (standardized fortification adding 5 g/100 mL of the commercially available multicomponent fortifier FM 85 [Nestlé Nutrition] resulting in supplementation of 1 g of bovine protein/100 mL of breast milk and an overall supply of about 3.5 g/kg/d of protein, assuming administration of 150 mL/kg/d of breast milk with about 1.3 g of protein/100 mL); (2) a higher-protein group further divided into 2 subgroups (a) standardized higher-protein supplementation using an investigational multicomponent fortifier and (b) individually adjusted fortification based on the individual human milk macronutrient content on top of standard fortification, as in group 1. Protein supplementation in the higher-protein group aimed at 4.5 g/kg/d of protein based on recent recommendations,[9] increasing protein intake by about 0.5 to 1.0 g/kg/d compared with standard care (lower-protein group). The investigational fortifier administered to group 2a contained 1.8 g of bovine protein/5 g of fortifier (10.01.DE.INF; Nestlé Nutrition) (for details see eTable 1 in Supplement 2).

In all 3 study groups, multicomponent fortifier was added in a fixed dose of 2.5 g/100 mL of breast milk at enteral intakes between 100 and 149 mL/kg/d and at 5 g/100 mL of breast milk once at least 150 mL/kg/d of enteral feeds had been reached and always thereafter.

For individually adjusted fortification (group 2b), additional bovine protein (Aptamil Eiweiß Plus; Milupa) was added according to breast milk content aiming for 4.5 g/kg/d of enteral protein if weight was less than 1500 g, or 4.0 g/kg/d of enteral protein if weight was 1500 g or greater.[9] In addition, fat (generic medium-chain triglyceride oil) was supplemented to ensure fat intakes greater than 4.8 g/kg/d.

The study interventions were continued according to initial allocation from randomization until definite discharge planning (<1 week before discharge), when standardized supplementation according to unit standards (identical to group 1) was resumed.

Parenteral and enteral feeding regimens were standardized by feeding guidelines, which remained unchanged during the

study period. In all infants, breast milk feeding was complemented by standard preterm formula containing 2.88 g of protein/100 mL (80 kcal) (Beba preterm infant formula, level 1; Nestlé Nutrition) if expressed breast milk supply of the infant's own mother did not meet required enteral feeding intake.

Outcome Variables

The primary outcome variable was weight gain measured as grams per kilogram per day from birth to the end of the intervention. It was calculated from day to day (including the first week of life) by the formula weight gain = (weight, day [n +1] − weight, day n)/weight, day n. Predefined secondary outcome variables were (1) head circumference growth from birth to the end of intervention; (2) weight, head circumference, and length at discharge and corresponding standard deviation score (SDS); (3) SDS differences for weight and head circumference between discharge and birth ($SDS_{discharge}$ − SDS_{birth}); and (4) lower leg longitudinal growth measured weekly in millimeters per week.

Human Milk Macronutrient Content Measurements

In all 3 groups, unfortified breast milk's macronutrient content was determined as the mean of 3 individual measurements of single breast milk samples twice weekly by midinfrared spectroscopy using a human milk analyzer (Miris AB), as previously described.[10,11] All measurements were preceded by internal validation using a check solution according to the manufacturer's instructions. Caregivers were blinded to human milk macronutrient content throughout the study. For retrospective calculation of actual macronutrient intake, human milk macronutrient content on days between measurements was assumed by linear interpolation of adjacent values.

Actual Nutritional Intakes

Actual nutritional intakes were recorded daily including de facto administered feeding volumes, feeding type, and supplements. Protein, fat, and energy intakes were calculated from the volume of milk ingested, the measured protein, fat, and energy content of the individual breast milk and the manufacturer's information on nutrient content for fortifiers and formula.

Anthropometric Measures and Clinical Data Collection

Infant weight was determined daily on an electronic scale, and fronto-occipital head circumference was measured weekly using a nonstretchable tape measure. Lower leg longitudinal growth was measured by knemometry, as described previously.[12] Length at discharge was determined using a recumbent length board (Ulmer Stadiometer).

