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Higher versus lower protein intake in formula-fed low birth weight infants (Review)

Fenton TR, Premji SS, Al-Wassia H, Sauve RS

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[Intervention Review]

Higher versus lower protein intake in formula-fed low birth weight infants

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ABSTRACT

Background

The ideal quantity of dietary protein for formula-fed low birth weight infants is still a matter of debate. Protein intake must be sufficient to achieve normal growth without negative effects such as acidosis, uremia, and elevated levels of circulating amino acids.

Objectives

To determine whether higher (≥ 3.0 g/kg/d) versus lower (< 3.0 g/kg/d) protein intake during the initial hospital stay of formula-fed preterm infants or low birth weight infants (< 2.5 kilograms) results in improved growth and neurodevelopmental outcomes without evidence of short- and long-term morbidity.

To examine the following distinctions in protein intake.

1. Low protein intake if the amount was less than 3.0 g/kg/d.
2. High protein intake if the amount was equal to or greater than 3.0 g/kg/d but less than 4.0 g/kg/d.
3. Very high protein intake if the amount was equal to or greater than 4.0 g/kg/d.

If the reviewed studies combined alterations of protein and energy, subgroup analyses were to be carried out for the planned categories of protein intake according to the following predefined energy intake categories.

1. Low energy intake: less than 105 kcal/kg/d.
2. Medium energy intake: greater than or equal to 105 kcal/kg/d and less than or equal to 135 kcal/kg/d.
3. High energy intake: greater than 135 kcal/kg/d.

As the Ziegler-Fomon reference fetus estimates different protein requirements for infants based on birth weight, subgroup analyses were to be undertaken for the following birth weight categories.

1. < 800 grams.

2. 800 to 1199 grams.
3. 1200 to 1799 grams.
4. 1800 to 2499 grams.

Search methods

The standard search methods of the Cochrane Neonatal Review Group were used. MEDLINE, CINAHL, PubMed, EMBASE, and the Cochrane Central Register of Controlled Trials (CENTRAL; *The Cochrane Library*) were searched.

Selection criteria

Randomized controlled trials contrasting levels of formula protein intake as low (< 3.0 g/kg/d), high (≥ 3.0 g/kg/d but < 4.0 g/kg/d), or very high (≥ 4.0 g/kg/d) in formula-fed hospitalized neonates weighing less than 2.5 kilograms were included. Studies were excluded if infants received partial parenteral nutrition during the study period or were fed formula as a supplement to human milk. Studies in which nutrients other than protein also varied were added in a post-facto analysis.

Data collection and analysis

The standard methods of the Cochrane Neonatal Review Group were used.

Main results

Five studies compared low versus high protein intake. Improved weight gain and higher nitrogen accretion were demonstrated in infants receiving formula with higher protein content while other nutrients were kept constant. No significant differences were seen in rates of necrotizing enterocolitis, sepsis, or diarrhea.

One study compared high versus very high protein intake during and after an initial hospital stay. Very high protein intake promoted improved gain in length at term, but differences did not remain significant at 12 weeks corrected age. Three of the 24 infants receiving very high protein intake developed uremia.

A post-facto analysis revealed further improvement in all growth parameters in infants receiving formula with higher protein content. No significant difference in the concentration of plasma phenylalanine was noted between high and low protein intake groups. However, one study ([Goldman 1969](#)) documented a significantly increased incidence of low intelligence quotient (IQ) scores among infants of birth weight less than 1300 grams who received a very high protein intake (6 to 7.2 g/kg).

Authors' conclusions

Higher protein intake (≥ 3.0 g/kg/d but < 4.0 g/kg/d) from formula accelerates weight gain. However, limited information is available regarding the impact of higher formula protein intake on long-term outcomes such as neurodevelopmental abnormalities. Available evidence is not adequate to permit specific recommendations regarding the provision of very high protein intake (> 4.0 g/kg/d) from formula during the initial hospital stay or after discharge.

PLAIN LANGUAGE SUMMARY

Higher versus lower protein intake in formula-fed low birth weight infants

Dietary protein is needed for normal growth and development. The protein intake required for growth of the low birth weight infant has been estimated by the growth rate of the fetus to be 3.5 to 4.0 g/kg/d. Controlling the amount of protein given to low birth weight babies (less than 2.5 kg) fed with formula is important. Too much protein can raise blood urea and amino acid (phenylalanine) levels, and this may harm neurodevelopment. Too low protein intake may limit the growth of these infants. The review authors searched the medical literature to identify studies that compared protein intake as follows: between 3 and 4 g of protein per kg of infant body weight each day versus less than 3.0 g/kg/d or greater than 4.0 g/kg/d by low birth weight infants fed formula during their initial hospital stay. Increased protein intake resulted in greater weight gain of around 2.0 g/kg/d. Based on increased body incorporation of nitrogen, this was associated with increased lean body mass. The present conclusion was based on six studies that changed only the protein content of the formula and was supported by three additional studies that made changes in other nutrients as well. No significant difference in the concentration of plasma phenylalanine was noted between infants fed high or low protein content formula. The review was limited in the conclusions made because differences in protein content among comparison groups in some of the individual trials were small and formulas differed substantially across studies; some studies included healthier and more mature premature infants. Study periods varied from eight days to two years, so information on long-term outcomes was limited. Existing research is not adequate to allow specific recommendations regarding formula with protein content that provides more than 4.0 g/kg/d.

BACKGROUND

Description of the condition

Good nutrition is essential for optimal growth and development of the preterm infant (Raiha 2001). Protein is an important component of adequate nutrition, as it provides essential amino acids required for protein synthesis, which is necessary for growth. Hence, the quantity of protein consumed is an important consideration (Raiha 2001). The protein requirement for preterm infants can be estimated in two ways: estimates based on the protein intake of breast-fed infants or estimates based on theoretical calculations (the factorial approach). A preterm infant fed own mother's milk receives approximately 1.4 g/100 mL (Gomella 1999) or about 2.5 g/kg/d of protein (Carlson 1998). The factorial approach is a theory-based calculation that sums the requirements for growth and those for replacement of inevitable losses in urine, feces, and skin (Fomon 1991). It is difficult to estimate requirements for protein intake in premature infants because they may have a high rate of protein turnover and breakdown (Pencharz 1981) as a result of immaturity or illness (Hay 1996; Kalhan 2000). Preterm infants have a very rapid rate of growth and protein accretion. Based on the factorial approach using the "reference fetus," Ziegler and Fomon estimated the protein intake required for preterm infant growth and nitrogen accretion (Ziegler 1976; Ziegler 1981) to be 4 g/kg/d of enteral protein for infants with a birth weight of less than 1200 grams, and 3.5 g/kg/d for infants with a birth weight of 1200 to 1800 grams (AAP 1998). Formulas currently available for preterm infants in North America contain 3 g of protein per 100 kcal. If energy intakes are maintained at the recommended range (CPS 1995), formula-fed infants would receive about 3.2 to 4.2 g/kg/d of protein. A disparity is evident between what is provided in own mother's milk versus estimated protein intake based on the factorial approach using the Ziegler-Fomon reference and what is contained in preterm formula.

Description of the intervention

Putative risks of higher protein intake include increased concentrations of amino acids, hydrogen ions, and urea as a result of immaturity of amino acid metabolic pathways in preterm infants (Senterre 1983). Premature infants may not be able to handle higher protein intake efficiently; hence metabolic acidosis and higher plasma levels of amino acids such as tyrosine and phenylalanine concentrations may result (Micheli 1999). Theoretically, these metabolic changes could lead to mental retardation. Additionally, adaptive responses of endocrine and metabolic homeostasis resulting from early nutrition may lead to "metabolic programming," which alters long-term outcomes of chronic diseases. Renal hypertrophy accompanied by a significant rise in kidney tissue and circulating insulin-like growth factor-1 has been reported secondary to high protein intake (Murray 1993). High protein intake in early life may increase risks later in life of obesity (Rolland-Cachera 1995; Scaglioni 2000) and other pathology (Rolland-Cachera 1995) such as diabetes mellitus (Raiha 2001). Therefore, long-term consequences of early nutrition need to be considered.

