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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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Table 1. Current Nutrient Recommendations for Enteral Feeding Preterm Infants.

	Birth Weight	Protein Intake (g/kg/day)	Energy Intake (kcal/kg/day)
American Academy of Pediatrics[10]	800-1200 g	4.0	105-130
	1200-1800 g	3.5	105-130
Canadian Pediatric Society[13]	<1000 g	3.5-4.0	105-135
	>1000 g	3.0-3.6	105-135
Tsang et al,[14] USA, "growing"—clinically stable and gaining weight	ELBW	3.8-4.4	130-150
	VLBW	3.4-4.2	110-130
European Society for Paediatric Gastroenterology, Hepatology and Nutrition[3]	<1000 g	4.0-4.5	110-135
	1000-1800 g	3.5-4.0	110-135

Abbreviations: ELBW, extremely low birth weight (<1000 g); VLBW, very low birth weight (<1500 g).

<2000 g, the use of human milk fortifiers (HMFs) is standard clinical practice.[3]

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;[11] however, the protein was of poor quality, and recent reviews suggest this may no longer apply to current practice.[2,12]

Cochrane systematic reviews of growth in "high" versus "low" protein formula fed infants[12] and infants fed fortified versus unfortified HM have been published.[15] The former[12] 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[15] 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)[14] 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 • Birth weight <2500 g • Protein intakes intentionally different between 2 or more groups • Reports comparison of change between groups in any or all of the following: weight, length, head circumference	• Protein intakes not reported • Studies investigating differences in parenteral feeding solutions • Energy difference >10% relative composition or shown to be statistically significantly different • Energy intake of any group <100 kcal/kg • Protein intakes between 2 or more groups are shown to be not statistically significantly different