For weight and head circumference, SDSs were computed using LMSgrowth software (version 2.14; http://www.healthforallchildren.com/?product=lmsgrowth). The reference population was the British 1990 growth reference[13,14] fitted by maximum penalized likelihood, as previously described.[13] For weight and head circumference, SDS-differences for different intervals during hospitalization (eg, SDS_{day28} − SDS_{birth}) were calculated to illustrate in-hospital postnatal growth.

Biochemical Analyses

Determinations of clinical chemical parameters (albumin, cystatin C, and urea) were scheduled at days 14 ± 2 and 28 ± 4

Figure 1. Participant Flow Through the Trial
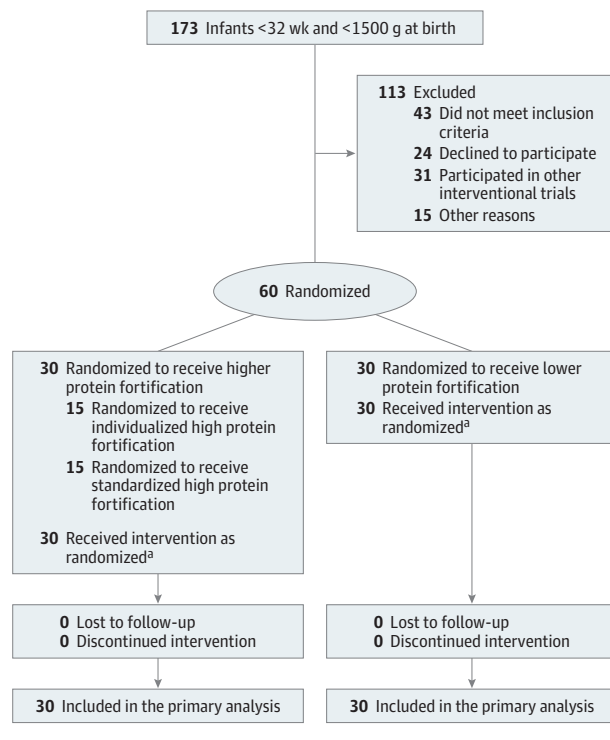
a In 2 infants (1 in the lower-protein group and 1 in the higher-protein group), human milk feedings were discontinued during the interventional period due to ceasing supply of breast milk from the infants' own mothers. According to the study protocol, these infants received supplementary preterm infant formula until discharge and were analyzed according to intention to treat.

after randomization and were performed on the ADVIA XPT clinical chemistry analyzer (Siemens Healthineers).

Sample Size and Statistical Analyses

Based on a mean (SD) postnatal weight gain of 12.8 (3.0) g/kg/d in very preterm infants born in the study hospital in 2008, we estimated that a sample size of 46 infants (23 allocated to lower-protein intake [group 1] vs 23 to higher-protein intake [groups 2a and 2b]) would detect a difference in weight gain of 3 g/kg/d with a power of 90% and a 2-sided significance level of 5%. This difference in weight gain was achieved in a recent study[15] by increasing protein supply by about 0.5 g/kg/d, resembling the difference in protein intake of 0.5 to 1.0 g/kg/d anticipated for our study groups. To compensate for potential dropouts (eg, early transfer or death) and insufficient breast milk supply, total sample size was set to 60 infants.

According to our hierarchical study design, the subordinate hypothesis was to be tested only if the superordinate research hypothesis was proven. Sample size calculation for the subordinate hypothesis revealed that 15 infants each in of groups 2a and 2b would be sufficient to prove a difference in postnatal weight gain of 4 g/kg/d between different strategies of higher protein supplementation with 80% power and 5% alpha (which does not require adjustment for multiple comparisons based on the hierarchical design).

Table 1. Infant Characteristics[a]