How the intervention might work

Putative benefits of higher protein intake include adequacy of protein for growth of lean tissue, growth of bone and blood constituents, turnover of tissues, synthesis of hormones and enzymes, and maintenance of oncotic pressure (Fomon 1993). In

an animal study, higher protein intake was shown to accelerate maturation of the renal tubules (Jakobsson 1990). Deficiency of protein in infants leads to growth failure and, when extreme, can result in edema and lower resistance to infection (Nayak 1989).

Why it is important to do this review

Sufficient energy and other nutrients are needed to allow protein to be used for anabolism (Kashyap 1994) rather than as a fuel source. When energy availability is limited, nitrogen balance and protein utilization for tissue synthesis are limited. When protein is used for energy, the amino groups are cleaved and are converted primarily to urea, which is excreted, while the carbon skeleton enters the citric acid cycle to be used as the energy source. When protein is used as an energy source, optimal protein synthesis cannot occur (Kashyap 1994). Consequently, protein intake needs to be evaluated in relation to energy intake for a direct comparison of alleged benefits and risks of higher protein intake.

Protein intake also needs to be evaluated in relation to that of other nutrients, as differences in intake of other nutrients may influence infant growth rates (Castillo-Duran 2003; Musoke 2001). If studies provide variable amounts of protein and other nutrients at the same time, it is not possible to attribute study findings solely to the difference in protein intake. If formulas vary by more than 10% in any constituent other than protein, a direct comparison of outcomes may not be valid.

A related Cochrane review by Kuschel and Harding (Kuschel 2000) concluded that protein supplementation of human milk in relatively well preterm infants offers certain short-term benefits, including increases in weight gain, linear growth, and head growth. Although urea levels were higher in patients receiving protein supplementation, this was thought to reflect adequate rather than excessive dietary protein intake. Long-term effects and adverse effects of protein supplementation of human milk could not be evaluated in Kuschel and Harding's systematic review (Kuschel 2000) because of an absence of relevant data.

The balance between supposed benefits and risks of higher protein intake for formula-fed low birth weight infants weighing < 2.5 kilograms remains unclear.

OBJECTIVES

To determine whether higher (≥ 3.0 g/kg/d) versus lower (< 3.0 g/kg/d) protein intake during the initial hospital stay of formula-fed preterm infants or low birth weight infants (< 2.5 kilograms) results in improved growth and neurodevelopmental outcomes without evidence of short- and long-term morbidity.

To examine the following distinctions in protein intake.

1. Low protein intake if the amount was less than 3.0 g/kg/d.
2. High protein intake if the amount was equal to or greater than 3.0 g/kg/d but less than 4.0 g/kg/d.
3. Very high protein intake if the amount was equal to or greater than 4.0 g/kg/d.

If the reviewed studies combined alterations of protein and energy, subgroup analyses were to be carried out for the planned categories of protein intake according to the following predefined energy intake categories.

1. Low energy intake: less than 105 kcal/kg/d.
2. Medium energy intake: greater than or equal to 105 kcal/kg/d and less than or equal to 135 kcal/kg/d.
3. High energy intake: greater than 135 kcal/kg/d.

As the Ziegler-Fomon reference fetus estimates different protein requirements for infants based on birth weight, subgroup analyses were to be undertaken for the following birth weight categories.

1. < 800 g.
2. 800 to 1199 g.
3. 1200 to 1799 g.
4. 1800 to 2499 g.

METHODS

Criteria for considering studies for this review

Types of studies

Randomized controlled trials. Quasi-randomized trials were not considered.

Types of participants

Infants who weighed less than 2.5 kilograms at birth, whether appropriate- or small-for-gestational-age (AGA or SGA), and were studied during their initial hospital stay. They were exclusively fed formula and did not receive parenteral nutrition during the study.

Types of interventions

The interventions comprised different levels of protein intake during the initial hospital stay, which were categorized as follows: low protein intake if the amount was less than 3.0 g/kg/d, high protein intake if the amount was equal to or greater than 3.0 g/kg/d but less than 4.0 g/kg/d, and very high protein intake if the amount was equal to or greater than 4.0 g/kg/d. Contrasting levels of protein intake were compared over different periods of time.

Types of outcome measures

Primary outcomes

1. Growth parameters, including weight gain (g/kg/d or g/d), linear growth (cm/wk), and head growth (cm/kg/wk or cm/wk), expressed in absolute terms or relative to intrauterine standards or Centers for Disease Control and Prevention (CDC) growth charts once the infant is term corrected age.
2. Nitrogen utilization as reflected by blood urea (mmol/L).
3. Nitrogen accretion, expressed in absolute terms such as g/kg/d or relative to fetal accretion rate.
4. Intelligence quotient (IQ) scores and Bayley score at 18 months and/or later.
5. Abnormal phenylalanine levels.
6. Growth failure (weight for age < 10% based on intrauterine standards or CDC growth charts once the infant is term corrected age).

Secondary outcomes

1. Decreased gastric motility (number of episodes of abdominal distention experienced per day).

2. Days to full feedings (days from initiation of feedings to achievement of 120 mL/kg/d).
3. Feeding intolerance (number of feeding interruptions related to feeding intolerance experienced per day).
4. Necrotizing enterocolitis (Bell's stage II or greater).
5. Metabolic acidosis (pH, base excess).
6. Serum albumin (g/L).
7. Sepsis (number of babies who developed confirmed sepsis-positive blood culture and the organism(s) identified).
8. Diarrhea (number of babies who developed episodes of stools considered to have abnormal water loss).

Search methods for identification of studies

Electronic searches

Several databases were searched, including MEDLINE back to 1966, CINAHL back to 1982, PubMed back to 1966, EMBASE back to 1980, and the Cochrane Central Register of Controlled Trials (CENTRAL, 2013, Issue 5). MeSH headings, including infant, newborn, low birth weight, small for gestational age, very low birth weight, premature, amino acids, dietary proteins, milk proteins, milk, infant food, food, and formulated, and text words, including formula and protein, were used for the computerized searches. No language restrictions were applied.

Computerized searches were updated by two review authors up to June 2013.

Searching other resources

Abstracts and conference and symposia proceedings from the Society of Pediatric Research and the American Academy of Pediatrics were also identified. Cross-references were reviewed independently for additional relevant titles and abstracts for articles up to 50 years old. Experts were contacted to identify other studies relevant to the area. No language restrictions were applied.

Clinical trials registries were searched for ongoing and recently completed trials (clinicaltrials.gov; controlled-trials.com; who.int/ictrp).

Data collection and analysis

The standard methods of the Cochrane Neonatal Review Group were employed.

Selection of studies

All articles retrieved from the complete search were assessed for relevance independently by two review authors. Randomized controlled trials testing contrasting levels of formula protein intake during initial hospital stay were considered if they met the following criteria for relevance.

1. Study participants weighed less than 2.5 kilograms at birth.
2. Study participants were not receiving parenteral nutrition at the time of randomization.
3. Study participants were exclusively formula-fed.
4. Energy, Na, K, P, Zn, and essential fatty acid intakes did not differ significantly (by no more than 10% relative concentration).

Given the small number of trials that met all of the criteria, and some larger and important studies that met the first three but

not the last criterion, three review authors decided to include these studies in a post-facto analysis of the primary outcomes to provide readers with a more comprehensive and clinically relevant systematic review.

If all of the protein intake groups within a study fell inside one of the predesignated protein intake criteria, this study was excluded.

Data extraction and management

Data were extracted independently by two review authors. Differences were resolved by discussion and upon consensus of three review authors. Efforts were made to contact investigators for data, additional information, and/or clarification regarding ten studies (Bhatia 1991; Cooke 2006; Embleton 2005; Hillman 1994; Kashyap 1986; Mimouni 1989; Nichols 1966; Svenningsen 1982; Thom 1984; Wauben 1995).

Assessment of risk of bias in included studies

The standard methods of the Cochrane Neonatal Review Group were employed. The methodological quality of the studies was assessed using the following key criteria: allocation concealment (blinding of randomization), blinding of intervention, completeness of follow-up, and blinding of outcome measurement/assessment. For each criterion, assessment was yes, no, or can't tell. Two review authors separately assessed each study (SSP, TRF). Disagreements were resolved by discussion. This information was added to the [Characteristics of included studies](#) table.

