Protein Intake and Growth in Preterm Infants: A Systematic Review

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Abstract

Objective. This review aimed to investigate the relationship between varying levels of enteral protein intake and growth in preterm infants, regardless of feeding method. Data Sources. Electronic databases were searched for relevant studies, as were review articles, reference lists, and text books. Study Selection. Trials were included if they were randomized or quasirandomized, participants were <37 weeks gestation at birth, and protein intakes were intentionally or statistically different between study groups. Trials reporting weight, length, and head circumference gains in infants fed formula, human milk, or fortified human milk were included. Data Extraction. Studies were categorized by feeding-type and relevant data were extracted into summary tables by one reviewer and cross-checked by a second. Data Synthesis. A meta-analysis could not be conducted due to extensive variability among studies; thus, results were synthesized graphically and narratively. Twenty-four trials met the inclusion criteria and were included in a narrative synthesis and 19 in a graphical synthesis of study results. Conclusions. There was extensive variability in study design, participant characteristics, and study quality. Nonetheless, results are fairly consistent that higher protein intake results in increased growth with graphical representation indicating a potentially linear relationship. Additionally, intakes as high as 4.5 g/kg/day were shown to be safe in infants weighing >1000 g.

Keywords

infant, premature, human milk, dietary proteins, growth

The incidence of preterm births has increased in developed countries over the past decade, and due to technological advances, the survival rate of marginally viable infants has also increased.1,2 Feeding these very small infants is a challenge. Those infants born as early as 22 weeks gestation spend the entirety of the last trimester of pregnancy outside the intrauterine environment.1,2 To match intrauterine growth, very low birth weight (<1500 g) infants have high nutritional requirements.3 However, the immaturity of their organ systems can limit the safety of providing high nutrient intakes.2 Preterm infants experience postnatal growth delay, with the resulting growth deficit often not recovered during hospital admission.4 Clinical studies comparing growth curves of preterm infants with those of infants in utero show a higher proportion of preterm infants small for gestational age (weight <10th percentile) at discharge.4-6 The neonatal admission period is increasingly being shown to be the critical time for neurodevelopment.7-9 Early nutritional practices, specifically increased protein intake, and improved short-term growth outcomes during this time have been associated with beneficial long-term growth and neurodevelopment.7,9

Current opinion suggests the aim of feeding preterm infants is to replicate the growth and body composition seen in utero.3,10 Parenteral nutrition is initiated within the first hour and enteral nutrition within the first days of life, with an aim to achieve full enteral feeding as soon as is clinically possible.3 Both infant formulas and human milk (HM) are used in enteral feeding. As HM has inadequate energy, protein, and bone minerals to support optimal growth in preterm infants weighing

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<2000 g, the use of human milk fortifiers (HMFs) is standard clinical practice. The quantity of dietary protein required to enable optimal growth in preterm infants remains a contentious issue. Recommendations for protein intake vary between key bodies (Table 1) and have been revised up over the last decade. Early research with protein intakes of 6.0 to 7.0 g/kg/day resulted in metabolic acidosis, uremia, and hyperaminoacidaemia; however, the protein was of poor quality, and recent reviews suggest this may no longer apply to current practice.

Cochrane systematic reviews of growth in “high” versus “low” protein formula fed infants and infants fed fortified versus unfortified HM have been published. The former concluded infants receiving formula with higher protein content had improved weight gain. The review compared “high” (3.0–4.0 g/kg/day) with “low” (<3.0 g/kg/day) protein intakes and excluded trials where comparison groups fell within the same range. In a review comparing infants receiving fortified versus unfortified HM, Kuschel and Harding found improved weight, length, and head circumference (HC) growth. However, the review included trials comparing non-isocaloric feeds, thereby making it difficult to separate the effects of protein and energy. Additionally, neither of these Cochrane reviews included studies published since 1995; therefore, an updated review including the most recent research is required. Randomized controlled trials (RCTs) comparing the effects of HMFs with different protein concentrations on growth have shown inconsistent findings. Additionally, many neonatal units use mixed feeding and provide preterm formula to infants when the mother’s milk supply is not adequate. A comprehensive systematic review investigating increased protein and growth including all feeding methods and reflecting the mixed feeding approach in neonatal units is yet to be published.

The objective of this review is to investigate the relationship between enteral protein intake and growth in preterm infants.

Methods

Types of Studies

Randomized or quasi-randomized controlled trials were considered for inclusion in this review.

Types of Participants, Interventions, and Outcome Measures

Trials that included preterm infants with birth weight less than 2.5 kg were included in this review. Trials that compared varying protein intakes in formula, unfortified, or fortified HM fed infants were included. Trials primarily investigating parenteral nutrition and quality of enteral protein intake were beyond the scope of this review. To investigate the relationship between protein intake and growth independent of energy, only studies that held energy constant between groups were included. Similarly, only studies that provided infants with adequate energy to allow protein to be used for tissue accretion (ie, >100 kcal/kg) were included. Trials that reported outcomes of weight gain, length gain, or HC gain were included. The inclusion and exclusion criteria are summarized in Table 2. In trials with >2 groups, any groups not meeting the review criteria were excluded from analysis.

Search Method and Data Extraction

Computerized searches were conducted up to March 30, 2013. Databases, search terms, and filters used are summarized in Figure 1, and in addition the clinical trials registers, “clinicaltrials.gov,” and Australian New Zealand...
Table 2. Inclusion and Exclusion Criteria for Literature Searches.

| Inclusion Criteria                                                                 | Exclusion Criteria                                                                 |
|-----------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| • Gestational age at birth <37 weeks                                              | • Protein intakes not reported                                                      |
| • Birth weight <2500 g                                                            | • Studies investigating differences in parenteral feeding solutions                   |
| • Protein intakes intentionally different between 2 or more groups                 | • Energy difference >10% relative composition or shown to be statistically significantly different |
| • Reports comparison of change between groups in any or all of the following: weight, length, head circumference | • Energy intake of any group <100 kcal/kg                                             |
| • Protein intakes between 2 or more groups are shown to be not statistically significantly different |

439 Records identified from database search
122 From Web of Knowledge
139 From Scopus
144 From Medline Ovid
34 From Cochrane Library (CENTRAL)

369 Records excluded through title and abstract screening
151 Duplicates
218 Not relevant (animal studies, did not relate to either outcome or exposure, not preterm infants, interventions post term, clearly not RCTs or other publication types)

71 Potentially relevant articles identified for full-text screening

1 Article identified through hand searching of reference lists

48 Articles excluded through full-text screening using inclusion & exclusion criteria
4 Multiple reports of the same trial
16 Protein intakes not different
13 No reported protein intakes
10 Not isocaloric
3 Intervention was parenteral
1 No reported growth data
1 Randomisation not cited

24 Trials included in qualitative synthesis
12 Formula Studies
  5 Weight only
  2 Weight & length
  5 Weight, length & HC
  5 Unfortified/Fortified HM
  5 Weight, length & HC
7 Comparison of HMFs
7 Weight, length & HC

Figure 1. Flow diagram of search methods.