Characteristic	Lower-Protein Group	Higher-Protein Group		
		Standardized	Individualized	Total
No. of infants	30	15	15	30
Birth weight, g	1215 (1065-1393)	1115 (950-1220)	1245 (1130-1360)	1193 (984-1326)
Gestational age, wk	30.0 (29.0-31.1)	28.6 (27-30.7)	30 (29.4-31.1)	29.7 (27.9-31.0)
Female sex, No. (%)	19 (63)	5 (33)	9 (60)	14 (47)
Birth weight, No. (%)				
<1000 g	6 (20)	6 (40)	2 (13)	8 (27)
<10th percentile for gestational age	11 (37)	6 (40)	4 (27)	10 (33)
Singleton birth, No. (%)	15 (50)	12 (80)	6 (40)	18 (60)
Apgar score at 5 min	8 (8-9)	8 (8-9)	8 (8-10)	9 (8-9)
CRIB score	1 (1-2)	1 (1-2)	1 (1-3)	1 (1-2)
Age at study entry, d	7 (6-7)	7 (6-10)	7 (6-8)	7 (6-8)
Length of intervention period, d	43 (32-51)	56 (30-62)	38 (30-51)	40 (30-58)
Postmenstrual age at the end of study intervention, wk	36.6 (35.8-38.5)	36.7 (35.6-37.9)	36.6 (35.6-38)	36.7 (35.6-37.9)
Length of hospital stay, d	52 (42-65)	65 (36-72)	49 (37-61)	52 (37-70)

Abbreviations: CRIB, clinical risk index for babies; IQR, interquartile range.

[a] Unless otherwise indicated, data are reported as median (IQR).

Data are presented as mean (SD) if normally distributed, or as median (interquartile range [IQR]) if not. Comparisons were performed using parametric or nonparametric tests, as appropriate, and Fisher exact tests in categorical outcomes. In a post hoc multivariate regression model, effects of interventions and actual protein intake on weight gain were adjusted for gestational age at birth, sex, and birth weight SDS. Analyses were performed with JMP software, version 12.2.0 (SAS Institute Inc). All analyses were conducted in the intention-to-treat population.

Results

Participants

Sixty infants were enrolled and evaluated as shown in **Figure 1**. Infants' birth weight ranged from 580 to 1495 g in the lower-protein group and from 490 to 1470 g in the higher-protein group; gestational age ranges at birth were 25.9 to 32.0 weeks and 25.4 to 31.7 weeks for the lower- and higher-protein groups, respectively.

No statistically significant group differences were detected with respect to birth characteristics (**Table 1**) and postnatal age at randomization. The median (IQR) proportion of cumulative total enteral feeding volume provided as breast milk of the infants' own mother during the intervention period was similar between groups: 92% (79%-98%) in the lower-protein group vs 94% (62%-99%) in the higher-protein group (*P* = .89).

There were no deaths and no relevant differences between study groups with regard to typical neonatal morbidities, ventilatory support, supplemental oxygen, or medication use (**Table 2**).

Primary Outcome and Macronutrient Supply

While the amount of protein administered in the higher-protein group exceeded that administered in the lower-protein group by 0.6 g/kg/d, the average weight gain from birth to the end of the intervention was similar for lower- and higher-protein study groups (**Table 3**). Also, energy intake was similar (Table 3).

Table 2. Neonatal Morbidities and Respiratory Support

Characteristic	Protein Supplementation		P Value
	Lower	Higher	
Necrotizing enterocolitis (any stage), No.	0	0	NA
Focal intestinal perforation, No.	0	0	NA
Retinopathy of prematurity (maximum disease stage), No.	0	1 (1)	>.99[a]
Bronchopulmonary dysplasia, No. (%)[b]	0	2 (7)	.49[a]
Intraventricular hemorrhage (maximum grade), No.	3 (1)	0	.24[a]
Periventricular leukomalacia	0	0	NA
Mechanical ventilation via endotracheal tube, median (IQR), d	0 (0-2)	1 (0-2)	.54[c]
Nasal CPAP, median (IQR), d	8 (3-32)	23 (4-41)	.16[c]
Supplemental oxygen, median (IQR), d	0 (0-4)	1 (0-24)	.33[c]

Abbreviations: CPAP, continuous positive airway pressure; IQR, interquartile range; NA, not applicable.

[a] Fisher exact test, 2 tailed.

[b] Defined as need for supplementary oxygen or positive airway pressure at a postmenstrual age of 36 weeks.

[c] Wilcoxon test.