In addition, the following issues were evaluated and were entered into the [Risk of bias in included studies](#) table.

1. Sequence generation: Was the allocation sequence adequately generated?
2. Allocation concealment: Was allocation adequately concealed?
3. Blinding of participants, personnel, and outcome assessors: Was knowledge of the allocated intervention adequately prevented during the study? At study entry? At the time of outcome assessment?
4. Incomplete outcome data: Were incomplete outcome data adequately addressed?
5. Selective outcome reporting: Are reports of the study free of the suggestion of selective outcome reporting?
6. Other sources of bias: Was the study apparently free of other problems that could put it at high risk of bias?

Measures of treatment effect

Statistical analyses were performed using Review Manager software. Categorical data were analyzed using risk ratio (RR), risk difference (RD), and the number needed to treat for an additional beneficial outcome (NNTB). Continuous data were analyzed using weighted mean difference (WMD). The 95% confidence interval (CI) was reported on all estimates. A standardized statistical method was used to handle three-arm trials in which two groups fell within one predesignated protein intake group (Rosner 2000).

Assessment of heterogeneity

A statistical test for heterogeneity (I^2 test) included in the graphical output of Cochrane reviews was used to assess variability in treatment effects evaluated by different trials.

Data synthesis

Meta-analysis was performed using Review Manager software (RevMan 5) supplied by The Cochrane Collaboration.

For meta-analysis, WMDs and 95% CIs are reported for continuous variables, and typical estimates for RR, RD, and 95% CIs are reported for categorical outcomes. Fixed-effect models were assumed.

Subgroup analysis and investigation of heterogeneity

If the reviewed studies combined alterations of protein and energy, subgroup analyses were to be carried out for the planned categories of protein intake according to the following predefined energy intake categories.

1. Low energy intake: less than 105 kcal/kg/d.
2. Medium energy intake: greater than or equal to 105 kcal/kg/d and less than or equal to 135 kcal/kg/d.
3. High energy intake: greater than 135 kcal/kg/d.

As the Ziegler-Fomon reference fetus estimates different protein requirements for infants based on birth weight, subgroup analyses were to be undertaken for the following birth weight categories.

1. < 800 grams
2. 800 to 1199 grams
3. 1200 to 1799 grams
4. 1800 to 2499 grams

RESULTS

Description of studies

The literature search identified 49 studies, of which 13 were non-randomized controlled studies. A total of 36 randomized studies were scrutinized for criteria of relevance, of which 19 studies were excluded for the following reasons.

1. In seven studies, the protein intake groups fell inside one of the predesignated protein intake criteria.
2. In eight studies, the intervention being examined was different from that proposed in this systematic review (eg, studies examining quality of protein).
3. In three studies, infants received parenteral nutrition during the study period.
4. In one study, the experimental protocol was modified during the study period.

Details of reasons for exclusion are listed in the [Characteristics of excluded studies](#) table.

Six studies (Bhatia 1991; Embleton 2005 (partial data); Hillman 1994; Kashyap 1986; Svenningsen 1982; Wauben 1995) met all inclusion criteria. Four studies (Cooke 2006; Goldman 1969; Kashyap 1988; Raiha 1976) differed in one or more nutrients by more than 10% in either direction; however, they were included in a post-facto analysis for the primary outcomes. Details of studies that met the inclusion criteria and of those included for the post-facto analysis are presented in the [Characteristics of included studies](#) table. Three studies (Mimouni 1989; Nichols 1966; Thom 1984) provided incomplete data.

Studies meeting all a priori inclusion criteria

Bhatia 1991 randomly assigned 26 AGA and SGA infants of birth weight less than 1550 grams to one of three formulas that were identical in composition, except for the protein content. Three infants were withdrawn from the study. Infants were given study formula when they were tolerating 60 kcal/kg/d of a standard premature infant formula. The study formulas were continued for two weeks after intake reached 100 kcal/kg/d. Growth, biochemical parameters, necrotizing enterocolitis, and neonatal behavior were assessed. Data for two groups in the high protein category were combined in this review.

Embleton 2005 randomly assigned 77 AGA and SGA infants of gestational age less than 35 weeks and birth weight less than 1750 grams in two strata (< 1250 grams, 1250 to 1750 grams) to one of three study formulas. The study began when infants were tolerating ≥ 150 mL/kg/d of enteral intake for ≥ 48 hours and current weight was ≥ 1000 grams. Infants received the study formula until term plus 12 weeks corrected age. Data for two groups in the high protein category were combined in this review. Growth, body composition, and biochemical parameters were assessed.

Hillman 1994 randomly assigned 27 infants weighing less than 1500 grams at birth in three weight group strata (< 1000 grams, 1000 to 1250 grams, 1250 to 1500 grams) to one of three study formulas before feedings were initiated in the first week of life. All infants completed two-week and four-week assessments of growth, biochemical parameters, and bone mineral content; however, 14 of the 27 infants were discharged before the six-week assessment was performed. Data for two groups in the high protein category were combined in this review.

Kashyap 1986 randomly assigned 34 AGA and SGA low birth weight infants weighing 900 to 1750 grams at birth to receive one of three formulas. One group of nine infants received increased energy intake, so they were not included in this review. Growth, biochemical parameters, necrotizing enterocolitis, diarrhea, and nutrient balance were assessed. Data on energy expenditure and energy balance were collected for a subset of infants in this study and were published by **Schulze 1987**.

Svenningsen 1982 randomly allocated 48 AGA and SGA very low birth weight and premature infants in the third week of life to one of three groups. One group received human milk and was not eligible for this review. The other two groups received formulas with or without the addition of a commercial product "protinpur" to produce high or low protein intake. **Svenningsen 1982a** reported long-term follow-up growth parameters and neurodevelopmental outcomes up to two years of age.

Wauben 1995 randomly allocated 16 healthy AGA premature infants between 28 and 35 weeks gestational age to two formulas with differing protein content and conducted a modified three-day protein and energy balance study. The study began once infants were receiving full enteral feedings of 160 mL/kg/d.

Studies comparing formulas with differences in other nutrients

In **Cooke 2006**, 18 preterm infants of birth weight ≤ 1500 g and gestational age ≤ 32 weeks received both standard (3.0 g/100 kcal) and high protein (3.6 g/100 kcal) formulas over two one-week comparison periods with the sequence of formula feeding randomly determined in a balanced cross-over design. The higher

protein formula had a 20% higher percentage of medium chain triglycerides (MCT), 16% more sodium, 13% more potassium, 12% more chloride, 19% less copper, and 13% more magnesium compared with the standard protein formula. Anthropometry was performed at the beginning and at the end of each study period. Nutrient balance and plasma amino acids were determined at the end of each week.

Goldman 1969 randomly assigned 304 AGA and SGA infants of birth weight less than 2000 grams in three birthweight strata—< 1000 grams, 1000 to 1499 grams, and 1500 to 2000 grams—to two study formulas. Infants > 1000 grams were further stratified based on gender, and twins were assigned separately. Infants were followed from the first few days of life until 2200 grams was achieved. The study compared high (3.0 to 3.6 g/kg/d) versus very high (6.0 to 7.2 g/kg/d) protein intake. The higher protein formula had a 17% higher concentration of minerals. Growth and biochemical and neurological parameters were assessed. Two separate papers (**Goldman 1971, 1974**) on the same study reported neurodevelopmental outcomes at three and five to seven years of age.

Kashyap 1988 randomly assigned 50 AGA and SGA low birth weight infants weighing 900 to 1750 grams at birth to receive one of three formulas until study end when the infants reached 2200 grams. One group of 15 infants who received increased energy intake was not included in this review. Formula in the high protein groups had 14% more potassium, 15% more calcium, and 20% more magnesium compared with formula in the low protein groups. Growth, biochemical parameters, necrotizing enterocolitis, and nutrient balance were assessed before study end when the infants reached 2200 grams.