Clinical trials registry were searched for trials in progress. A combination of MeSH terms (infant, newborn; infant, premature; infant, low birth weight; human milk; dietary proteins; infant food; growth) and keywords (preterm; neonate; breast milk; protein) were utilized in searches. English language filters were applied; however, no limits
were placed on year of study. Hand-searching of reference lists was conducted and review articles and text books were used to identify further relevant studies. Studies were screened for relevance according to the selection criteria (Table 2). Studies were categorized by feed-type to facilitate comparison between studies with somewhat similar protein quality, and relevant data were extracted into summary tables by one reviewer and cross-checked by a second. A meta-analysis could not be conducted due to extensive variability among studies; thus, results have been synthesized graphically and narratively.

**Methodological Quality**

Trials were evaluated for risk of bias according to the Academy of Nutrition and Dietetics Quality Criteria Checklist for primary research. Briefly, this assesses trials for relevance to practice and scientific rigour. Individual trials were assessed against quality criteria specific for RCTs, with “Yes” or “No” being assigned to each criterion, or “Unclear” if the study report lacked adequate detail for assessment. A summary outcome of “Positive,” “Negative,” or “Neutral” is produced.

**Results**

The search strategy yielded 439 titles; 71 full-text articles were reviewed (Figure 1). Forty-eight of these were excluded. Characteristics of excluded studies are summarized in Figure 1. Twenty-four trials met the inclusion criteria for this review. Twelve trials compared the growth of infants fed formula with varying protein intakes; 5 compared infants fed unfortified HM with protein fortified HM, and 7 trials compared infants fed different HMFs resulting in varying protein intakes. All studies were published between May 1976 and October 2012. Trials involving formula-fed infants have been carried out throughout this entire period. Conversely, trials assessing the adequacy of unfortified HM were conducted between 1985 and 1990, after which time it was thought to be unethical to conduct these comparisons, and those comparing HMFs or fortification methods have occurred since then (1995-2012). Characteristics of included studies are summarized in Tables 3, 4 and 5.

**Trials Comparing Groups of Formula-Fed Infants**

**Summary of Studies.** Twelve of the included trials compared the growth of infants fed formula with varying protein intakes (Table 3). Protein intakes ranged from 1.6 g/kg/day to 4.7 g/kg/day (Table 3). Five trials found no statistically significant differences between groups for any growth outcomes. Cooke et al and Darling et al found that infants with increased protein intakes (in both studies an additional 0.8 g/kg/day) had a greater rate of daily weight gain compared to controls (8 and 7 g/day greater than the control group, respectively). Five further studies showed higher protein intake groups had greater rates of fractional weight gain compared with controls (3-6 g/kg/day greater than controls). Kashyap et al and Darling et al found increased rate of HC growth in infants with higher protein intakes (0.4 and 0.1 cm/week, respectively, more than controls). Darling et al demonstrated increased growth in the higher protein intake group for all outcome measures (weight and HC reported above, additional 0.2 cm/week length gain; P < .01). Three trials also included a reference group of HM-fed infants and compared their growth with that of formula-fed infants. Bell et al and Svenningsen et al found no statistically significant difference in any outcome measures between the HM- and formula-fed groups, while Raiha et al found significantly increased weight gain in formula-fed infants compared with HM-fed controls (+5 g/day, P < .05).

**Critique of Studies.** Random sequence generation and allocation concealment were typically poorly reported in trials finding an effect compared with those showing no effect. Conversely, 5 of these trials used a standard operating procedure for anthropometric measurements, thus ensuring consistency and accuracy, compared with no clear description of measurement methods in all trials showing no effect. Furthermore, only 2 trials conducted an intention-to-treat analysis. The study duration (>28 days) was a strength of 6 trials. Longer trial duration limits the effect of daily fluid fluctuations on weight gain, enabling meaningful changes in length and HC to be observed. The difference in sodium content of the formula between comparison groups is a limitation of the trials by Cooke et al and Bell et al. The change in weight seen in these trials may have been due to the influence of sodium on fluid balance rather than tissue growth. Supporting this, neither trial showed a significant difference in length or HC gain (Table 3). The small sample sizes of the trials by Costa-Orvay et al and Bhatia et al may have limited their ability to show a significant difference between groups, as both trials showed a trend toward increased growth in infants with higher protein intakes. The trials by Costa-Orvay et al and Embleton and Cooke did not reach the required sample size, thus making them vulnerable to Type II error (see Supplementary Table 1, available online at http://gph.sagepub.com/supplemental).
Table 3. Data Summary of Trials Comparing Growth in Infants Fed Isocaloric Formulas With Varying Protein Content.

| Study | Study Description | Intervention | Outcomes and Results |
|-------|-------------------|--------------|----------------------|
|       | Study Participants | Study Intakes | Study end, mean (SD) |
|       | Study Intervention | Intakes       | Growth               |
|       | Study Outcomes     |               | Quality              |
|       | Study Results      |               |                      |
|       | Study Description  | Study Intakes | Study end, mean (SD) |
|       | Study Participants | Intakes       | Growth               |
|       | Study Intervention |               | Quality              |
|       | Study Outcomes     | Study Intakes | Study end, mean (SD) |
|       | Study Results      |               | Growth               |
|       | Study Description  | Study Intakes | Study end, mean (SD) |
|       | Study Participants | Intakes       | Growth               |
|       | Study Intervention |               | Quality              |
|       | Study Outcomes     | Study Intakes | Study end, mean (SD) |
|       | Study Results      |               | Growth               |
|       | Study Description  | Study Intakes | Study end, mean (SD) |
|       | Study Participants | Intakes       | Growth               |
|       | Study Intervention |               | Quality              |
|       | Study Outcomes     | Study Intakes | Study end, mean (SD) |
|       | Study Results      |               | Growth               |

(continued)
Table 3. (continued)