Exploratory analysis of the subordinate research hypothesis revealed similar mean (SD) weight gain from birth to the end of intervention for the standardized higher-protein group vs the individualized higher-protein group: 16.0 (2.4) g/kg/d vs 16.1 (2.6) g/kg/d; mean difference, 0.1 (95% CI, −2.0 to 1.8) g/kg/d. For these subgroups, the mean (SD) protein supply from birth to the end of the intervention was higher in the standardized higher-protein group: 4.5 (0.3) g/kg/d vs 4.2 (0.2) g/kg/d; mean difference, 0.3 (95% CI, 0.1-0.5) g/kg/d, whereas mean (SD) energy intake was similar: 137 (7) kcal/kg/d vs 140 (6) kcal/kg/d; mean (SD) difference, 3 (95% CI, −8 to 2) kcal/kg/d. In the individually supplemented high-protein group, protein intake approximated targeted intake levels better and showed less variability than in the standardized higher-protein group (eFigure in Supplement 2).

Table 3. Growth Outcomes and Macronutrient Supply

Characteristic	Protein Supplementation		Mean Difference (95% CI)	P Value
	Lower (n = 30)	Higher (n = 30)		
Weight gain, mean (SD), g/kg/d				
From birth to end of intervention[a]	16.3 (2.2)	16.0 (2.5)	−0.2 (−1.5 to 1.0)[b]	.70[c]
During intervention period	18.3 (2.7)	18.3 (2.3)	0.0 (−1.3 to 1.3)	.97[c]
Lower leg longitudinal growth from birth to end of intervention, mean (SD), mm/wk[d]	4.1 (0.9)	3.9 (0.8)	−0.2 (−0.6 to 0.3)	.42[c]
Discharge data, mean (SD)				
Weight, g	2611 (420)	2669 (445)	58 (−166 to 282)	.60[c]
Head circumference, cm	32.9 (1.3)	32.9 (1.4)	0.0 (−0.7 to 0.7)	.98[c]
Length, cm	45.9 (2.7)	46.9 (2.5)	1.0 (−0.4 to 2.3)	.17[c]
Below 10th percentile, No. (%)				
Weight	7 (23)	4 (13)		.50[e]
Head circumference	3 (11)	2 (7)		.67[e]
Length	13 (46)	8 (38)		.27[e]
Actual protein intake, mean (SD), g/kg/d[d]				
Total, from birth to end of intervention	3.8 (0.24)	4.3 (0.27)	0.6 (0.4 to 0.7)[b]	<.001[c]
Enteral, randomization to day 28	3.5 (0.35)	4.1 (0.39)	0.6 (0.4 to 0.7)	<.001[c]
Actual energy intake, mean (SD), kcal/kg/d				
Total, birth to end of intervention	137 (9.2)	138 (6.4)	1 (−3 to 5)	.56[c]
Enteral, randomization to day 28	129 (11.3)	130 (9.2)	1 (−4 to 7)	.66[c]

[a] Primary outcome.

[b] The apparent discrepancy is due to rounding: more precise weight gain, 16.25 g/kg/d in the lower-protein group and 16.02 g/kg/d in the higher-protein group; mean difference, −0.23 g/kg/d. Total protein intake, 3.76 g/kg/d in the lower-protein group and 4.33 g/kg/d in the higher-protein group; mean difference, 0.57 g/kg/d.

[c] t Test, 2-tailed.

[d] Mean (SD) of individual means.

[e] Fisher exact test, 2 tailed.