Raiha 1976 randomly assigned 106 AGA infants of birth weight 2100 grams or less to one of four isocaloric formulas that varied in both quantity (2.25 and 4.5 g/kg/d) and type (whey:casein ratios) of protein in the first week of life. Infants were grouped into three categories: 28 to 30 weeks, 31 to 33 weeks, and 34 to 36 weeks. Potassium varied by 17%, calcium by 15%, and phosphorus by 12% in relative concentration in the whey predominant formulas between low and very high protein groups. Sodium varied by 28% and magnesium by 12% in relative concentration in the casein predominant formulas. Study formulas were provided until hospital discharge. Three separate papers on this study have been published (**Rassin 1977, 1977, and Gaull 1977**) and have reported different outcomes.

Risk of bias in included studies

Infants were allocated to assigned treatment by randomization in all studies included in this updated review. Only three studies (**Bhatia 1991; Embleton 2005; Hillman 1994**) reported adequate concealment of allocation and blinding of randomization. Eight studies (**Bhatia 1991; Cooke 2006; Embleton 2005; Goldman 1969; Hillman 1994; Kashyap 1986; Kashyap 1988; Raiha 1976**) reported that the intervention was blinded to caregivers and/or investigator(s). Two studies (**Bhatia 1991; Raiha 1976**) reported blinding of outcome. Two studies reported an intention-to-treat analysis (**Cooke 2006; Wauben 1995**).

Effects of interventions

High versus low protein intake (restricted to studies meeting all a prior inclusion criteria) (comparison 1)

Primary outcomes

Growth parameters (Outcome 1.1)

Weight gain (g/kg/d) (Outcome 1.1.1)

Bhatia 1991 and Svenningsen 1982 found no significant differences in weight gain between groups. However, Hillman 1994, Kashyap 1986, and Wauben 1995 found that infants receiving high protein intake had significantly greater weight gain. The overall analysis revealed a significant difference in weight gain (WMD 2.36 g/kg/d, 95% CI 1.31 to 3.40) in favor of the high protein group.

Linear growth (cm/wk) (Outcome 1.1.2)

Kashyap 1986 found that infants receiving high protein intake had significantly greater linear growth, and Svenningsen 1982 observed no significant differences between groups. The overall analysis did not reveal a significant difference (WMD 0.16 cm/wk, 95% CI -0.02 to 0.34).

Head growth (cm/wk) (Outcome 1.1.3)

Kashyap 1986 found that infants receiving high protein intake had significantly greater head growth. Bhatia 1991, Hillman 1994, and Svenningsen 1982 reported no significant differences in head growth. However, data were missing, so these three studies were not included in the meta-analysis.

Nitrogen utilization (Outcome 1.2)

Blood urea nitrogen (mg/dL) (Outcome 1.2.1)

Bhatia 1991, Kashyap 1986, and Svenningsen 1982 report higher blood urea nitrogen levels among infants receiving high protein intake. Svenningsen 1982 did not find a significant difference in blood urea nitrogen at the third and fifth weeks of life, although at seven weeks, levels were significantly higher among infants receiving higher protein intake (third week P value 0.85, fifth week P value 0.375, and seventh week P value 0.0005). Blood urea nitrogen levels were measured by Svenningsen 1982 at different time points than in the other studies, so this study was not included in the meta-analysis. When data from the two studies that measured blood urea nitrogen at the two-week point were combined, significantly higher levels were noted in infants in the high protein intake group (WMD 1.92 mg/dL, 95% CI 1.00 to 2.84) compared with the low protein intake group.

Nitrogen balance (Outcome 1.3)

Nitrogen accretion (mg/kg/d) (Outcome 1.3.1)

Kashyap 1986 and Wauben 1995 reported statistically significantly higher protein accretion in the high protein formula groups. The meta-analysis revealed significantly higher nitrogen accretion (WMD 143.7 mg/kg/d, 95% CI 128.7 to 158.8) in infants receiving formula with high protein content compared with infants given low protein formula. Of note, significant heterogeneity of treatment effect was evident; consequently, these data should be interpreted prudently.

IQ score and Bayley score at 18 months and/or later

No study primarily addressed these outcomes; however, Bhatia 1991 and Svenningsen 1982 reported neurodevelopmental outcomes for infants enrolled in their studies. Bhatia 1991 assessed behavior in a subset of 15 infants within five days of completing

the feeding study. The infants were approximately 36 to 37 weeks at the time of testing. A certified child psychologist, blinded to the feeding history of the infants, administered the Neonatal Behavior Assessment Scale. Infants receiving formula with higher protein intake performed significantly better on the orientation (P value 0.0003), habituation (P value 0.003), and autonomic stability (P value 0.01) clusters of the Neonatal Behavior Assessment Scale. No differences between groups were noted in the remaining behavioral clusters of motor (P value 0.7), range of state (P value 0.5), and regulation of state (P value 0.29). Svenningsen 1982 reported no significant differences in neurodevelopmental outcomes up to two years of age. Investigators assessed developmental performance indicators such as sitting, standing, walking, and talking at five to six, 10 to 11, 14 to 18, and 24 months of age for 46 of the 48 infants enrolled in the study. At 10 to 14 months, an audiometric test was also performed. The instruments used for these assessments were not identified.

Phenylalanine levels (Outcome 1.4)

Plasma phenylalanine concentration (μmol/dL) (Outcome 1.4.1)

Bhatia 1991 and Kashyap 1986 tested phenylalanine levels and found no significant differences between low and high protein formula groups. Bhatia 1991 measured phenylalanine concentrations at the end of the two-week study period. Kashyap 1986 monitored plasma amino acid concentrations before feedings were started, then weekly once the target intake was achieved. Different approaches were used to report data, so a meta-analysis could not be undertaken.

Growth failure

No study addressed outcomes using this term.

Secondary outcomes

Decreased gastric motility (number of episodes of abdominal distention)

No study addressed this outcome.

Days to full feedings (from initiation of feedings to achievement of 120 mL/kg/d)

Kashyap 1986 defined full intake as 180 mL/kg/d, which was maintained throughout the study. No significant differences between groups were noted with respect to the age at which feedings were started and the age at which full feeding was attained. None of the other studies provided information on when full feedings were achieved.

Feeding Intolerance (number of episodes per day)

No study addressed this outcome.

Necrotizing enterocolitis (Bell's stage II or greater) (Outcome 1.5)

Svenningsen 1982 and Wauben 1995 reported no incidence of necrotizing enterocolitis in high or low protein intake groups. However, it is uncertain what criteria were used to define necrotizing enterocolitis in these studies. For the purpose of this systematic review, necrotizing enterocolitis was defined as Bell's stage II or greater. The overall analysis showed no significant effect of protein intake on necrotizing enterocolitis (typical RD 0.00, 95% CI -0.12 to 0.12).

Metabolic acidosis (pH, base excess) (Outcome 1.6)

[Kashyap 1986](#) reported blood acid-base status and found pH and base excess to be within normal limits for all infants enrolled in the study regardless of group assignment.

Serum albumin (g/L) (Outcome 1.7)

[Kashyap 1986](#) reported albumin as approximately 3 g/dL, and [Hillman 1994](#) and [Svenningsen 1982](#) reported albumin as 3 mg/dL and 30 g/mL, respectively. We attempted to clarify the units with the latter two study authors without success. [Hillman 1994](#) measured albumin values at four and six weeks of age. [Svenningsen 1982](#) measured albumin levels at approximately zero, two, and four weeks of the study. The values reported for each time period were not significantly different between low and high protein formula groups. [Kashyap 1986](#) reported prealbumin (mg/dL) (ie, transthyretin) levels and found a significant difference between low and high protein formula groups, favoring the high protein formula group. A meta-analysis could not be undertaken, given the discrepancy in the units used to report findings and differences in the time frames used to measure serum albumin.

Sepsis: incidence, number of episodes (Outcome 1.8)

Although [Svenningsen 1982](#) reported no differences in rates of septicemia between groups, supporting data were not provided. Additionally, it is uncertain what constituted septicemia (eg, positive blood culture, positive cerebrospinal fluid). [Hillman 1994](#) indicated that five of the 27 infants enrolled in this study failed to complete at least four weeks of the study because the infant became unwell (eg, sepsis) or because the infant was transferred to another hospital. The exact number of infants who developed infection was not specified.