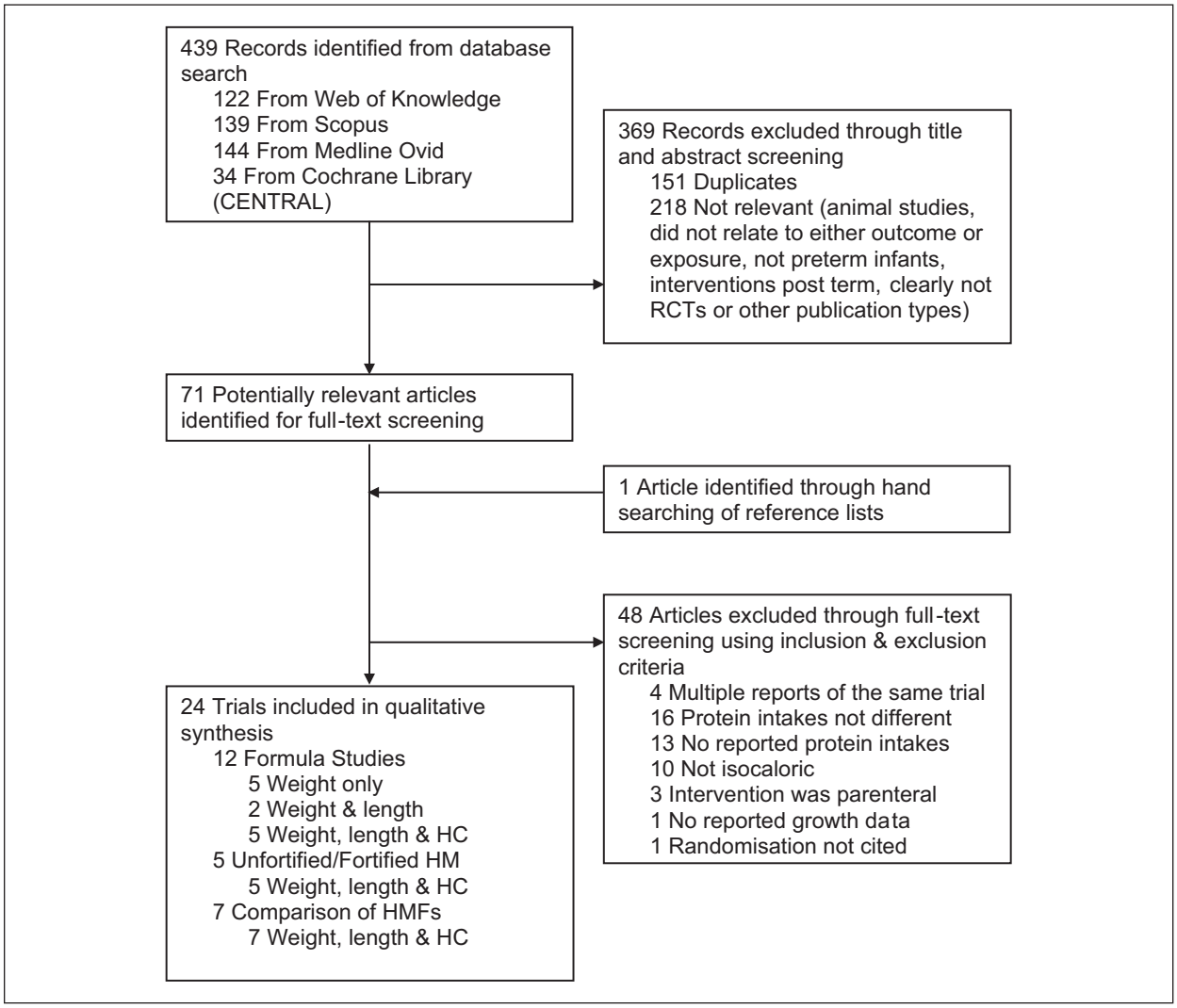

Figure 1. Flow diagram of search methods.

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.[16] Briefly, this assesses trials for relevance to practice and scientific rigour.[16] 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).[17-28] 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.[17,19,22,27,28] Cooke et al[18] and Darling et al[26] 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).[20,21,23-25] Kashyap et al[25] and Darling et al[26] 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[26] 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.[24,27,28] Bell et al[24] and Svenningsen et al[27] found no statistically significant difference in any outcome measures between the HM- and formula-fed groups, while Raiha et al[28] 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,[20,23-26] compared with no clear description of measurement methods in all trials showing no effect.[18,19,21,22,27,28] Furthermore, only 2 trials conducted an intention-to-treat analysis.[18,19] The study duration (>28 days) was a strength of 6 trials.[17,19,21,26-28] 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[18] and Bell et al.[24] 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[17] and Bhatia et al[22] 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[17] and Embleton and Cooke[19] 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 Description			Outcomes and Results		
Study		Participants	Intervention	Intakes	Growth	Quality
Costa-Orvay et al[17] (2011), Spain	EN n = 38	BW < 1500 g	Alprem	Study period, average	Study end, mean (SD)	Positive
	DO n = 4	GA ≤ 32 wk	Group A excluded	Energy, kcal/kg/d	Weight, g; Group B: 1998 (146), Group C: 2154 (202), *P* = .62	
	SS: on enteral nutrition, IV ceased	AGA	Not isocaloric	Group B:150, Group C: 150, *P* N/D	Length, cm; Group B: 44.5 (1.2), Group C: 45.6 (1.8), *P* N/D	
	SE: 28 days from study start	Healthy infants	Group B (n = 12):	Protein, g/kg/d	HC, cm; Group B: 32.3 (1.2), Group C: 32.3 (0.8), *P* = .74	
			Promod 0.7 g/kg/d	Group B: 4.2, Group C: 4.7, *P* N/D		
			Duocal 3.7 g/kg/d Group C (n = 12): Promod 1.3 g/kg/d Duocal 3.3 g/kg/d			
Cooke et al[18] (2006), United Kingdom	EN n = 18	BW ≤ 1500 g	RegPro:	Days 1-14, mean (SD)	Study period, mean (SD)	Neutral
	DO n = 0	GA ≤ 32 wk	Protein 3.0 g/100 kcal	Energy, kcal/kg/d; HiPro: ~120*, RegPro: ~120*, *P* N/D	Δ Weight, g/d; HiPro: 35 (9), RegPro: 27 (6), *P* = .005	
	SS: N/D	Clinically stable	HiPro:	Protein, g/kg/d; HiPro: 4.6 (0.4), RegPro: 3.8 (0.2), *P* < .001	Δ Weight, g/kg/d; HiPro: 23 (7), RegPro: 17 (6), *P* N/D	
	SE: 14 days after start (7 days crossover)	No steroids, diuretics, or oxygen therapy at balance study	Protein 3.6 g/100 kcal	*Calculated from target volume of 150 mL/kg/d		
			Titrated using CHO Vit and min content same			
Embleton and Cooke[19] (2005), United Kingdom	EN n = 77	BW ≤ 1750 g	Group A (n = 25):	Study period, mean (SD)	Enrolment–discharge, mean (SD)	Positive
	DO n = 3	GA ≤ 34 wk	Protein 3.3 g/100 kcal	Energy, kcal/kg/d; Group A: 131 (23), Group B: 125 (23), Group C: 129 (25), *P* N/D	Δ Weight, g/d; Group A: 42 (7), Group B: 37 (6), Group C: 40 (7), *P* > .05	
	SS: full enteral feeds (150 mL/kg/d)	Intake ≥ 150 mL/kg/d for ≥48 h	Group B (n = 26):	Protein, g/kg/d; A > B by 0.5, *P* < .001. and B > C by 0.2, *P* < .05	Δ Length, cm/wk; Group A: 1.3 (0.3), Group B: 1.2 (0.3), Group C: 1.3 (0.3), *P* > .05	
	SE: term + 12 weeks corrected age	Healthy infants -Weight≥1000g	Protein 3.0 g/100 kcal Group C (n = 26): Protein 2.7 g/100 kcal Vit and min content same Titrated using fat			