Figure 2. Standard Deviation Score (SDS) Difference for Weight and Head Circumference During Intervention
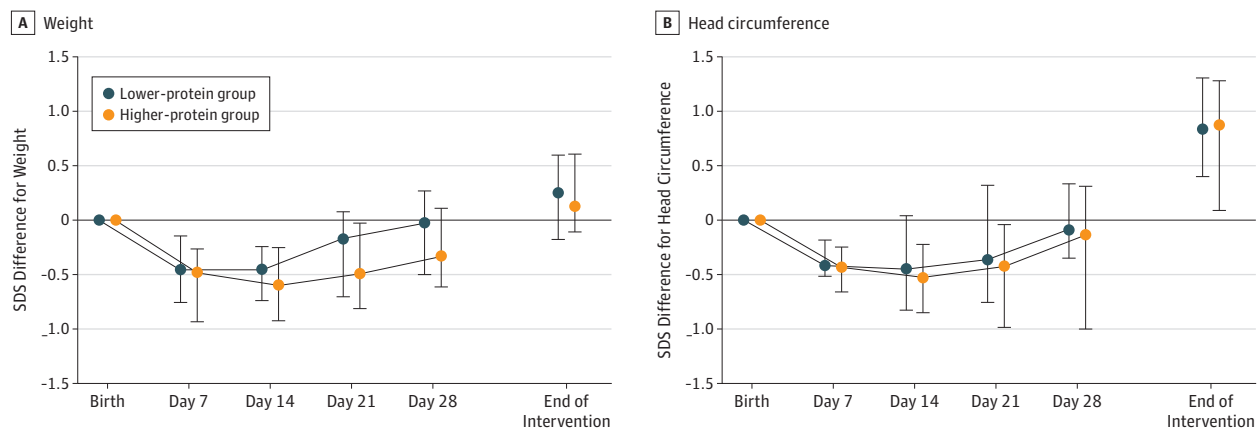
Plots for data points in time are separated only to enhance clarity of data presentation. Each data point represents the median value; error bars, interquartile range. Study interventions were continued for a median (interquartile range) of 41 (30-57) days and until definite discharge planning.

Secondary Growth Outcomes

There were no differences in change of weight and head circumference SDS during hospitalization (**Figure 2**). Seventy percent of all study infants (n = 42), 67% in the lower-protein group (n = 20) and 73% in the higher-protein group (n = 22), regained their birth centile for weight or crossed centiles upwards until the end of the intervention. In addition, no differences in lower leg longitudinal growth (Table 3) and in head circumference and length at discharge (Table 3) were detected between lower- and higher-protein groups.

A post hoc multivariate regression model adjusting for gestational age at birth, sex, and birth weight SDS revealed no significant differences for weight gain among the 3 treatment groups or for actual protein intake.

In the overall study population, the proportion of infants below the 10th centile for weight decreased remarkably from birth to discharge (35% [n = 21] vs 18% [n = 11], P < .001). This was also true for head circumference (37% [n = 22] vs 8% [n = 5], P = .06).

Adverse Events

No adverse events related to study fortifier or applied nutritional interventions were detected, and no differences in tolerance of the 2 fortifiers became evident. Full enteral feedings were reached by postnatal day (IQR) 7 (6-8) in the lower-protein group and by postnatal day 6 (6-7) in the higher-protein group (P = .17). No case of necrotizing enterocolitis or focal intestinal perforation occurred. No infant in the lower-protein group and 2 in the higher-protein group developed

blood culture–proven nosocomial sepsis (P = .49). A trend toward longer time on nasal continuous positive airway pressure in the higher-protein group (Table 2) may be explained by slightly more immature infants among infants who weighed less than 1000 g in this group (eTable 2 in Supplement 2).

Additional Findings
Serum concentrations of urea, cystatin C, and albumin are reported in eTable 3 in Supplement 2 showing higher urea concentrations in the higher-protein group. Breast milk macronutrient data are reported in eTable 4 in Supplement 2.

Discussion

The primary aim of this randomized clinical trial was to assess the effect on preterm infants' growth of a higher enteral protein intake, either through standardized fortification using a new fortifier with higher protein content, or an individualized fortification procedure adjusting human milk supplementation to actual protein and fat content. To our knowledge, this study is unique with respect to the high-protein content of the study fortifier and the secondary comparison of different fortification strategies, one with standardized high-protein supplementation, the other with individually tailored high-protein fortification on the basis of human milk analysis. This study shows that an additional supply of 0.6 g/kg/d of protein, resulting in an average protein intake of 4.3 g/kg/d over an interventional period of approximately 6 weeks, did not improve weight gain (Table 3). This was also true for the admittedly small proportion of infants who weighed less than 1000 g (eTable 2 in Supplement 2). Both study groups experienced remarkably good growth during postnatal hospitalization and showed near-fetal growth rates (Figure 2).