Diarrhea (number of episodes per day per baby) (Outcome 1.9)

[Kashyap 1986](#) addressed the outcome of diarrhea using a categorical rather than a continuous level of measurement. [Kashyap 1986](#) indicated that of seven infants withdrawn from the study (n = 34 infants), one developed diarrhea. This infant belonged in the group that differed in energy intake rather than protein intake and therefore was not included in this review.

Subgroup analyses

Stratification based on energy intake

No study addressed this outcome.

Distinction in birth weight categories

Although [Hillman 1994](#) assigned infants enrolled in this study within three overlapping weight group strata (< 1000 grams, 1000 to 1250 grams, and 1250 to 1500 grams), data were not presented for each weight category but rather were based on protein group assignment. No other study reported data for birth weight categories. Consequently, subgroup analyses for birth weight categories were not undertaken.

Very high versus low protein intake (restricted to studies meeting all a priori inclusion criteria) (comparison 2)

No study addressed this outcome.

Very high versus high protein intake (restricted to studies meeting all a priori inclusion criteria) (comparison 3)

Primary outcomes

Growth parameters at discharge (Outcome 3.1)

Weight gain (g/d) (Outcome 3.1.1)

[Embleton 2005](#) found that infants receiving very high protein intake compared with high protein intake had greater weight at discharge, and this approached a statistically significant difference (P value 0.05).

Linear growth (cm/wk) (Outcome 3.1.2)

[Embleton 2005](#) observed no significant differences between groups in linear growth at discharge (P value 1.0).

Head growth (cm/wk)

No study addressed this outcome.

Growth parameters at term (Outcome 3.2)

Weight gain (g/d) (Outcome 3.2.1)

[Embleton 2005](#) detected no differences in weight gain between infants receiving very high protein intake and those with high protein intake from study enrollment until term (P value 0.2).

Linear growth (cm/wk) (Outcome 3.2.2)

[Embleton 2005](#) found that infants receiving very high protein intake from study enrollment to term had significantly greater gain in length (cm/wk) at term (P value 0.04).

Head growth (cm/wk)

No study addressed this outcome.

Growth parameters between discharge and term plus 12 weeks corrected age (Outcome 3.3)

Weight gain (g/d) (Outcome 3.3.1)

[Embleton 2005](#) observed no significant differences in weight gain between groups receiving study formula between the time of discharge and term plus 12 weeks corrected age (P value 0.88).

Linear growth (cm/wk) (Outcome 3.3.2)

[Embleton 2005](#) found no significant differences in linear growth between groups receiving study formula between the time of discharge and term plus 12 weeks corrected age.

Head growth (cm/wk)

No study addressed this outcome.

Nitrogen utilization

Blood urea nitrogen (mM)

[Embleton 2005](#) tested serum for urea nitrogen levels (mM) and found that three of the 24 infants receiving very high protein intake developed uremia, defined as serum urea nitrogen level greater than 6 mM. Increase in serum urea nitrogen levels was associated with the level of protein intake; levels normalized without intervention within two to three days in two infants and within 10 days in another infant. As data were not reported, it was not feasible to determine whether group differences were significant when groups with high protein intake were combined.

Nitrogen balance

Nitrogen accretion (mg/kg/d)

No study addressed this outcome.

IQ score and Bayley score at 18 months and/or later

No study addressed this outcome.

Phenylalanine levels

Plasma phenylalanine concentration (μmol/dL)

No study addressed this outcome.

Growth failure

No study addressed this outcome.

Secondary outcomes

Decreased gastric motility (number of episodes of abdominal distention)

No study addressed this outcome.

Days to full feedings (from initiation of feedings to achievement of 120 mL/kg/d)

[Embleton 2005](#) did not define full feedings. No significant differences between the three groups were noted with respect to the age at which feedings were started and the age at which full feeding was attained. As data were skewed, median and range were reported; it was not feasible to combine data for the groups representing high protein intake and compare them with data for the groups representing very high protein intake.

Feeding intolerance (number of episodes per day)

No study addressed this outcome.

Necrotizing enterocolitis (Bell's stage II or greater)

No study addressed this outcome.

Metabolic acidosis (pH, base excess)

[Embleton 2005](#) reported acid-base status within normal limits for infants in the very high protein intake group who developed uremia (ie, serum blood urea nitrogen levels greater than 6 mM).

Serum albumin (g/L)

[Embleton 2005](#) reported no significant differences in total albumin among infants in the three groups. As data were not reported, it is not feasible to combine data for the groups representing high protein intake and compare them with data for the groups representing very high protein intake.

Sepsis: incidence, number of episodes

No study addressed this outcome.

Diarrhea (number of episodes per day per baby)

No study addressed this outcome.

Subgroup analysis

Stratification based on energy intake

No study addressed this outcome.

Distinction in birth weight categories

Although [Embleton 2005](#) stratified infants enrolled in this study into two birth weight categories (< 1250 grams and 1250 to 1750 grams), data were not presented for each weight category but rather were based on protein group assignment. Consequently, subgroup analyses for birth weight categories were not undertaken.

Post-facto analysis

High versus low protein intake (adding studies comparing formulas with differences in other nutrients) (comparison 4)

Primary outcomes

Growth parameters (Outcome 4.1)

Weight gain (g/kg/d) (Outcome 4.1.1)

[Kashyap 1988](#) found weight gain to be significantly lower in the low protein intake formula group. Inclusion of this study in the overall analysis revealed improvement in weight gain (WMD 2.53 g/kg/d, 95% CI 1.62 to 3.45), beyond that revealed in the a priori analysis, in infants receiving formula with high protein content.

Linear growth (cm/wk) (Outcome 4.1.2)

[Kashyap 1988](#) and [Svenningsen 1982](#) found no significant differences in linear growth between groups. These findings differed from those of [Kashyap 1986](#), who noted a significant increase in linear growth among infants receiving higher protein intake. Inclusion of the [Kashyap 1988](#) study in the meta-analysis revealed a significant difference (WMD 0.16 cm/wk, 95% 0.03 to 0.30) and greater linear growth with high protein intake compared with low protein intake.

Head growth (cm/wk) (Outcome 4.1.3)

[Kashyap 1988](#) found that infants receiving high protein intake had significantly greater head growth (P value 0.027). With inclusion of this study, a meta-analysis revealed significantly greater head growth among those in the high protein intake group (WMD 0.23 cm/wk, 95% 0.12 to 0.35) compared with the low protein intake group.

Nitrogen utilization (Outcome 4.2)

Blood urea nitrogen (mg/dL) (Outcome 4.2.1)

[Kashyap 1988](#) found significantly higher blood urea nitrogen levels with increased protein intake. These findings are consistent with those of [Svenningsen 1982](#) and [Bhatia 1991](#). [Kashyap 1986](#) reported low levels of blood urea nitrogen in all groups, but levels were significantly lower in the low protein group. As both [Kashyap 1986](#) and [Kashyap 1988](#) reported results that were measured weekly, a meta-analysis was possible for both of these studies. A significant increase in blood urea nitrogen levels was evident in the high protein intake group (WMD 3.22 mg/dL, 95% CI 2.48 to 3.96). Of note, significant heterogeneity of treatment effect was evident; consequently, the data should be interpreted with caution.

Nitrogen balance (Outcome 4.3)

Nitrogen accretion (mg/kg/d) (Outcome 4.3.1)

[Kashyap 1988](#) found that protein intake exerted a positive effect on nitrogen retention. These findings are consistent with those of [Kashyap 1986](#) and [Wauben 1995](#). With inclusion of this

study, the meta-analysis continued to show significantly higher nitrogen accretion (WMD 112.6 mg/kg/d, 95% CI 101.4 to 123.8) in infants receiving formula with higher protein content. Significant heterogeneity of treatment effect was evident; consequently, the data should be interpreted with caution.

IQ score and Bayley score at 18 months and/or later

No study addressed this outcome.