(continued)

Table 3. (continued)

Study	Study Description			Outcomes and Results		Quality
		Participants	Intervention	Intakes	Growth	
Wauben et al[20] (1995), Netherlands	EN n = 16	BW: AGA	F1.5 (n = 8):	Days 4-7, mean (SD)	Study days 1-8, mean (SD)	Neutral
	DO n = 0	GA: 28-35 wk	Energy 68 kcal/100 mL	Energy, kcal/kg/d; F1.5: 118 (9), F2.0: 117 (11), $P > .05$	Δ Weight, g/kg/d; F1.5: 12 (3), F2.0: 16 (4), $P < .05$	
	SS: enteral volume 160 mL/kg/d	Healthy infants	Protein 1.5 g/100 mL	Protein, g/kg/d; F1.5: 2.7 (0.3), F2.0: 3.4 (0.3), $P < .05$		
	SE: 8 days		F2.0 (n = 8): Energy 70 kcal/100 mL Protein 2.0 g/100 mL Vit and min content same			
Hillman et al[21] (1994), United States	EN n = 32	BW: <1500 g	Group A (n = 9):	Study period, average	Birth to 30 days, mean (SD)	Neutral
	DO n = 5	GA: N/D	Protein 3.0 g/100 kcal	Energy, kcal/kg/d; Groups A, B, and C: Aim 120, *P* N/D	Δ Weight, g/kg/d; Group A*: 19 (4), Group B: 16 (3), Group C*: 13 (5), *$P < .05$	
	SS: N/D	Not receiving TPN or diuretics	Group B (n = 9):	Protein, g/kg/d; Group A: 3.6, Group B: 3.2, Group C: 2.8, *P* N/D	Gain in HC and length not different (data N/D)	
	SE: 30 days		Protein 2.7 g/100 kcal Group C (n = 9): Protein 2.2 g/100 kcal Vit and min content same Titrated using CHO			
Bhatia et al[22] (1991), United States	EN n = 26	BW: <1550 g	High (n = 8):	Day 1 to study end, mean (SD)	Day 1 to study end, mean (SD)	Neutral
	DO n = 3	GA: N/D	Protein 3.0 g/100 kcal	Energy, kcal/kg/d; Low: 117 (4), Mid: 120 (3), High: 118 (6), *P* N/D	Δ Weight, g/kg/d; Low: 19 (1), Mid: 20 (3), High: 21 (2), $P > .05$	
	SS: when enteral energy intake reached 100 kcal/kg/d	Enteral feeds by 14 days age	Mid (n = 8):	Protein, g/kg/d; Low: 2.6 (0.1), Mid: 3.1 (0.1), High: 3.8 (0.2), *P* N/D	Gain in HC and length not different (data N/D)	
	SE: 2 weeks from study day 1	Energy intake 100 kcal/kg/d by 21 days age	Protein 2.7 g/100 kcal			
		Healthy infants	Low (n = 7): Protein 2.2 g/100 kcal Vit and min content same Titrated using CHO			

(continued)

Table 3. (continued)

Study	Study Description			Outcomes and Results		Quality
		Participants	Intervention	Intakes	Growth	
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 mLkg/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 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 Energy 67 kcal/100 mL Minimal vit and min diff Titrated with fat and CHO Group 3 excluded Not isocaloric		Δ HC, 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)	Study period, mean (SD)	Study period, mean (SD)	Neutral
	DO n = 2	GA: N/D	Group B (n = 25):	Energy, kcal/kg/d; Group B: 128 (14), Group C: 128 (15), HM: 127 (21), *P* NS	Δ Weight, g/kg/d; Group B*: 19 (4), Group C*: 16 (4), HM: 16 (5), *P < .05	
	SS: enteral intake 150 mL/kg/day and IV ceased	Gender: both	Protein 2.4 g/100 mL	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: 1.1 (0.4), *P* > .05	
	SE: weight >2000 g (average duration of study N/D)	Healthy infants	Energy 79 kcal/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	
			Group C (n = 25): Protein 2.1 g/100 mL Energy 74 kcal/100 mL HM (n = 10): Protein 1.5 g/100 mL Energy 70 kcal/100 mL Titrated with fat and CHO			

(continued)

Table 3. (continued)