| Study | Study Description | Intervention | Outcomes and Results |
|-------|-------------------|--------------|----------------------|
|       |                   | Intakes      | Growth               | Quality   |
|       |                   | Days 4-7, mean (SD) | Study days 1-8, mean (SD) | Neutral |
| Wauben et al²⁰ (1995), Netherlands | EN n = 16 BW: AGA | F1.5 (n = 8): Energy 68 kcal/100 mL | Δ Weight, g/kg/d; F1.5: 118 (9), F2.0: 117 (11), P > .05 Protein, g/kg/d; F1.5: 2.7 (0.3), F2.0: 3.4 (0.3), P < .05 | Neutral |
|       | DO n = 0 GA: 28-35 wk | Energy 70 kcal/100 mL Protein 2.0 g/100 mL | Δ Weight, g/kg/d; F1.5: 12 (3), F2.0: 16 (4), P < .05 | Neutral |
|       | SS: enteral volume 160 mL/kg/d | Δ Weight, g/kg/d; F1.5: 12 (3), F2.0: 16 (4), P < .05 | Δ Weight, g/kg/d; F1.5: 12 (3), F2.0: 16 (4), P < .05 | Neutral |
|       | SE: 8 days Healthy infants | Δ Weight, g/kg/d; F1.5: 12 (3), F2.0: 16 (4), P < .05 | Δ Weight, g/kg/d; F1.5: 12 (3), F2.0: 16 (4), P < .05 | Neutral |
|       |                   | Δ Weight, g/kg/d; F1.5: 12 (3), F2.0: 16 (4), P < .05 | Δ Weight, g/kg/d; F1.5: 12 (3), F2.0: 16 (4), P < .05 | Neutral |
| Hillman et al²¹ (1994), United States | EN n = 32 BW: <1500 g | Group A (n = 9): Protein 3.0 g/100 kcal | Gain in HC and length not different (data N/D) | Neutral |
|       | DO n = 5 GA: N/D | Energy, kcal/kg/d; Groups A, B, and C: Aim 120, P N/D | Group A*: 19 (4), Group B: 16 (3), Group C*: 13 (5), P < .05 | Neutral |
|       | SS: N/D Not receiving TPN or diuretics | Δ Weight, g/kg/d; Group A*: 19 (4), Group B: 16 (3), Group C*: 13 (5), P < .05 | Δ Weight, g/kg/d; Group A*: 19 (4), Group B: 16 (3), Group C*: 13 (5), P < .05 | Neutral |
|       | SE: 30 days | Protein 2.7 g/100 kcal Group C (n = 9): Protein 2.2 g/100 kcal | Group B: 3.6, Group C: 2.8, P N/D | Neutral |
|       |                   | Δ Weight, g/kg/d; Group A*: 19 (4), Group B: 16 (3), Group C*: 13 (5), P < .05 | Δ Weight, g/kg/d; Group A*: 19 (4), Group B: 16 (3), Group C*: 13 (5), P < .05 | Neutral |
| Bhatia et al²² (1991), United States | EN n = 26 BW: <1550 g | High (n = 8): Energy intake 100 kcal/kg/d by 21 days age Healthy infants | Gain in HC and length not different (data N/D) | Neutral |
|       | DO n = 3 GA: N/D | Day 1 to study end, mean (SD) | Gain in HC and length not different (data N/D) | Neutral |
|       | SS: when enteral energy intake reached 100 kcal/kg/d | Day 1 to study end, mean (SD) | Gain in HC and length not different (data N/D) | Neutral |
|       | SE: 2 weeks from study day 1 Enteral feeds by 14 days age | Day 1 to study end, mean (SD) | Gain in HC and length not different (data N/D) | Neutral |
|       |                   | Protein 2.7 g/100 kcal | Gain in HC and length not different (data N/D) | Neutral |

(continued)
### Table 3. (continued)

| Study | Participants | Intervention | Outcomes and Results |
|-------|--------------|--------------|----------------------|
| Study Description | | | Intakes | Growth | Quality |
| Kashyap et al\(^{23}\) (1988), United States | EN n = 50, BW: 900-1750 g | Group 1 (n = 16): | Study period, mean (SD) | Study period, mean (SD) | Neutral |
| | DO n = 6, GA: N/D | Protein 1.6 g/100 mL | Energy kcal/kg/d: Group 1: 119 (2), Group 2: 120 (2), P N/D | Δ Weight, g/kg/d: Group 1: 16 (2), Group 2: 19 (3), P < .05 | |
| | SS: intake 180 mL/kg/d | Healthy infants | Energy 66 kcal/100 mL | Protein g/kg/d: Group 1: 2.8 (<0.1), Group 2: 3.8 (<0.1), P N/D | Δ Δ Length, cm/wk: Group 1: 1.0 (0.2), Group 2: 1.2 (0.3), P > .05 |
| | SE: until infant weight 2200 g (average duration of study N/D) | Group 2 (n = 16): | Protein 2.1 g/100 mL | Δ Δ Length, cm/wk: Group 1: 1.0 (0.1), Group 2: 1.2 (0.3), P > .05 | |
| | | Protein 2.1 g/100 mL | Energy kcal/100 mL | Minimal vit and min diff Titrated with fat and CHO Group 3 excluded Not isocaloric | |
| | | Energy 67 kcal/100 mL | Δ Δ Length, cm/wk: Group 1: 1.0 (0.1), Group 2: 1.2 (0.3), P > .05 | |
| Bell et al\(^{24}\) (1986), Ireland | EN n = 75 (10 HM enrolled separately), BW: <1800 g | Group A excluded: Increased energy intake compared with B and C (P < .05) | Energy kcal/kg/d: Group B: 128 (14), Group C: 128 (15), HM: 127 (21), P NS | Δ Weight, g/kg/d: Group B: 1.9 (4), Group C: 1.6 (4), HM: 1.5 (5), P > .05 | Neutral |
| | DO n = 2, GA: N/D | Group B (n = 25): | Protein g/kg/d: Group B: 3.9 (0.4), Group C: 3.6 (0.5), HM: 2.6 (0.3), P < .001 | Δ Length, cm/wk: Group B: 1.4 (0.7), Group C: 1.5 (0.5), HM: 1.5 (0.4), P > .05 | |
| | SS: enteral intake 150 mL/kg/day and IV ceased | Gender: both | Protein 2.4 g/100 mL | Δ OFC, cm/wk: Group B: 1.1 (0.3), Group C: 1.1 (0.3), HM: 1.1 (0.2), P > .05 | |
| | SE: weight >2000 g (average duration of study N/D) | Healthy infants | Energy 79 kcal/100 mL | Group C (n = 25): | |
| | | Protein 2.1 g/100 mL | Energy 74 kcal/100 mL | Δ Weight, g/kg/d: Group B: 1.9 (4), Group C: 1.6 (4), HM: 1.5 (5), P > .05 | |
| | | Energy 70 kcal/100 mL | Titrated with fat and CHO | Group C: 1.1 (0.3), Group C: 1.1 (0.3), HM: 1.1 (0.2), P > .05 | |
| Study | Participants | Intervention | Study Description | Intakes | Outcomes and Results | Growth | Quality |
|-------|--------------|--------------|-------------------|---------|----------------------|--------|---------|
| Kashyap et al. (1986), United States | EN n = 34 | GA: 27-37 wk | Group 1 (n = 11): | Study period, mean (SD) | Δ Weight, g/kg/d; Group 1: 115 (1), Group 2: 114 (1), P N/D | Δ Length, cm/wk; Group 1: 0.9 (0.2), Group 2: 1.2 (0.3), P < .05 | Neutral |
| | DO n = 7 | BW: 900-1750 g | Protein 1.3 g/100 mL | Energy, kcal/kg/d; Group 1: 63 kcal/100 mL, Group 2: 63 kcal/100 mL, P < .05 | Δ Weight, g/kg/d; Group 1: 14 (3), Group 2: 18 (3), P < .05 | Δ Length, cm/wk; Group 1: 0.9 (0.2), Group 2: 1.2 (0.3), P < .05 | Neutral |
| | SS: intake reached 180 mL/kg/d | Healthy infants | Energy 63 kcal/100 mL | Protein, g/kg/d; Group 1: 2.2 (0.0), Group 2: 3.6 (0.0), P N/D | Δ Weight, g/kg/d; Group 1: 14 (3), Group 2: 18 (3), P < .05 | Δ Length, cm/wk; Group 1: 0.9 (0.2), Group 2: 1.2 (0.3), P < .05 | Neutral |
| | SE: weight 2200 g | Group 2 (n = 11): | Protein 2.0 g/100 mL | Δ Weight, g/kg/d; Group 1: 14 (3), Group 2: 18 (3), P < .05 | Δ Length, cm/wk; Group 1: 0.9 (0.2), Group 2: 1.2 (0.3), P < .05 | Neutral |
| | | | Energy 63 kcal/100 mL | Δ Length, cm/wk; Group 1: 0.9 (0.2), Group 2: 1.2 (0.3), P < .05 | Neutral |
| | | | Vit and min content same | Neutral |
| | | | Titrated with fat and CHO | Neutral |
| | | | Group 3 excluded | Neutral |
| | | | Not isocaloric | Neutral |
| | | | Protein 2.0 g/100 mL | Δ Weight, g/kg/d; Group 1: 14 (3), Group 2: 18 (3), P < .05 | Δ Length, cm/wk; Group 1: 0.9 (0.2), Group 2: 1.2 (0.3), P < .05 | Neutral |
| Darling et al. (1985), Canada | EN n = 15 | BW: 1300-1600 g | Group 1 (n = 5): | Study period, mean (SEM) | Δ Weight, g/d; Group 1*: 36 (3), Group 2*: 29 (2), Group 3*: 30 (3),*P = .03 | Δ Length, cm/wk; Group 1*: 1.1 (0.1), Group 2*: 0.8 (0.0), Group 3: 0.9 (0.0),*P < .01 | Neutral |
| | DO n = N/D | GA: N/D | Protein 1.9 g/100 mL | Energy, kcal/kg/d; Group 1: 149 (9), Group 2: 153 (6), Group 3: 147 (7), P NS | Δ Weight, g/d; Group 1*: 36 (3), Group 2*: 29 (2), Group 3*: 30 (3),*P = .03 | Δ Length, cm/wk; Group 1*: 1.1 (0.1), Group 2*: 0.8 (0.0), Group 3: 0.9 (0.0),*P < .01 | Neutral |
| | SS: at initiation of enteral feeding | AGA | Energy 72 kcal/100 mL | Protein, g/kg/d; Group 1: 4.3 (0.2), Group 2*: 3.5 (0.1), Group 3*: 4.4 (0.2),*P = .03 compared with groups 1 and 3 | Δ Weight, g/d; Group 1*: 36 (3), Group 2*: 29 (2), Group 3*: 30 (3),*P = .03 | Δ Length, cm/wk; Group 1*: 1.1 (0.1), Group 2*: 0.8 (0.0), Group 3: 0.9 (0.0),*P < .01 | Neutral |
| | SE: discharge at 2200 g, feeding continued on same formula until 3 months | No hemolytic disease | Whey-casein 60:40 | Δ OFC, cm/wk; Group 1: 0.8 (0.0), Group 2: 0.7 (0.0), Group 3: 0.8 (0.0), P < .05 | Δ OFC, cm/wk; Group 1: 0.8 (0.0), Group 2: 0.7 (0.0), Group 3: 0.8 (0.0), P < .05 | Δ OFC, cm/wk; Group 1: 0.8 (0.0), Group 2: 0.7 (0.0), Group 3: 0.8 (0.0), P < .05 | Neutral |
| | | No hyaline membrane disease | Group 2 (n = 5): | Δ OFC, cm/wk; Group 1: 0.8 (0.0), Group 2: 0.7 (0.0), Group 3: 0.8 (0.0), P < .05 | Δ OFC, cm/wk; Group 1: 0.8 (0.0), Group 2: 0.7 (0.0), Group 3: 0.8 (0.0), P < .05 | Δ OFC, cm/wk; Group 1: 0.8 (0.0), Group 2: 0.7 (0.0), Group 3: 0.8 (0.0), P < .05 | Neutral |
| | | No notable respiratory distress | Protein 1.5 g/100 mL | Δ OFC, cm/wk; Group 1: 0.8 (0.0), Group 2: 0.7 (0.0), Group 3: 0.8 (0.0), P < .05 | Δ OFC, cm/wk; Group 1: 0.8 (0.0), Group 2: 0.7 (0.0), Group 3: 0.8 (0.0), P < .05 | Δ OFC, cm/wk; Group 1: 0.8 (0.0), Group 2: 0.7 (0.0), Group 3: 0.8 (0.0), P < .05 | Neutral |
| | | | Energy 70 kcal/100 mL, Whey-casein 20:80 | Δ OFC, cm/wk; Group 1: 0.8 (0.0), Group 2: 0.7 (0.0), Group 3: 0.8 (0.0), P < .05 | Δ OFC, cm/wk; Group 1: 0.8 (0.0), Group 2: 0.7 (0.0), Group 3: 0.8 (0.0), P < .05 | Δ OFC, cm/wk; Group 1: 0.8 (0.0), Group 2: 0.7 (0.0), Group 3: 0.8 (0.0), P < .05 | Neutral |