Phenylalanine levels (Outcome 4.4)

Plasma phenylalanine concentration (μmol/dL) (Outcome 4.4.1)

[Kashyap 1988](#) found no significant difference in concentrations of plasma phenylalanine between infants fed high versus low protein intake. When data from this study were included with those of [Kashyap 1986](#), the meta-analysis showed no significant difference (WMD 0.25 μmol/dL, 95% CI -0.20 to 0.70) in concentrations of plasma phenylalanine between groups.

Growth failure

No study addressed this outcome.

Metabolic acidosis

No study addressed this outcome.

Serum albumin

No study addressed this outcome.

Very high versus low protein intake (adding studies comparing formulas with differences in other nutrients) (comparison 5)

Primary outcomes

Weight gain (g/wk) (Outcome 5.1)

[Raiha 1976](#) reported rate of weight gain in g/wk measured from the time birth weight was regained to 2400 grams based on gestational age category. No significant differences in the rate of weight gain were noted between low and very high protein intake groups of any gestational age.

Linear growth (cm/wk) (Outcome 5.2)

[Raiha 1976](#) reported rate of growth in crown-rump length (cm/wk) from regaining birth weight to attaining 2400 grams based on gestational age category. No significant differences were noted between low and very high protein intake groups in any gestational age strata.

Head growth (cm/wk)

[Raiha 1976](#) reported no significant differences in rate of growth of head circumference from regaining birth weight to 2400 grams between low and very high protein intake groups of any gestational age. No numerical data were documented.

Nitrogen utilization

Blood urea nitrogen (mg/dL)

[Raiha 1976](#) reported a significant difference in blood urea nitrogen levels between infants fed very high versus low protein formulas when data from the three gestational ages were combined. Blood urea nitrogen levels varied directly with the quantity of protein in the diet; levels were greater than the normal range in infants receiving very high protein intake. Investigators report progressive elevation in blood urea nitrogen levels and metabolic acidosis in

two infants receiving very high protein intake—one given whey predominant formula (5%) and one given casein predominant formula (5%). Graphical data rather than numerical values were presented.

Nitrogen balance

Nitrogen accretion (mg/kg/d)

No study addressed this outcome.

IQ score and Bayley score at 18 months and/or later

No study addressed this outcome.

Phenylalanine levels (Outcome 5.3)

Plasma phenylalanine concentration (μmol/dL) (Outcome 5.2.1)

[Raiha 1976](#) found that infants fed formula providing higher protein intake had higher concentrations of plasma phenylalanine, particularly infants fed the casein predominant formula, when data from the three gestational ages were combined.

Growth failure

No study addressed outcomes using this term.

Very high versus high protein intake (adding studies comparing formulas with differences in other nutrients) (comparison 6)

Primary outcomes

Growth parameters (Outcome 6.1)

Weight gain (g/kg/d) (Outcome 6.1.1)

[Cooke 2006](#) reported significantly higher weight gain in the very high protein formula group compared with the high protein group (23.1±7 vs 16.7±6 g/kg/d, P value 0.04). [Goldman 1969](#) reported not weight gain (g/kg/d) but rather number of days from regaining birth weight to 2200 grams. Based on regression curves calculated for infants < 1500 grams and > 1500 grams, more infants in the very high protein intake group took longer than the calculated period of time to reach 2200 grams (P < 0.01).

Weight gain (g/d) (Outcome 6.1.2)

In the analysis of weight gain in g/d, when the results of [Cooke 2006](#) and [Embleton 2005](#) were combined, weight gain was greater in the very high protein formula group (WMD 3.9 g/d, 95% CI 1.04 to 6.77).

Linear growth (cm/wk)

No study addressed this outcome.

Head growth (cm/wk)

No study addressed this outcome.

Nitrogen utilization (Outcome 6.2)

Blood urea nitrogen (mg/dL) (Outcome 6.2.1)

[Cooke 2006](#) reported a significantly higher blood urea level in the very high protein formula group compared with the high protein group (WMD 1.4 mg/dL, 95% CI 0.4 to 2.4).

Nitrogen balance (Outcome 6.3)

Nitrogen accretion (mg/kg/d) (Outcome 6.3.1)

[Cooke 2006](#) found significantly higher nitrogen accretion in infants fed the very high protein formula (WMD 88 mg/kg/d, 95% CI 25.17 to 150.83).

IQ score and Bayley score at 18 months and/or later (Outcomes 6.4 and 6.5)

Two separate papers on the study by [Goldman 1969](#) (Goldman 1971, 1974) reported incidences of low Stanford-Binet test scores in infants at three and five to seven years of life, respectively. Of the 80% of infants from the original study who were assessed at three years (corrected age and chronological age), a similar incidence of IQ scores below 90 was observed among infants fed very high and high protein formulas. Of the 81% of infants from the original study who were assessed at five to seven years, a similar incidence of IQ scores below 90 was reported in both groups. At both the three-year evaluation and the five- to seven-year evaluation, a significantly higher incidence of IQ scores below 90 is reported among infants of birth weight less than 1300 grams who received very high protein intake compared with those fed high protein intake.

Phenylalanine levels (Outcome 6.6)

Plasma phenylalanine concentration (μmol/dL) (Outcome 6.6.1)

[Cooke 2006](#) reported a non-significantly higher plasma phenylalanine concentration in infants fed very high protein formula (WMD 0.3 μmol/dL, 95% CI -0.67 to 1.27).

Metabolic acidosis (mEq/L) (Outcome 6.7)

[Cooke 2006](#) found no significant difference in base excess between the two groups (WMD 0.5 mEq/L, 95% CI -1.19 to 2.19).

Serum albumin (g/L) (Outcome 6.8)

[Cooke 2006](#) reported serum albumin levels and found no significant differences between the two groups (31 ± 3 vs 31 ± 3 g/L).

Growth failure

No study addressed outcomes using this term.

DISCUSSION

Although a large number of studies ($n = 41$) were identified, upon close inspection only six studies ([Bhatia 1991](#); [Embleton 2005](#) (partial data); [Hillman 1994](#); [Kashyap 1986](#); [Svenningsen 1982](#); [Wauben 1995](#)) were found to be suitable for inclusion in this systematic review. Most studies were excluded because they did not compare sufficiently different protein intakes, or they examined a different intervention (eg, studies examining quality of protein). Methodological limitations of the included trials that may have introduced bias and therefore pose a threat to the validity of the analysis are as follows.

1. Only two studies ([Bhatia 1991](#); [Hillman 1994](#)) had adequately concealed allocation.
2. Differences in protein content among comparison groups in some of the individual trials may be too small (range 0.56 to 1.36 g/kg/d) to illustrate the potential effects of changes in protein intake.
3. Formulas differed substantially across studies.
4. The duration of the interventions and/or study periods varied from eight days ([Wauben 1995](#)) to two years ([Svenningsen 1982](#)).
5. Characteristics of participants varied across studies, with some studies including healthier and more mature premature infants.

These limitations may explain some of the differences in treatment effects and the statistical heterogeneity evident in measures of weight gain and nitrogen accretion.

For this review to be comprehensive and more clinically relevant, studies that varied in nutrient content other than protein were included only in a post-facto analysis. Four studies ([Cooke 2006](#); [Goldman 1969](#); [Kashyap 1988](#); [Raiha 1976](#)) were considered, although only two of these studies ([Cooke 2006](#); [Kashyap 1988](#)) could be included in the meta-analysis.

Weight gain (g/kg/d) was the most commonly reported outcome. An overall increase in weight gain was reported in infants randomly assigned to the high protein intake group compared with those in the low protein intake group (WMD 2.36 g/kg/d, 95% CI 1.31 to 3.40 for the overall analysis; WMD 2.53 g/kg/d, 95% CI 1.62 to 3.45 for the post-facto analysis). The most desirable level of protein intake is that which contributes to infant growth at the infant's predetermined genetic potential without negative consequences. The ideal composition of weight gain for the preterm infant is not known. It is generally considered that the lower lean tissue and higher fat gain of these infants relative to the fetus may not be desirable ([Schulze 1987](#)). Significantly greater nitrogen accretion (WMD 143.7 mg/kg/d, 95% CI 128.7 to 158.8 for the overall analysis; WMD 112.6 mg/kg/d, 95% CI 101.4 to 123.8 for the post-facto analysis) was observed in infants randomly assigned to the high protein intake groups. This greater nitrogen accretion suggests that some or all of the increment in weight is due to gains in lean body mass. These findings indicate that higher protein intake may help correct the non-optimal body composition seen in preterm infants at term adjusted age ([Atkinson 2000](#)). Statistical heterogeneity was noted in nitrogen accretion; hence the data should be interpreted cautiously. Potential sources of heterogeneity might include clinical diversity (eg, variability in participants, interventions, and outcomes) and methodological variability (eg, differences in trial design).