Study	Study Description			Outcomes and Results		Quality
		Participants	Intervention	Intakes	Growth	
Kashyap et al[25] (1986), United States	EN n = 34	GA: 27-37 wk	Group 1 (n = 11):	Study period, mean (SD)	Study period, mean (SD)	Neutral
	DO n = 7	BW: 900-1750 g	Protein 1.3 g/100 mL	Energy, kcal/kg/d; Group 1: 115 (1), Group 2: 114 (1), *P* N/D	Δ Weight, g/kg/d; Group 1: 14 (3), Group 2: 18 (3), *P* < .05	
	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 Length, cm/wk; Group 1: 0.9 (0.2), Group 2: 1.2 (0.3), *P* > .05	
	SE: weight 2200 g		Group 2 (n = 11):		Δ HC, cm/wk; Group 1: 0.9 (0.2), Group 2: 1.2 (0.3), *P* < .05	
			Protein 2.0 g/100 mL Energy 63 kcal/100 mL Vit and min content same Titrated with fat and CHO Group 3 excluded Not isocaloric			
Darling et al[26] (1985), Canada	EN n = 15	BW: 1300-1600 g	Group 1 (n = 5):	Study period, mean (SEM)	Study period, mean (SEM)	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	
	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 Length, cm/wk; Group 1*: 1.1 (0.1), Group 2*: 0.8 (0.0), Group 3: 0.9 (0.0), *P* < .01	
	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	
		No hyaline membrane disease	Group 2 (n = 5):			
		No notable respiratory distress	Protein 1.5 g/100 mL			
			Energy 70 kcal/100 mL Whey–casein 20:80 Group 3 (n = 5): Protein 2.0 g/100 mL Energy 74 kcal/100 mL Titrated with fat and CHO Vit and min content same			

(continued)

Table 3. (continued)

Study	Study Description			Outcomes and Results		Quality
		Participants	Intervention	Intakes	Growth	
Svenningsen et al[27] (1982), Sweden	EN n = 48	Mean BW: 1385 ± 343 g	HM-Group (n = 18):	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 F2-Group (n = 16): Protein 3.0 g/100 kcal Titration N/D			
Raiha et al[28] (1976), Finland	EN n = 106	BW ≤ 2100 g	F1 (n = 21):	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*: 22 (2), F1*: 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	F2 (n = 20): Protein 3.0 g/100 mL Whey–casein 60:40 F3 and F4 excluded Differed from F1 and F2 in protein quality only Pooled HM (n = 22): Pooled banked milk Similar vit and mins Titrated using lactose		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.

Table 4. Data Summary of Trials Comparing Growth in Infants Fed Unsupplemented HM With Those Fed Protein-Supplemented HM.

	Study Description			Outcomes and Results		
Study		Participants	Intervention	Intakes	Growth	Quality
Kashyap et al[29] (1990), United States	EN n = 66	BW: 900-1750 g	Group 1 (n = 14):	Study period, mean (SD)	Study period, mean (SD)	Neutral
	DO n = 24	GA: N/D	Mothers HM	Energy, kcal/kg/d; Group 1: 129 (11), Group 2: 131 (12), *P* N/D	Δ Weight, g/kg/d; Group 1: 17 (2), Group 2: 21 (2), *P* < .01	
	CP n = 27	SGA and AGA infants included	Group 2 (n = 13):	Protein, g/kg/d; Group 1: 2.5 (0.5), Group 2: 3.2 (0.4), *P* N/D	Δ Length, cm/wk; Group 1: 0.9 (0.2), Group 2: 1.3 (0.5), *P* NS	
	SS: enteral feedings 180 mL/kg/d	Healthy infants	Mothers HM + protein (1.1 g/kg/d), Ca (3.7 mmol/kg/d), P (2.1 mmol/kg/d), Na (1.1 mmol/kg/d)		Δ HC, cm/wk; Group 1: 1.0 (0.2), Group 2: 1.2 (0.2), *P* NS	
	SE: infant weight 2200 g		Reports groups 1 and 2 isocaloric due to variation in composition of HM			
			Group 3 excluded			
			Not isocaloric			
			Target volume: 180 mL/kg/d			
			HM composition: daily samples pooled for weekly analysis			
Polberger et al[30] (1989), Sweden	EN n = 34	BW: <1500 g	HM excluded	Study period, mean (SD)	Study period, mean (SD)	Neutral
	DO n = 6	GA: N/D	Not isocaloric	Energy, kcal/kg/d; HMF: 121 (10)	Δ Weight, g/kg/d; HMF: 16 (2), HMP: 20 (1), *P* N/D	
	CP n= 5 (mothers milk), n = 10 (little mothers milk)	AGA	HM + HMF (n = 8):	HMP: 117 (9), *P* N/D	Δ Length, cm/wk; HMF: 0.9 (0.2), HMP: 1.3 (0.1), *P* N/D	
	SS: stable on 170 mL/kg/d	Tolerance of complete enteral feeding	HM with human milk fat (1 g/100 mL)	Protein, g/kg/d; HMF: 2.1 (0.3)	Δ HC, cm/wk; HMF: 1.1 (0.2), HMP: 1.2 (0.1), *P* N/D	
	SE: 2200 g or breastfeeding initiated	Healthy infants	HM + HMP (n = 9):	HMP: 3.6 (0.2), *P* N/D		
			HM with human milk protein (1 g/100 mL)			
			HM + HMPF excluded			
			Not isocaloric			
			Groups HM + HMP and HM + HMF isocaloric due to addition of fat			
			Target volume: 170 mL/kg/d			
			HM composition: daily aliquot of milk pooled for weekly analysis			
Greer and McCormick[31] (1988), United States	EN n = 38	BW: <1600 g	HM (n = 10): Mothers HM	First 6 weeks enteral feeds, mean (SD)	First 6 weeks enteral feeds, mean (SD)	Neutral
	DO n = N/D	GA: <32 weeks	FHM (n = 10):	Energy, kcal/kg/d; HM: 112 (10), FHM: 105 (15), *P* NS	Δ Weight, g/kg/d; HM: 13 (1), FHM: 17 (2), *P* < .01	
	CP n= N/D	Healthy infants	Mothers HM + protein (0.9 g/100 mL), Ca (90 mg/100 mL), P (45 mg/100 mL)	Protein, g/kg/d; HM: 3.3 (0.6), FHM: 4.2 (0.5), *P* < .01	Δ Length, cm/wk; HM: 0.8 (0.2), FHM: 1.1 (0.2), *P* < .01	
	SS: full oral feedings achieved (120 kcal/kg/d)	AGA	HM groups similar energy intake due to higher volume feeds in HM group		Δ HC, cm/wk; HM: 0.8 (0.2), FHM: 1.1 (0.2), *P* < .02	
	SE: 6 weeks from study start		Formula groups excluded			