(continued)
### Table 3. (continued)

| Study | Participants | Intervention | Outcomes and Results |
|-------|--------------|--------------|----------------------|
| **Quality Participants Intervention Intakes Growth** | **Intakes** | **Growth** | **Quality** |
| **Svenningsen et al**<sup>27</sup> (1982), Sweden | EN n = 48 | Mean BW: 1385 ± 343 g | 3-7 weeks age, average | 3-7 weeks age, mean (SD) | Negative |
| DO n = N/D | Mean GA: 30.8 ± 2.9 wk | Protein 1.6 g/100 kcal | Energy, kcal/kg/d; HM-Group: 116, F1-Group: 117, F2-Group: 118, P N/D | Δ Weight, g/kg/d; HM-Group: 13 (3), F1-Group: 13 (4), F2-Group: 14 (4), P NS |
| SS: 3rd week life | Infants with respiratory distress, septicemia were included | F1-Group (n = 14): | Protein, g/kg/d; HM-Group: 1.9, F1-Group: 2.5, F2-Group: 3.2, P N/D | Δ Length, cm/wk; HM-Group: 1.0 (N/D), F1-Group: 1.0 (N/D), F2-Group: 1.0 (N/D), P NS |
| SE: 7th week life | Protein 2.3 g/100 kcal | F1-Group (n = 16): | Δ Length, cm/wk; HM-Group: 1.0 (N/D), F1-Group: 1.0 (N/D), F2-Group: 1.0 (N/D), P NS |
| **Raiha et al**<sup>28</sup> (1976), Finland | EN n = 106 | BW ≤ 2100 g | Study period, average | Regained birthweight to study end, mean (SEM) (g/wk divided by 7, Time 3 shown) | Neutral |
| DO n = 7 | GA: 28-36 wk | Protein 1.5 g/100 mL | Energy, kcal/kg/d; HM: 114, F1: 118, F2: 116, P N/D | Δ Weight, g/d; HM<sup>*</sup>: 22 (2), F1<sup>*</sup>: 27 (1), F2: 26 (2), *P NS |
| SS: feedings started before 24 hours life | AGA | Whey-casein 60:40 | Protein, g/kg/d; HM: 1.6, F1: 2.3, F2: 4.5, P N/D | No significant difference between any groups in mean rate of HC gain |
| S | SE: weight 2400 g (>28 days) | Healthy infants | Pooled HM (n = 22): | Data N/D |