Two studies ([Kashyap 1986](#); [Kashyap 1988](#)) attempted to determine whether utilization of protein was enhanced by higher energy intake. These studies compared medium energy intake (120 kcal/kg/d) versus high energy intake (142 kcal/kg/d). [Kashyap 1986](#) found that higher energy intake did not enhance protein utilization. This was evident in the similarities noted between groups in quantities of nitrogen retention, albumin, and prealbumin, as well as concentrations of blood urea nitrogen and most plasma amino acids. In contrast, in a later study, [Kashyap 1988](#) reported improvements in nitrogen retention and blood urea nitrogen levels with higher energy intake.

Three studies reported that blood urea nitrogen levels were higher among infants fed high protein intake compared with those fed low protein intake ([Bhatia 1991](#); [Kashyap 1986](#); [Svenningsen 1982](#)). Although detectable, some of these differences may not be clinically significant. Three studies ([Bhatia 1991](#); [Kashyap 1986](#); [Kashyap 1988](#)) reported no significant differences in phenylalanine levels between low and high protein intake groups. Inclusion of the two [Kashyap](#) studies in the post-facto meta-analysis resulted in no significant difference (WMD 0.25, 95% CI -0.20 to 0.75) in concentrations of plasma phenylalanine between high and low protein intake groups.

Although the [Kashyap](#) studies ([Kashyap 1986](#); [Kashyap 1988](#)) reported acid-base status within normal limits, other trials raised concerns regarding metabolic acidosis among infants receiving high protein intake ([Raiha 1976](#); [Svenningsen 1982](#)). [Raiha 1976](#) noted that infants receiving very high protein intake (4.5 g/kg/d) developed metabolic acidosis that resolved once the infants were

removed from the study and fed breast milk. In the [Svenningsen 1982](#) study, late metabolic acidosis occurred in 25% and 7%, respectively, of infants in high and low protein intake groups. It is possible that the supplement "protinpur" that they added to their low protein formula to prepare the high protein formula had a poor biological value.

Very high protein intake may be poorly tolerated in infants with very low birth weight and extreme prematurity. Studies have not adequately evaluated short- and long-term adverse sequelae of very high protein intake. The maximal utilizable protein limits for infants in different weight and gestational age categories are unknown. In recent years, preterm infant formulas used in North America have changed such that if infants are fed at energy intake that exceeds 133 kcal/kg/d, protein intake will exceed 4 g/kg/d. In this systematic review, only one study ([Embleton 2005](#)) compared high (≥ 3.0 g/kg/d but < 4.0 g/kg/d) versus very high protein intake (≥ 4.0 g/kg/d) both during and after initial hospital stay. Very high protein intake promoted significantly improved gain in length (cm/wk) at term, but differences did not remain significant at 12 weeks corrected age. Three of the 24 infants receiving very high protein intake developed uremia, which was associated with the level of protein intake and normalized without intervention within two to three days in two infants and within 10 days in another infant. Three studies ([Cooke 2006](#); [Goldman 1969](#); [Raiha 1976](#)) in the post-facto analysis assessed protein intake above 4.0 g/kg/d. The quantity of protein intake in these studies was 4.6 g/kg/d ([Cooke 2006](#)), 6 to 7.2 g/kg/d ([Goldman 1969](#)), and 4.5 g/kg/d ([Raiha 1976](#)). The findings of two studies ([Goldman 1969](#); [Raiha 1976](#)) could not be included in the meta-analysis, as comparisons made within these studies were unique. A post-facto meta-analysis of two studies ([Cooke 2006](#); [Embleton 2005](#)) demonstrated significantly greater weight gain in the very high protein group compared with the high protein group (WMD 3.9 g/d, 95% CI 1.04 to 6.77).

Other potential adverse effects of high protein intake were assessed by reporting neurodevelopmental outcomes, days to full feedings, necrotizing enterocolitis, sepsis, and diarrhea. However, limited information could be obtained regarding these potential risks. Although a meta-analysis was carried out for necrotizing enterocolitis, the findings presented should be interpreted cautiously because (1) uncertainty about the definition of necrotizing enterocolitis used by some studies continues, and (2) small numbers of infants were included in the two groups (N = 49 receiving high protein intake and N = 38 receiving low protein intake).

Neurodevelopmental outcomes of early nutrition were evaluated by three studies ([Bhatia 1991](#); [Goldman 1969](#); [Svenningsen 1982](#)) included in this systematic review. [Svenningsen 1982](#) did not report the tool used. [Bhatia 1991](#) used the Neonatal Behavioral Assessment Scale, which has known psychometric properties but has been validated for use only in term infants up to two months of age ([Brazelton 1995](#)). Results of [Bhatia 1991](#) suggest improvement in some of the parameters of neurodevelopmental outcome with high protein intake compared with low intake. [Goldman 1969](#), who administered the Stanford-Binet test at three and five to seven years of age, noted a significant increase in the incidence of low IQ among infants with birth weight < 1300 g fed very high protein intake of 6 to 7.2 g/kg/d during their initial hospitalization.

AUTHORS' CONCLUSIONS

Implications for practice

This systematic review suggests that weight gain and nitrogen accretion can be promoted by regulating protein intake in "healthy" formula-fed preterm infants. The American Academy of Pediatrics ([AAP 1998](#)) and the Canadian Pediatric Society ([CPS 1995](#)) recommend 3 to 4 g/kg/d of protein for preterm infants. Increased levels of blood urea nitrogen and metabolic acidosis may occur in some infants who receive protein intake above 3 g/kg/d but less than 4 g/kg/d. This review revealed benefits in weight gain and nitrogen accretion and no clear risks associated with this intake. Growth advantages of protein intake greater than 4 g/kg/d remain uncertain and require careful monitoring for protein overload (ie, serum urea nitrogen levels). The exact protein intake that safely promotes optimal growth and development of low birth weight infants remains uncertain.

Implications for research

Future research should determine the precise protein requirements of preterm infants according to birth weight and gestational age. Moreover, unanswered research questions remain regarding protein requirements according to postnatal age and the presence of short-term and long-term growth and neurodevelopmental morbidities. The question of whether clinically significant risks are associated with moderately elevated blood urea nitrogen and metabolic acidosis warrants study. Given the current state of evidence, protein intake above 4 g/kg/d should be considered experimental.

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* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES

Characteristics of included studies [ordered by study ID]

Bhatia 1991

Methods	<p>RCT: numbered envelopes</p> <p>Blinding of randomization: yes</p> <p>Blinding of intervention: yes (medical and nursing staff)</p> <p>Complete follow-up: no</p> <p>Blinding of outcome: yes (to psychologist)</p> <p>Three of 26 participants (12%) were withdrawn for provision of human milk and necrotizing enterocolitis</p> <p>Eight of 23 participants (22%) were lost to follow-up</p>
Participants	<p>26 infants</p> <p>Inclusion criteria: BW < 1500 g, no major congenital anomalies, no congestive heart failure, oxygen requirements < 40% on study entry, no supplemental oxygen on day one of study</p> <p>Study entry: when infants reached 60 kcal/kg/d</p> <p>Study day one: enteral intake reached 100 kcal/kg/d within 21 days of life</p> <p>To stay in study: enteral feeding to begin by 14 days of age, and infant to achieve enteral intake of 100 kcal/kg/d by 21 days of life</p>
Interventions	<p>High protein intake: 3.8 g/kg/d (n = 8) and 3.1 g/kg/d (n = 8). Data for these two groups were combined in this review</p>

Bhatia 1991 (Continued)

Low protein intake: 2.6 g/kg/d (n = 7)