Our results are in line with data recently reported by Miller et al,[16] who also found no influence of increased enteral protein intake (4.2 g/kg/d vs 3.6 g/kg/d) on weight gain, albeit in more immature infants (mean gestational age at birth, 27.5 weeks in the higher-protein group and 28 weeks in the lower-protein group). By contrast, Arslanoglu et al[15] showed a significant improvement in weight gain associated with enhanced protein supply adjusted according to level of serum urea nitrogen, albeit at a considerably lower level of protein intake (2.8 vs 3.4 g/kg/d in the third week). Moya et al[17] enhanced protein supply by 0.6 g per 100 mL of fortified human milk, but also showed no significant effect on weight gain. Unfortunately, actual protein and energy content of the administered breast milk was not measured by Moya et al.

We hypothesize that our findings point to a potential ceiling effect for enteral protein supply, at least for the population studied, indicating that an enteral protein intake exceeding 3.5 to 4.0 g/kg/d might not further improve weight gain in this population. This hypothesis is supported by the finding of a post hoc multivariate regression model in all 60 infants that revealed no effect of actual protein intake on weight gain after adjustment for gestational age at birth, sex, and birth weight SDS. Increased concentrations of serum urea and identical cystatin C levels in the higher-protein group (eTable 3 in Supplement 2) indicate that additional protein was absorbed,

but instead of being used for body protein synthesis, this additional protein was metabolized to urea, supporting the hypothesis of a ceiling effect. Such a ceiling effect might be induced by other nutrient deficiencies that impede further growth improvement (a "next limiting nutrient") or reflects a situation where maximal protein effects on infant growth have been achieved in both groups.

Furthermore, there was no apparent benefit of individualized higher-protein fortification based on twice-weekly human milk analysis with regard to weight gain compared with standardized higher-protein supplementation. This is true even though results of milk analysis confirmed known high variability of human milk protein and fat content (eTable 4 in Supplement 2). Admittedly, the hierarchical design of our study does not allow firm conclusions in this respect, and individualized supplementation may be important at lower overall protein intakes. To fully assess potential benefits of targeted fortification, larger prospective trials on clinically relevant outcomes are warranted, including determination of macronutrient content in 24-hour pooled human milk samples on a daily basis.[1]

In contrast to data reported by Moya et al,[17] who showed slightly increased linear growth, our study intervention had no significant impact on lower leg longitudinal growth and length at discharge (Table 3; secondary outcome in both studies). Miller et al,[16] choosing linear growth as their primary outcome, found a trend toward greater length gain for infants receiving higher enteral protein intake. In agreement with other recent reports,[16,17] we found no significant effect on head growth in our study, while Morgan et al[18] showed improved head growth with higher parenteral and total protein supply. Importantly, the small increase in protein supply (0.3 g/kg/d) in the study by Morgan et al was administered at a lower level of total protein intake.

Strengths of the present study are as follows: (1) determination of actual macronutrient intake, taking into account actual human milk macronutrient content based on twice-weekly milk analyses in all study infants; (2) a long intervention period owing to very early exclusive enteral nutrition; and (3) high cumulative breast milk intakes.

Limitations
A limitation of our study is the small number of infants (n = 14) who weighed less than 1000 g at birth (23%). This occurred because we were contemporaneously recruiting infants younger than 28 weeks gestational age at birth for further interventional multicenter trials. Hence our results should not be extrapolated to extremely low-birth-weight infants who might particularly benefit from higher protein supply, although the studies of Miller et al[16] and Moya et al[17] do not support this. In addition, the approach to using standard preterm formula (and not banked breast milk) whenever the supply of milk of the infant's own mother was insufficient may have affected results. However, the mean cumulative breast milk intake during the intervention period was similarly high in both study groups (>90%). In both study groups, weight gain was substantially higher than originally anticipated and reached near-fetal growth rates. With almost identical weight gain in both groups, even a much larger sample size would not have yielded a statistically significant and clinically relevant difference. Finally, this study

did not evaluate effects of higher protein intake on other organ functions (eg, of the immune system).

Conclusions

A mean increase in actual protein intake of 0.6 g/kg/d to a mean intake of 4.3 g/kg/d did not enhance growth of very preterm infants with a median birth weight of 1200 g. This might point to a ceiling effect for enteral protein intake with respect to its influence on growth in this population. To (1) evaluate potential benefits on growth in extremely low-birth-weight infants, (2) verify effects on neurocognitive outcome, (3) detect smaller differences in weight gain, and (4) assess benefits of targeted fortification, larger randomized clinical trials are needed.

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