**Abbreviations:** AAs, amino acids; AGA, appropriate for gestational age; BW, birth weight; CHO, carbohydrate; DO, drop outs, that is, not included in growth outcomes; EN, enrolled; GA, gestational age; HC, head circumference; HM, human milk; LBW, low birth weight; MCT, medium-chain triglycerides; Na, sodium; NS, nonsignificant; N/D, not described; OFC, occipito-frontal circumference; PTF, preterm formula; SGA, small for gestational age; SD, standard deviation; SE, study end; SEM, standard error of the mean; SS, study start; TPN, total parenteral nutrition; VLBW, very low birth weight.
| Study | Study Description | Outcomes and Results |
|-------|-------------------|----------------------|
| Kashyap et al. (1990), United States | EN n = 66
DO n = 24
CP n = 27
SS: enteral feedings 180 mL/kg/d
SE: infant weight 2200 g | Study period, mean (SD)
Study period, mean (SD) Neutral
Energy, kcal/kg/d; Group 1: 129 (11), Group 2: 131 (12), P N/D
Protein, g/kg/d; Group 1: 2.5 (0.5), Group 2: 3.2 (0.4), P N/D
Δ Weight, g/kg/d; Group 1: 17 (2), Group 2: 21 (2), P < .01
Δ Length, cm/wk; Group 1: 0.9 (0.2), Group 2: 1.3 (0.5), P NS
Δ HC, cm/wk; Group 1: 1.0 (0.2), Group 2: 1.2 (0.2), P NS |
| Polberger et al. (1989), Sweden | EN n = 34
DO n = 6
CP n = 5 (mothers milk), n = 10 (little mothers milk)
SS: stable on 170 mL/kg/d
SE: 2200 g or breastfeeding initiated | Study period, mean (SD) Neutral
Energy, kcal/kg/d; HMF: 121 (10), HMP: 117 (9), P N/D
Protein, g/kg/d; HMF: 2.1 (0.3), HMP: 3.6 (0.2), P N/D
Δ Weight, g/kg/d; HMF: 16 (2), HMP: 20 (1), P N/D
Δ Length, cm/wk; HMF: 0.9 (0.2), HMP: 1.3 (0.1), P N/D
Δ HC, cm/wk; HMF: 1.1 (0.2), HMP: 1.2 (0.1), HMF: 2.1 (0.2), P N/D |
| Greer and McCormick (1988), United States | EN n = 38
DO n = N/D
CP n = N/D
SS: full oral feedings achieved (120 kcal/kg/d)
SE: 6 weeks from study start | First 6 weeks enteral feeds, mean (SD) Neutral
First 6 weeks enteral feeds, mean (SD)
Energy, kcal/kg/d; HM: 112 (10), FHM: 105 (15), P NS
Protein, g/kg/d; HM: 3.3 (0.6), FHM: 4.2 (0.5), P < .01
Δ Weight, g/kg/d; HM: 13 (1), FHM: 17 (2), P < .01
Δ Length, cm/wk; HM: 0.8 (0.2), FHM: 1.1 (0.2), P < .01
Δ HC, cm/wk; HM: 0.8 (0.2), FHM: 1.1 (0.2), P < .02 |

(continued)
### Table 4. (continued)

| Study | Participants | Intervention | Study Description | Outcomes and Results |
|-------|--------------|--------------|-------------------|----------------------|
|       |              |              |                   |                      |
| Putet et al (1987) | EN n = 16 BW: <1500 g | HM (n = 8); Pooled HM | Not isocaloric Target volume: 120-200 mL/kg/d HM composition: 5% daily aliquots of feeds pooled for weekly analysis | Study period (3 days), mean (SD) 7 days overlapping balance study, mean (SD) |
|       |              |              |                   |                      |
|       | DO n = 0 GA: N/D | HM-Pr (n = 8): | HM composition: 5% daily aliquots of feeds pooled for weekly analysis |                      |
|       | CP n = 16 Gender: male | Pooled HM + 1 g sup/100 mL, providing (100 g powder): | Neutral |                      |
|       | SS: N/D Healthy infants | Nitrogen: 13.2 g, lipid: 1.4 g, Ca: 2.5 g, P: 1.1 g, Na: 80 mg | Isocaloric due to higher volume feeds of HM group Target volume: N/D HM composition: one aliquot taken from entire pool of milk for study |                      |
|       | SE: 7 days after study start | | |                      |
|       |              |              |                   |                      |
| Ronnholm et al (1986) | EN n = 54 BW: <1500 g | HM (n = 23): | Neutral |                      |
|       | DO n = 10 GA: ≤36 wk | Unsupplemented HM | HM composition: 5 mL samples taken at beginning and end of each milking |                      |
|       | CP n = 44 SGA and AGA | HM-Pr (n = 21): | Energy, kcal/kg/d; HM: 111 (4), HM-Pr: 110 (4), P = .01 Protein*, g/kg/d; HM: 1.8 (0.1), HM-Pr: 3.2 (0.2), P = .12 |                      |
|       | SS: N/D Healthy infants | HM + HM protein (0.9 g/100 mL of milk) | Energy, kcal/kg/d; HM: 1.3 (3), HM-Pr: 1.3 (2), P = .01 Protein, g/kg/d; HM: 1.9 (0.0), HM-Pr: 3.7 (0.1), P = .13 |                      |
|       | SE: N/D | | |                      |

*Calculated as total Nitrogen × 6.25

Abbreviations: AGA, appropriate for gestational age; BW, birth weight; Ca, calcium; CP, completed study protocol; DO, dropouts, that is, not included in growth outcomes; EN, enrolled; GA, gestational age; HC, head circumference; HM, human milk; LBW, low birth weight; Na, sodium; NS, nonsignificant; N/D, not described; OFC, occipito-frontal circumference; P, phosphorous; SGA, small for gestational age; SD, standard deviation; SEM, standard error of the mean; SE, study end; SS, study start; VLBW, very low birth weight.
### Table 5. Data Summary of Trials Comparing Growth in Infants Fed HM Fortified With HMFs With Varying Protein Content.