(continued)

Table 4. (continued)

Study	Study Description			Outcomes and Results		Quality
		Participants	Intervention	Intakes	Growth	
			Not isocaloric Target volume: 120-200 mL/kg/d HM composition: 5% daily aliquots of feeds pooled for weekly analysis			
Putet et al[32] (1987)	EN n = 16	BW: <1500 g	HM (n = 8): Pooled HM	Study period (3 days), mean (SD)	7 days overlapping balance study, mean (SD)	Neutral
	DO n = 0	GA: N/D	HM-Pr (n = 8):	Energy, kcal/kg/d; HM: 107 (7), HM-Pr: 106 (14), *P* > .05	Δ Weight, g/kg/d; HM: 15 (3), HM-Pr: 17 (2), *P* > .05	
	CP n = 16	Gender: male	Pooled HM + 1 g sup/100 mL, providing (/100 g powder):	Protein*, g/kg/d; HM: 2.5 (0.4), HM-Pr: 3.8 (0.5), *P* < .01	Δ Length, cm/wk; HM: 1.1 (0.3), HM-Pr: 1.2 (0.3), *P* > .05	
	SS: N/D	Healthy infants	Nitrogen: 13.2 g, lipid: 1.4 g, Ca: 2.5 g, P: 1.1 g, Na: 8.0 mg	*Calculated as total Nitrogen × 6.25	Δ HC, cm/wk; HM: 1.0 (0.1), HM-Pr: 1.2 (0.2), *P* > .05	
	SE: 7 days after study start		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			
Ronnholm et al[33] (1986)	EN n = 54	BW: <1500 g	HM (n = 23):	2 weeks of age, mean (SEM)	Weeks 1-6, mean (SEM)	Neutral
	DO n = 10	GA: ≤36 wk	Unsupplemented HM	Energy, kcal/kg/d; HM: 111 (3), HM-Pr: 110 (4), *P* N/D	Δ Weight, g/kg/d; HM: 10 (1), HM-Pr: 13 (1), *P* < .01	
	CP n = 44	SGA and AGA	HM-Pr (n = 21):	Protein, g/kg/d; HM: 1.8 (0.1), HM-Pr: 3.2 (0.2), *P* N/D	Δ Length, cm/wk; HM: 0.8 (0.1), HM-Pr: 1.0 (0.1), *P* = .04	
	SS: N/D	Healthy infants	HM + HM protein (0.9 g/100 mL of milk)	6 weeks of age, mean (SEM)	Δ HC, cm/wk; HM: 0.6 (0.0), HM-Pr: 0.7 (0.0), *P* = .13	
	SE: N/D		All infants fed either pooled banked mature HM or mothers HM Reason for similarity of energy intake between groups N/D Target volume: 200 mL/kg/d HM composition: 5 mL samples taken at beginning and end of each milking	Energy, kcal/kg/d; HM: 130 (3), HM-Pr: 133 (2), *P* N/D Protein, g/kg/d; HM: 1.9 (0.0), HM-Pr: 3.7 (0.1), *P* N/D		

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 Description			Outcomes and Results		
Study		Participants	Intervention	Intakes	Growth	Quality
Miller et al[34] (2012), Australia	EN n = 92	GA: <31 wk	Higher protein (HP) (n = 43):	Study weeks 1-4, median (IQR)	Enrolment to study end, median (IQR)	Positive
	DO n = 0	BW: N/D	1.4 g protein/100 mL	Energy, kcal/kg/d; HP: 137 (119-149), SP: 137 (122-150), *P* N/D	Δ Weight, g/d; HP: 24 (20-28), SP: 26 (24-28), *P* = .33	
	CP n = 59 (64%)	Both healthy and unwell infants	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 Length, cm/wk; HP: 1.2 (1.1-1.2), SP: 1.1 (1.1-1.1), *P* = .08	
	SS: enteral intake ~80 mL/kg/day	SGA and AGA infants	1.0 g protein/100 mL		Δ HC, cm/wk; HP: 0.9 (0.9-1.0), SP: 1.0 (0.9-1.0), *P* = .56	
	SE: discharge, estimated due date		Titrated with CHO			
			Minimal diff in vits and mins			
			Unfortified HM composition			
			Weekly sample			
Brumberg et al[35] (2010), United States	EN n = 23	GA: N/D	FHM + P/E (n = 11):	Study weeks 1-4, mean (SD)	Study period, mean (SD)	Neutral
	DO n = 3	BW: ≤1250 g	¼ teaspoon/30 mL fluid	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	
	CP (all 4 weeks), n = 13	Postnatal age ≥14 days	0.3 g protein/100 mL	Protein, g/kg/d; P/E: 3.5 (0.3), MCT: 3.0 (0.5), *P* < .05	Δ Length, cm/wk; P/E: 1.1 (0.4), MCT: 0.8 (0.3), *P* > .05	
	SE: 28 days	Diet ≥75% ENT	FHM + MCT (n = 12):		Δ HC, cm/wk; P/E: 1.1 (0.3), MCT: 0.8 (0.4), *P* < .05	
		Failure to regain BWOR weight gain <15 g/kg/d after BW regained	2 mL/kg/d			
		Otherwise healthy infants	0 g protein			
			HM and Formula fed infants randomized to P/E or MCT			
			HM composition			
			Assumed values of 68 kcal and 1.0 g protein/100 mL			
Arslanoglu et al[36] (2006), Italy	EN n = 36	BW: 600-1750 g	ADJ fortification (n = 17):	Week 2, mean (SD)	Study period, mean (SD)	Positive
	DO n = 2	GA: 24-34 weeks	If BUN 9-14 mg/dL, no adjustment; <9 mg/dL, increase 1 level; >14 mg/dL, decrease 1 level	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	
	CP n = 36	Enteral intake 90 mL/kg/d	Levels (g/100 mL): 0 = standard, 1 = 6.25 fortifier, 2 = 6.25 HMF + 0.4 pro	Protein, g/kg/d; ADJ: 3.2 (0.4), STD: 2.9 (0.3), *P* = .05	Δ Length, cm/wk; ADJ: 1.3 (0.5), STD: 1.1 (0.4), *P* > .05	
	SS: feed volume 150 mL/kg/day	Singletons only	STD fortification (n = 17):	Week 3, mean (SD)	Δ HC, cm/wk; ADJ: 1.4 (0.3), STD: 1.0 (0.3), *P* < .05	
	SE: weight 2000 g	Healthy infants	5 g HMF/100 mL	Energy, kcal/kg/d; ADJ: 128 (8), STD: 121 (8), *P* > .05		
			Fortified HM composition: twice weekly sample	Protein, g/kg/d; ADJ: 3.4 (0.5), STD: 2.8 (0.2), *P* < .05		
			Same HMF (0.8 g protein/100 mL)			

(continued)

Table 5. (continued)