| Study | Participants | Study Description | Interventions | Intakes | Outcomes and Results | Quality |
|-------|--------------|------------------|---------------|---------|----------------------|---------|
| Miller et al<sup>34</sup> (2012), Australia | EN n = 92 GA: <31 wk | Study weeks 1-4, median (IQR) | Higher protein (HP) (n = 43): Energy, kcal/kg/d; HP: 137 (119-149), SP: 137 (122-150), P N/D (24-28), P = .33 | Δ Weight, g/d; HP: 24 (20-28), SP: 26 (24-28), P = .33 | Positive |
|  | DO n = 0 BW: N/D | Enrolment to study end, median (IQR) | 1.4 g protein/100 mL | Δ Length, cm/wk; HP: 1.2 (1.1-1.2), SP: 1.1 (1.1-1.1), P = .08 | |
|  | CP n = 59 (64%) Both healthy and unwell infants | Study period, mean (SD) | Standard protein (SP) (Control) (n = 49): Protein, g/kg/d; HP: 4.2 (3.6-4.7), SP: 3.6 (3.2-4.0), P N/D | Δ HC, cm/wk; HP: 0.9 (0.9-1.0), SP: 1.0 (0.9-1.0), P = .56 | |
|  | SS: enteral intake ~80 mL/kg/day | Study weeks 1-4, mean (SD) | Δ Weight, g/kg/d; HP: 17 (2), MCT: 12 (5), P < .01 | Δ Length, cm/wk; HP: 1.1 (0.4), MCT: 0.8 (0.3), P > .05 | |
|  | SE: discharge, estimated due date | Study period, mean (SD) | Protein, g/kg/d; P/E: 3.5 (0.3), MCT: 3.0 (0.5), P < .05 | Δ HC, cm/wk; P/E: 1.1 (0.3), MCT: 0.8 (0.4), P < .05 | |
| Brumberg et al<sup>35</sup> (2010), United States | EN n = 23 GA: N/D | Study weeks 1-4, mean (SD) | FHM + P/E (n = 11): Energy, kcal/kg/d; P/E: 128 (11), MCT: 124 (9), P > .05 | Δ Weight, g/kg/d; P/E: 17 (2), MCT: 12 (5), P < .01 | Neutral |
|  | DO n = 3 BW: ≤1250 g | Study period, mean (SD) | ¼ teaspoon/30 mL fluid | Δ Length, cm/wk; P/E: 1.1 (0.4), MCT: 0.8 (0.3), P > .05 | |
|  | CP (all 4 weeks), n = 13 Postnatal age ≥14 days | Study weeks 1-4, median (SD) | 0.3 g protein/100 mL | Δ HC, cm/wk; P/E: 1.1 (0.3), MCT: 0.8 (0.4), P < .05 | |
|  | SE: 28 days Diet ≥75% ENT | Study weeks 1-4, mean (SD) | FHM + MCT (n = 12): | Failure to regain BW OR weight gain < 2 mL/kg/d | |
|  | Otherwise healthy infants 0 g protein | Study period, mean (SD) | | < 15 g/kg/d after BW regained | |
| Arslanoglu et al<sup>36</sup> (2006), Italy | EN n = 36 BW: 600-1750 g | Study weeks 1-4, mean (SD) | ADJ fortification (n = 17): Energy, kcal/kg/d; ADJ: 126 (12), STD: 127 (12), P > .05 | Δ Weight, g/kg/d; ADJ: 18 (3), STD: 14 (3), P < .01 | Positive |
|  | DO n = 2 GA: 24-34 weeks | Study week 2, mean (SD) | If BUN 9-14 mg/dL, no adjustment; <9 mg/dL, increase 1 level; >14 mg/dL, decrease 1 level | Δ Length, cm/wk; ADJ: 1.3 (0.5), STD: 1.0 (0.3), P < .05 | |
|  | CP n = 36 Enteral intake 90 mL/kg/d | Study period, mean (SD) | Levels (g/100 mL): 0 = standard, 1 = 6.25 fortified; 2 = 6.25 HMF + 0.4 pro | Δ HC, cm/wk; ADJ: 1.4 (0.3), STD: 1.0 (0.3), P < .05 | |
|  | SS: feed volume 150 mL/kg/day | Study week 3, mean (SD) | HMF/100 mL | Protein, g/kg/d; ADJ: 3.2 (0.4), STD: 2.9 (0.3), P < .05 | |
|  | SE: weight 2000 g Healthy infants | Study period, mean (SD) | 5 g HMF/100 mL | Δ Length, cm/wk; ADJ: 1.3 (0.5), STD: 1.0 (0.3), P < .05 | |
|  | Assumed values of 68 kcal and 1.0 g protein/100 mL | Study week 3, mean (SD) | Same HMF (0.8 g protein/100 mL) | Protein, g/kg/d; ADJ: 3.4 (0.5), STD: 2.8 (0.2), P < .05 | |

(continued)
| Study                        | Participants | Intervention | Intakes | Outcomes and Results |
|-----------------------------|--------------|--------------|---------|----------------------|
| **Berseth et al**<sup>37</sup> (2004), Canada, United States | EN n = 185   | Trial HMF (HMF-T) (n = 96): | Energy<sup>*, kcal/kg/d; HMF-T: 118 (2), HMF-C: 115 (2), P = .07</sup> | Δ Weight, g/kg/d; HMF-T: 18 (1), HMF-C: 17 (1), P = .63<sup>a</sup> |
|                             | DO n = 4     | Protein 1.1 g/100 mL | Study period, mean (SE) | Study period, mean (SE) |
|                             | CP n = 94 (51%) | Control HMF (HMF-F) (n = 85): | Protein, g/kg/d; HMF-T: 3.8 (0.1), HMF-C: 3.6 (0.1), P < .01<sup>a</sup> |
|                             | SS: enteral intake >100 mL/kg/d | Protein 1.0 g/100 mL | Δ Weight, g/kg/d; HMF-T: 18 (1), HMF-C: 17 (1), P = .63<sup>a</sup> |
|                             | SE: study day 28 or discharge | Healthy infants | Protein 0.6 g/100 mL | Protein, g/kg/d; HMF-T: 18 (1), HMF-C: 17 (1), P = .63<sup>a</sup> |
| **Reis et al**<sup>38</sup> (2000), United States | EN n = 144   | Study fortifier (SF) (n = 74): | Energy<sup>*, kcal/kg/d; SF: 118 (13), CF: 118 (16), P < .05</sup> | Δ Weight, g/kg/d; SF: 18 (4), CF: 15 (3), P < .01<sup>a</sup> |
|                             | DO n = 25    | Protein 0.9 g/100 mL | Study period, mean (SD) | Study period, mean (SD) |
|                             | CP n = 89    | Contains MCT oil | Protein, g/kg/d; SF: 3.5 (0.4), CF: 3.1 (0.5), P < .01<sup>a</sup> |
|                             | SS: full strength fortification and enteral intake >100 mL/kg/d | Control fortifier (CF) (n = 70): | Δ HC, cm/wk; SF: 1.1 (0.3), CF: 1.0 (0.4), P = .03<sup>a</sup> |
|                             | SE: study day 29 or discharge | Protein 0.6 g/100 mL | Δ Weight, g/kg/d; SF: 18 (4), CF: 15 (3), P < .01<sup>a</sup> |
| **Porcelli et al**<sup>39</sup> (2000), United States | EN n = 90    | New HMF (n = 47): | Energy<sup>*, kcal/kg/d; New HMF: 115 (20), Std HMF: 125 (16), P N/D</sup> | Δ Length, cm/wk; New HMF: 1.0 (0.4), Std HMF: 0.8 (0.1), P < .05<sup>a</sup> |
|                             | DO n = 28    | Protein 1 g/100 mL | Study period, estimated (SD) | Study period, mean (SEM) |
|                             | CP n = 64    | Energy 13 kcal/100 mL | Protein, g/kg/d; New HMF: 4.3, Std HMF: 4.2, P N/D<sup>a</sup> |
|                             | SS: HMF introduced | Enteral intake >150 mL/kg/d HM | Δ OFC, cm/wk; New HMF: 1.0 (0.1), Std HMF: 0.8 (0.1), P = .04<sup>a</sup> |
|                             | HM composition | Assumed values of 66 kcal and 1.0 g protein/100 mL | Δ Length, cm/wk; New HMF: 1.0 (0.4), Std HMF: 0.8 (0.1), P = .04<sup>a</sup> |

<sup>a</sup>Calculated (kJ divided by 4.187)
### Study Description

| Study | Participants | Intervention | Intakes | Outcomes and Results | Quality |
|-------|--------------|--------------|---------|----------------------|---------|
| Moro et al (1995), Italy | EN n = 42, GA: N/D | Same HMF (0.8 g protein/100 mL HM) | Week 2, mean (SD) | Study period, mean (SD) | Neutral |
| DO n = 6, BW: 900-1500 g | ADJ fortification (n = 17): | Energy, kcal/kg/d; ADJ: 125 (7), FIX: 119 (7), P > .05 | Δ Weight, g/kg/d; ADJ: 19 (2), FIX: 18 (2), P > .05 |
| CP n = 36, Healthy infants | If CSUN 6.1-9.0 mg/100 mL, add 4.1 g fortifier/100 mL; 9.1-12.0 mg/100 mL, no adjustment; 12.1-15 mg/100 mL, add 2.9 g fortifier/100 mL | Protein, g/kg/d; ADJ: 4.0 (0.5), FIX: 3.5 (0.3), P < .01 | Δ Length, cm/wk; ADJ: 0.9 (0.3), FIX: 1.0 (0.4), P > .05 |
| SS: feeding volume 160 mL/kg/d | Fixed (FIX) fortification (n = 17): | Energy, kcal/kg/d; ADJ: 120 (7), FIX: 117 (7), P > .05 | Δ HC, cm/wk; ADJ: 0.9 (0.3), FIX: 0.9 (0.3), P > .05 |
| SE: discharge at ~2200 g | 3.5 g HMF/100 mL HM | Protein, g/kg/d; ADJ: 3.7 (0.3), FIX: 3.4 (0.4), P > .05 | *Calculated from mm/d |