Study	Study Description			Outcomes and Results		Quality
		Participants	Intervention	Intakes	Growth	
Berseth et al[37] (2004), Canada, United States	EN n = 185	GA: ≤33 wk	Trial HMF (HMF-T) (n = 96):	Study period, mean (SE)	Study period, mean (SE)	Neutral
	DO n = 4	BW: ≤1500 g	Protein 1.1 g/100 mL	Energy*, kcal/kg/d; HMF-T: 118 (2), HMF-C: 115 (2), *P* = .07	Δ Weight, g/kg/d; HMF-T: 18 (1), HMF-C: 17 (1), *P* = .63	
	CP n = 94 (51%)	Enteral intake >100 mL/kg/d	Control HMF (HMF-C) (n = 85):	Protein, g/kg/d; HMF-T: 3.8 (0.1), HMF-C: 3.6 (0.1), *P* < .01		
	SS: enteral intake >100 mL/kg/d	Healthy infants	Protein 1.0 g/100 mL	*Calculated (kJ divided by 4.187)		
	SE: study day 28 or discharge	Had consumed no HMF	Titrated with CHO and fat			
		Did not receive EPO, VD, minerals, or Fe on study day 0	Not equivalent in vits and mins			
			HM composition			
			Assumed values of 66 kcal and 1.0 g protein/100 mL			
Reis et al[38] (2000), United States	EN n = 144	GA: ≤33 wk	Study fortifier (SF) (n = 74):	Study period, mean (SD)	Study period, mean (SD)	Neutral
	DO n = 25	BW: ≤1600 g	Protein 0.9 g/100 mL	Energy, kcal/kg/d; SF: 118 (13), CF: 118 (16), *P* > .05	Δ Weight, g/kg/d; SF: 18 (4), CF: 15 (3), *P* < .01	
	CP n = 89	Healthy infants	Contains MCT oil	Protein, g/kg/d; SF: 3.5 (0.4), CF: 3.1 (0.5), *P* < .01	Δ Length, cm/wk; SF: 1.1 (0.3), CF: 1.0 (0.4), *P* = .03	
	SS: full strength fortification and enteral intake 100 mL/kg/d		Control fortifier (CF) (n = 70):		Δ HC, cm/wk; SF: 1.0 (0.2), CF: 0.9 (0.3), *P* = .07	
	SE: study day 29 or discharge		Protein 0.6 g/100 mL			
			No MCT oil			
			Titrated with CHO and fat			
			Not equivalent in vits and mins			
			HM composition			
			Assumed values of 67 kcal and 1.4 g protein/100 mL			
Porcelli et al[39] (2000), United States	EN n = 90	GA: 25-32 wk	New HMF (n = 47):	Study period, estimated (SD)	Study period, mean (SEM)	Neutral
	DO n = 28	BW: 600-1500 g	Protein 1 g/100 mL	Energy*, kcal/kg/d; New HMF: 115 (20), Std HMF: 125 (16), *P* N/D	Δ Weight, g/kg/d; New HMF: 20 (1), Std HMF: 17 (1), *P* = .04	
	CP n = 64	AGA	Energy 13 kcal/100 mL	Protein, g/kg/d; New HMF: 4.3, Std HMF: 4.2, *P* N/D	Δ Length, cm/wk; New HMF: 0.9 (0.1), Std HMF: 0.8 (0.1), *P* > .05	
	SS: HMF introduced	Enteral intake >150 mL/kg/d HM	Standard HMF (n = 43):	*Calculated using energy of HM 67 kcal/100 mL and reported adjusted intakes (mL/kg/d)	Δ OFC, cm/wk; New HMF: 1.0 (0.1), Std HMF: 0.8 (0.1), *P* = .04	

(continued)

Table 5. (continued)

Study	Study Description			Outcomes and Results		Quality
		Participants	Intervention	Intakes	Growth	
		SE: only consuming unsupplemented HM	Medically stable	Protein 0.7 g/100 mL		
			Not receiving PN, formula, diuretics, or corticosteroids	Energy 14 kcal/100 mL		
			Mother's milk >14 days postpartum	Fat and protein higher in New HMF		
				Not equivalent in vits and mins		
				HM composition		
				Assumed values of 67-72 kcal and 1.8 g protein/100 mL		
Moro et al[40] (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):	Week 3, mean (SD)	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	Energy, kcal/kg/d; ADJ: 120 (7), FIX: 117 (7), $P > .05$	*Calculated from mm/d	
			HMP group excluded	Protein, g/kg/d; ADJ: 3.7 (0.3), FIX: 3.4 (0.4), $P > .05$		
			Protein intake shown to be nonsignificantly different			
			Fortified HM composition daily sample forming weekly pools analyzed			

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; P/E, 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.[23-25] 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 also, 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[21] did not exclude infants with respiratory distress or on oxygen/ventilator support. Again, this limits the generalizability of this evidence to infants who experience multiple medical issues associated with premature birth.[41] A further difficulty encountered when comparing these studies is the variation in method for calculating rate of growth gain. Different calculation methods have been shown to produce varying results, with some more accurate than others.[42] However, many studies simply did not report their method for calculating growth rate.

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[19] to 2.3 g/kg/day.[28] 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.[29-33] These trials achieved similarity in energy intake between groups through increased volume[31,32] or fat[30] of unfortified HM feeds, or natural variation in composition of HM.[29] 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).[29,31,33] 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)[31,33] and one significantly increased HC growth (0.3 cm/week greater than controls, $P < .02$).[31]

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,[30] introducing the possibility of allocation bias. Furthermore, personnel and outcome blinding were only described in one trial;[30] 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 [29.31,33] (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.[32] 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.[29,31,32] Polberger et al[30] 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.[33] 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.[34-40] All trials used multicomponent 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.[37,40] 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).[35,36,38,39] Three of these trials also showed significantly increased gains in HC with higher protein intakes (0.2-0.4 cm/week greater than controls).[35,36,39] Two trials showed a trend toward better length growth;[34,38] however, in the study by Miller et al[34] 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[34] alone reported random sequence generation, while 3 trials reported adequate concealment of group allocation.[34,36,40] For some of these studies,[37-39] 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[34] 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/supple-mental).

All trials are strengthened by adequate study duration (range = 21-74 days). The trial by Miller et al[34] 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[34,36,40] reported accurate protein intakes through analysis of HM samples. As it has been shown that assumed intakes can deviate from actual intakes significantly,[43] 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[38] while others fully completed feeding protocols.[35] 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.