Abbreviations: ADJ, adjustable; AGA, appropriate for gestational age; BUN, blood urea nitrogen; BW, birth weight; CHO, carbohydrate; CP, completed study protocol; CSUN, corrected serum urea nitrogen; DO, dropouts, that is, not included in growth outcomes; EN, enrolled; ENT, enteral nutrition; EPO, erythropoietin; Fe, iron; FHM, fortified human milk; FIX, fixed; GA, gestational age; HC, head circumference; HM, human milk; HMF, human milk fortifier; IQR, interquartile range; MCT, medium-chain triglyceride; N/D, not described; OFC, occipito-frontal circumference; FE, protein and energy; PN, parenteral nutrition; SD, standard deviation; SE, study end; SEM, standard error of the mean; SGA, small for gestational age; STD, standard; SS, study start; VD, vitamin D; VLBW, very low birth weight.
Few trials showed significant improvements in multiple outcome measures, limiting the consistency of this evidence. Many of the trials showing significantly increased weight gain in higher-protein intake groups did show a trend for increased rates of growth in length and HC but failed to reach significance. It may be that these trials were underpowered to detect statistically significant differences in these growth measures as they are more variable than weight. Nine studies did not report a power calculation, and all trials that did based their sample size on expected effect size of other outcomes such as nitrogen or fat-free mass accretion.

Given the clinical heterogeneity among the trials, it is difficult to draw robust conclusions from this evidence. The maturity and size of the infants studied varied between trials. Reasonably mature infants were studied overall (range = 1130-1958 g). This limits the generalizability of this evidence to very immature infants (<1000 g). The selection criteria varied widely between trials as well, with some including infants with intrauterine growth failure or those small for gestational age, while others excluded these infants. However, the clinical stability of infants was relatively uniform. Almost all studies described their sample as “healthy” or “clinically stable” (Table 3). Only one trial did not exclude infants with respiratory distress or on oxygen/ventilator support. Again, this limits the generalizability of this evidence to very immature infants (<1000 g).

The variance in effect size seen may reflect other key differences between the trials. The difference in protein intake between comparison groups ranged from 0.2 g/kg/day to 2.3 g/kg/day. Nine trials compared groups with less than 1 g/kg difference in intake (Table 3). Thus, differences in protein intake between comparison groups may have been too small to show the possible effect of increased protein intake in some trials. Differences in the composition of trial formulas and quality of protein may further contribute to statistical heterogeneity. Additionally, the medical management of infants also likely varied between trials, as these trials were conducted steadily over a period of 35 years and standards of care in neonatal intensive care units continue to improve.

These trials provide some evidence that increased enteral protein intake (intakes between 3.5 and 4.5 g/kg/day) results in increased weight gain of 3 to 6 g/kg/day in formula-fed infants, but little evidence suggesting increased length or HC growth.

**Trials Comparing Infants Fed Unfortified HM With Those Fed Protein-Fortified HM**

**Summary of Studies.** Five trials compared infants fed unfortified HM with those fed protein-fortified HM. These trials achieved similarity in energy intake between groups through increased volume or fat of unfortified HM feeds, or natural variation in composition of HM. All trials showed a trend toward increased weight, length, and HC in infants fed protein-fortified HM compared with unfortified HM (Table 4). A statistically significantly greater increment of weight gain in infants fed higher protein intakes was shown in 3 trials (range of 3-4 g/kg/day greater than controls). Two of these also showed significantly increased length growth in infants with higher protein intakes (0.2 and 0.4 cm/week more than controls) and one significantly increased HC growth (0.3 cm/week greater than controls, $P < .02$).

**Critique of Studies.** The quality of these pre-1991 trials is difficult to assess due to lack of adequate reporting of trial methods. None reported using random sequence generation, and only one adequately concealed group allocation, introducing the possibility of allocation bias. Furthermore, personnel and outcome blinding were only described in one trial; thus, bias may be introduced during unblinded measurement of outcomes. However, it is difficult to blind a trial of this type without changing the caloric density of the control feed as nonnutritive substances should not be added to preterm infant feeds. Three trials limited measurement error through the use of one outcome assessor, standardized techniques, and repeated measures (see Supplementary Table 2, available online at http://gph.sagepub.com supplemental).

The 4 trials showing increased growth with increased protein intake measured protein intakes through analysis of pooled daily samples of each infant’s milk, strengthening their findings. The only study showing no effect measured milk only once, at the beginning of the trial. The sample size used in this trial was also small (16 infants) compared to the other trials (34-66 infants; Table 4), increasing vulnerability to Type II error. Furthermore, the short study duration (7 days) may be limiting the ability of the study to show a significant effect. The generalizability of this study is also questionable, as it investigated male infants only. All studies were strengthened by their achievement of a substantially different protein intake between groups (range =
0.7 g/kg/day to 1.8 g/kg/day) ensuring any potential effect of increased protein intake was likely to be seen. However, the results of 3 trials may be confounded by the inclusion of bone minerals in the HMF. Polberger et al. did not report P values for any group comparisons, limiting interpretation of these results.

This evidence is strongly consistent, with all trials showing a trend to increased growth in all outcomes measured, with multiple outcomes reaching statistical significance in 3 trials. This may in part be due to the clinical homogeneity between studies. All trials investigated healthy infants of similar size (mean birth weights = 1090-1435 g) and maturity at study start (Table 4). The effect size is also remarkably consistent between trials showing significantly increased growth (weight = +3.8 to +4.1 g/kg/day; length = +0.35 to +0.36 cm/week) with only one study deviating from this. This trial was conducted earlier than the others, with feed and fortifier quality likely to have improved since.

There are quality issues with this evidence, primarily due to the age of the trials. However, it is highly consistent; all trials show a trend to increased growth in all outcomes measures with none showing the opposite trend. Thus, this evidence suggests increased protein intake (addition of 0.9-1.0 g/100 mL milk) in HM-fed infants does result in increased weight, length, and HC growth.

**Trials Comparing HMFs Resulting in Different Protein Intakes**

**Summary of Studies.** Seven trials compared the growth of HM-fed infants fed HMFs or supplements resulting in different protein intakes. All trials used multi-component HMFs including protein, energy, bone minerals, and a variable selection of micronutrients. Berseth and Moro were the only 2 trials that showed no trend toward better growth in the higher protein intake groups. Four trials showed significantly increased rates of fractional weight gain in infants with higher protein intakes (range = 3-6 g/kg/day greater than controls). Three of these trials also showed significantly increased gains in HC with higher protein intakes (0.2-0.4 cm/week greater than controls). Two trials showed a trend toward better length growth, however, in the study by Miller et al. this did not reach statistical significance (0.1 cm/week greater than controls; P = .08).