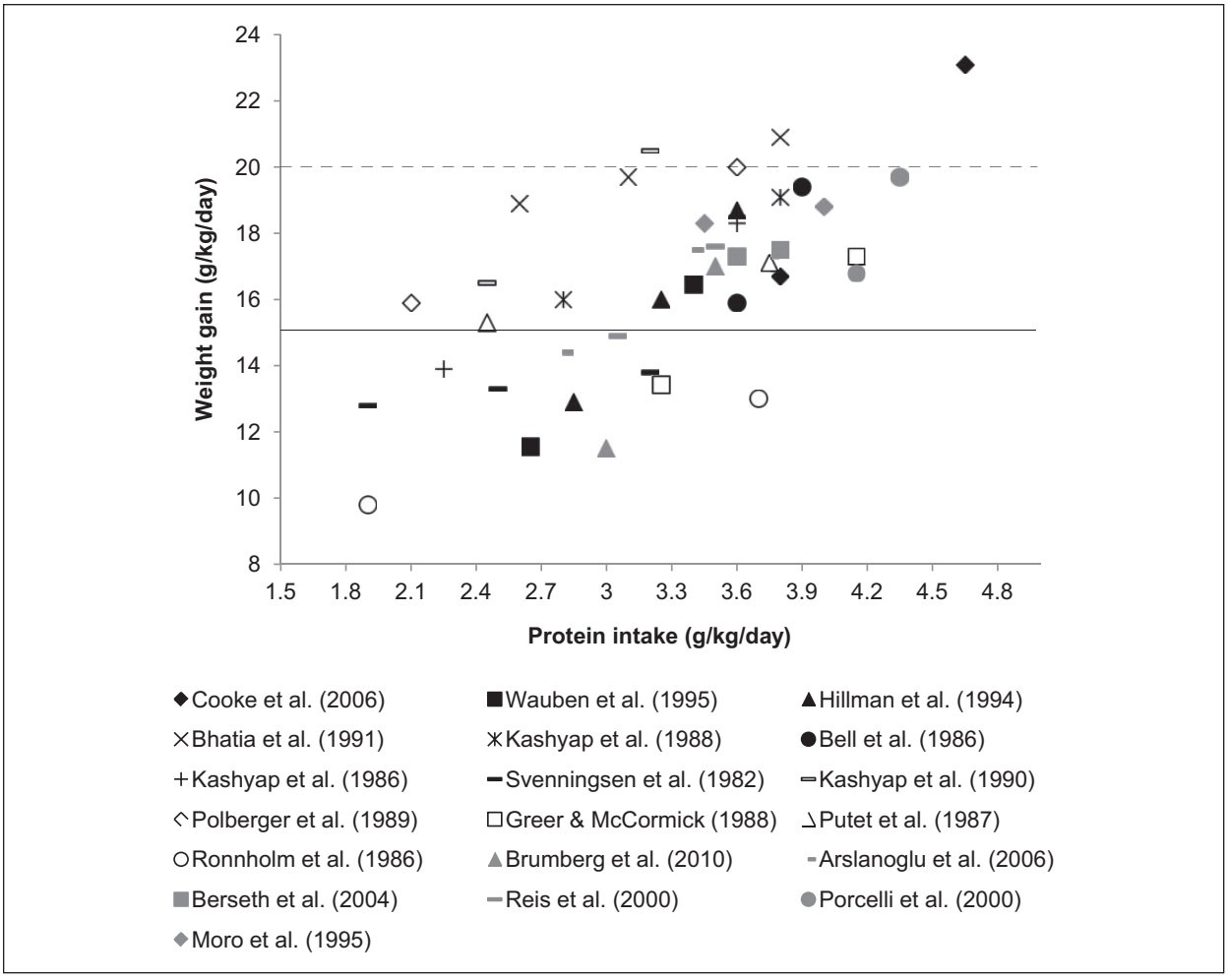

Figure 2. Relationship between protein intake and weight gain.
Five studies did not report weight gain in g/kg/day and thus were excluded.[17,19,26,28,34] Formula studies are indicated with black, unfortified versus fortified HM studies with white, and studies comparing different HMFs with grey markers. The solid black line represents the clinically used weight gain target of 15 g/kg/day.[45] The dashed line represents the recently updated weight gain target that accommodates catch-up growth, 20 g/kg/day.[46]

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,[22,27,29] and size for gestational age of infants studied[30,33] 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).[45] However, only 4 trials achieved the growth target[46] 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[18] to complex statistical modelling.[34] Patel et al[42] 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[27] reported late-onset metabolic acidosis in 5 infants (4 in higher protein intake group), and Raiha et al[28] 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.[2] Seven other trials reported either higher serum urea or elevated plasma amino acid concentration in infants with higher protein int akes.[17,19,24,25,31,32,34] 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[47] 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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