**Critique of Studies.** The trials are of varying quality. Miller et al. alone reported random sequence generation, while 3 trials reported adequate concealment of group allocation. For some of these studies, study quality was primarily limited by inadequate reporting of random sequence generation and allocation concealment. Only 3 trials were satisfactorily blinded, possibly introducing bias during outcome assessment. However, all but one study reported groups to be similar at baseline (Miller et al. had uneven multiple births between groups). Furthermore, 4 trials conducted statistical analysis on an intention-to-treat basis. This ensured groups remained balanced and thus similar at baseline, strengthening their results. Three of these trials found a significant increase in growth in the higher protein intake group (see Supplementary Table 3, available online at http://gph.sagepub.com/supplementary).

All trials are strengthened by adequate study duration (range = 21-74 days). The trial by Miller et al. was the most generalizable as it included healthy, sick, and small for gestational age infants. All other trials investigated “healthy” infants only. Furthermore, 3 trials reported accurate protein intakes through analysis of HM samples. As it has been shown that assumed intakes can deviate from actual intakes significantly, the use of assumed HM composition values limits the accuracy of the protein intakes reported by the other trials, and thus the results. The differences in protein intake between groups were small (range = 0.2-0.6 g/kg/day), and may not have been large enough to show a significant effect, despite satisfying the selection criteria to be included in this review. However, as many of these trials reported protein intakes that meet current recommendations (Table 1), assessing the effect of smaller increases in protein intake is clinically relevant.

There is some clinical heterogeneity among these trials. The birth weight of infants varied widely (range = 862-1407 g), as did clinical condition and maturity at study initiation (13-25 days postmenstrual age). Furthermore, compliance to feeding protocol within and between trials was wide-ranging, some infants receiving none of the assigned intervention while others fully completed feeding protocols. This may partly explain the variance in effect size seen between trials (Table 5). Variations in fortifier composition, different fortification methods, and diverse standards of care may also contribute. Overall, however, this evidence is reasonably consistent, as all trials showing significantly improved growth rate in one outcome variable also show a trend to improved growth in all outcome measures (Table 5). Thus, it is unlikely to be simply changes in fluid and fat mass confounding the results.

These trials provide evidence that increased protein intake (additional 0.2-0.6 g/kg/day) results in small weight, length, and HC gains in infants fed fortified HM.
Discussion

All 3 study categories show increased weight gain in infants fed higher protein intakes. When considered together and represented graphically, a somewhat linear dose–response relationship can be seen (Figure 2). However, weight gain increases from below intrauterine rates to above are larger in the trials comparing infants fed unfortified with fortified HM (Figure 2). This likely indicates protein intakes of unfortified HM-fed infants are inadequate for growth. This is consistent with the Cochrane review of the area, which also concluded unfortified HM is inadequate for infants <1500 g.15 Conversely, in infants fed formula or fortified HM, the growth of most comparison groups fell between 15 g/kg/day and 20 g/kg/day (Figure 2). This may indicate that generally protein intakes were adequate; thus, overall these studies compare adequate intakes with intakes supporting optimal growth. The findings of the Cochrane review investigating this in formula-fed infants are consistent with those of the present review: increased weight gain with higher protein intake, but little evidence for increased length or HC growth.12 Overall, statistically significant improvement in length or HC growth was shown in only 10 of the 18 studies investigating these outcomes. This may be due to the duration of the trials, as changes in these outcomes take longer to observe compared with weight gain.44

Comparing these trials is limited by variation in protein quality, micronutrient composition, and nonnutritive effects on growth of different feed types. This variation, along with differing medical management,27 energy intakes, race,
clinical stability, size for gestational age of infants studied may explain the spread of results seen in Figure 2. This comparison is very clinically relevant however, as mixed feeding is a reality in clinical practice. The growth achieved in many trials met the clinical growth target of 15 g/kg/day (Figure 2). However, only 4 trials achieved the growth target required for adequate catch-up growth, to prevent the disparity seen in the number of infants small for gestational age at discharge (Figure 2). This suggests that many of the protein intakes studied remain inadequate for truly optimal growth. However, the impact of the substantial discrepancies between studies in the calculation of rate of weight gain should not be underestimated. Methods used ranged from the simplest average of weight over time to complex statistical modelling. Patel et al showed large differences in the growth estimates produced using different calculation methods; thus, this undoubtedly contributes to the spread of results seen in Figure 2.

Any benefits of increased protein intake need to be balanced with potential adverse effects due to the immature organ systems of these infants. Two formula trials withdrew participants due to perceived adverse effects of higher protein intake. Svenningsen et al reported late-onset metabolic acidosis in 5 infants (4 in higher protein intake group), and Raiha et al reported 2 infants (both higher protein intake group) developed progressive nitrogen retention and metabolic acidosis. This may be plausibly explained by the age of these trials and therefore likely poorer protein quality of feeds. This effect was not shown in the more recent trials with even higher protein intakes. Additionally, medical management of preterm infants has advanced such that greater clinical and metabolic stability can be achieved during feeding. Seven other trials reported either higher serum urea or elevated plasma amino acid concentration in infants with higher protein intakes. These authors report, however, that although higher than in control infants, elevated biochemical parameters were not clinically affecting the health of the infant, or resolved without intervention. No studies reported increased incidence of necrotizing enterocolitis, patent ductus arteriosus, or sepsis in higher protein intake groups. The present evidence suggests, therefore, that in very low birth weight infants protein intakes up to 4.5 g/kg/day are well tolerated and do not result in adverse outcome. However, this evidence does not assess the safety of such intakes in the smallest and sickest infants.

The evidence base presented in this review is satisfactory, as RCTs with moderate risk of bias are included. The consistency and generalizability of the evidence is good as the included trials represent a number of geographical regions and thus are highly applicable to health care internationally. The outcomes measured represent increments of growth. Therefore, the small improvements shown accumulate over the hospital admission to have substantial implications for the infant’s overall growth. These results satisfactorily show that infants fed higher protein intakes achieve small improvements in weight in the order of 3 to 6 g/kg/day, length of 0.2 to 0.4 cm/week, and HC of 0.1 to 0.4 cm/week over infants receiving lower protein. Thus, preterm infants with birth weight <1750 g fed HM should have it fortified with a multicomponent fortifier including protein. It may also be beneficial to increase the protein content of HMFs to 1.4 g/100 mL milk, and of formulas to 2.4 to 2.9 g/100 mL as standard, as no adverse effects of these protein intakes were shown.

The evidence presented here is of less than high quality, as many of these trials were conducted before clear guidelines for reporting of RCTs were established. Thus, any future research needs to be done using adequately randomized and blinded trials, with large sample sizes. The smallest and sickest infants should be included, as currently very little research includes this group of preterm infants. Furthermore, trials involving HM-fed infants must accurately measure protein intakes through HM composition analysis. Importantly, a standardized method for calculating rate of weight gain needs to be adopted by all researchers in the field to facilitate comparison of growth velocity between studies. This evidence suggests increased enteral protein intake results in increased growth in preterm infants. Thus, future research should aim to determine the protein intakes that provide not only adequate but also truly optimal growth, with a focus on safety.

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