Outcomes	Weight, length, head circumference, skin-fold thickness, serum total protein, prealbumin, retinol-binding protein, urea nitrogen, plasma amino acids, and Neonatal Behavior Assessment Scale (orientation, habituation, stability, regulation, range, and motor) in subset of infants (n = 18, 69%) within five days of completing the feeding study
Notes	Did not mention total parenteral nutrition Carbohydrates added to make the energy level identical between formulas

Risk of bias

Bias	Authors' judgement	Support for judgement
Allocation concealment (selection bias)	Low risk	Blinding of randomization: yes, numbered envelopes
Blinding (performance bias and detection bias) All outcomes	Low risk	Blinding of intervention: yes (medical and nursing staff) Blinding of outcome: yes (to psychologist)
Incomplete outcome data (attrition bias) All outcomes	High risk	Complete follow-up: no Three of 26 participants (12%) were withdrawn for provision of human milk and necrotizing enterocolitis Eight of 23 participants (22%) were lost to follow-up

Cooke 2006

Methods	RCT: balanced cross-over design Blinding of randomization: no Blinding of intervention: yes Complete follow-up: yes Blinding of outcome: outcomes objective, and study described as double blind
Participants	Eighteen infants Inclusion criteria: Preterm infants with birth weight $\leq$ 1500 g and gestational age $\leq$ 32 weeks were eligible if they were clinically stable and had received feeding volumes of at least 130 mL/kg/d, had not received postnatal steroids or diuretics, and had received no oxygen therapy by the time the first balance study was due
Interventions	Nine infants were first fed the RgPro (3.0 g/100 kcal), and nine infants were first fed the HighPro (3.6 g/100 kcal), with a minimum equilibration period of 72 hours and a balance period of 48 hours
Outcomes	Nitrogen balance, metabolic status and growth
Notes	Differences between formulas were noted in total amounts of sodium (20%), copper (19%), potassium (13%), magnesium (23%), and chloride (11%), and in the proportions of carbohydrates provided by lactose and maltodextrin, and in the proportions of fats as medium-chain triglycerides

Risk of bias

Higher versus lower protein intake in formula-fed low birth weight infants (Review)

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Cooke 2006 (Continued)

Bias	Authors' judgement	Support for judgement
Blinding (performance bias and detection bias) All outcomes	Low risk	Described as double blind
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcomes objective
Selective reporting (reporting bias)	Low risk	All results reported
Other bias	Unclear risk	No concerns

Embleton 2005

Methods	<p>RCT: numbered envelopes</p> <p>Blinding of randomization: yes</p> <p>Blinding of intervention: yes (bottle color coded by manufacturer)</p> <p>Complete follow-up: no</p> <p>Blinding of outcome: cannot tell</p> <p>88 infants eligible, 77 enrolled, nine sets of parents refused and two sets of parents not available to obtain consent</p> <p>One infant died postdischarge because of sudden unexpected death in infancy. Two infants were readmitted to the hospital and were mechanically ventilated because of acute viral illness. These three infants were included in the analysis when data were available. At term, corrected age data were available on only 74 infants (96% follow-up), and at 12 weeks corrected age data were available on only 73 infants (95% follow-up). 68 of 77 (88%) infants had body composition measured (using DEXA scan) two weeks postdischarge. 55 infants (71%) had body composition measured at 12 weeks post-term. No explanation was provided with regard to infants lost to follow-up at various time points</p>
Participants	<p>88 infants</p> <p>Inclusion criteria: ≤ 34 weeks gestation, $BW \leq 1750$ g, AGA or SGA, enteral intake ≥ 150 mL/kg/d for ≥ 48 hours, and weight at enrollment ≥ 1000 g</p> <p>Exclusion: infants who were ventilated, oxygen requirement $\geq 40\%$ or on corticosteroid therapy, major congenital anomalies, gastrointestinal pathology, or neurological abnormality</p> <p>Study entry: when infants reached enteral intake ≥ 150 mL/kg/d for ≥ 48 hours</p>
Interventions	<p>Very high protein intake: 4.26 g/kg/d (n = 25)</p> <p>High protein intake: 3.8 g/kg/d (n = 26) and 3.5 g/kg/d (n = 26). Data for these two groups were combined in this review</p>
Outcomes	<p>Cumulative calorie and protein deficit; weight; crown-heel length; occipitofrontal circumference; serum biochemistry including serum urea nitrogen, creatinine, total serum protein, serum albumin, pH, and base deficit; body composition (measured using whole-body DEXA scan) including lean mass, fat mass, percentage fat mass, bone mineral content, and bone mineral density</p>

Embleton 2005 (Continued)

Notes Formulas identical in osmolality and in mineral and vitamin content. Taurine, nucleotides, and long-chain polyunsaturated fats included in formulas

Risk of bias

Bias	Authors' judgement	Support for judgement
Allocation concealment (selection bias)	High risk	Blinding of randomization: yes, numbered envelopes
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Blinding of intervention: yes (bottle color coded by manufacturer) Blinding of outcome: cannot tell
Incomplete outcome data (attrition bias) All outcomes	High risk	Complete follow-up: no 88 infants eligible, 77 enrolled, nine sets of parents refused and two sets of parents not available to obtain consent One infant died postdischarge because of sudden unexpected death in infancy. Two infants were readmitted to hospital and were mechanically ventilated because of acute viral illness. These 3 infants were included in the analysis when data were available. At term corrected age, data were available on only 74 infants (96% follow-up), and at 12 weeks corrected age, data were available on only 73 infants (95% follow-up). 68 of 77 (88%) infants had body composition measured (using DEXA scan) two weeks postdischarge. 55 infants (71%) had body composition measured at 12 weeks post-term. No explanation was provided with regard to infants lost to follow-up at various time points

Goldman 1969

Methods	<p>RCT: random sequence</p> <p>Infants assigned within the following birth weight groups: < 1000 g, 1000 to 1499 g male, 1000 to 1499 g female, 1500 to 2000 g male, 1500 to 2000 g female, and twins</p> <p>Blinding of randomization: cannot tell</p> <p>Blinding of intervention: yes (physician, nurses, and others)</p> <p>Complete follow-up: no</p> <p>Blinding of outcome: no (one physician aware of code for translation and did assessments of infants)</p> <p>Five infants (1.6%) were withdrawn from the study: two infants (0.7%) in the low protein intake group died as the result of apneic episodes, two infants (1.3%) in the high protein group and one infant (0.7%) in the low protein intake group were withdrawn from the study after they developed diarrhea</p>
Participants	<p>304 infants < 2000 g birth weight</p> <p>Inclusion criteria: no major congenital anomalies, intestinal obstruction, Rh disease</p> <p>Exclusion criteria: infants more than 3 days of age on admission to the nursery and infants who died in the first few days of life</p>
Interventions	<p>Very high protein intake: 6 to 7.2 g/kg/d (n = 152). High protein intake: 3 to 3.6 g/kg/d (n = 152)</p> <p>Formulas differed in nutrient content; very high protein formula was 17% higher in minerals</p>

Goldman 1969 (Continued)

Feeding initiated within 72 hours after birth with the initial two feedings of 5% dextrose water. Formula increased gradually to 150 to 180 mL/kg/d

Outcomes	<p>Axillary temperature, weight, edema, lethargy, nipple feeding efforts, cyanosis, central nervous system symptoms, apnea, abdominal distention, diarrhea, and serum albumin</p> <p>Goldman 1971 reported outcomes at three years of life: physical abnormalities, incidence of strabismus, and Stanford-Binet test of IQ</p> <p>Goldman 1974 reported five- to seven-year follow-up outcomes on survivors (81%): interval history, physical exam, Stanford-Binet IQ, and strabismus</p>
Notes	Study took place before routine use of parenteral nutrition

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	<p>Random sequence</p> <p>Infants assigned within the following birth weight groups: < 1000 g, 1000 to 1499 g male, 1000 to 1499 g female, 1500 to 2000 g male, 1500 to 2000 g female, and twins</p>
Allocation concealment (selection bias)	Unclear risk	Blinding of randomization: cannot tell
Blinding (performance bias and detection bias) All outcomes	High risk	<p>Blinding of intervention: yes (physician, nurses, and others)</p> <p>Blinding of outcome: no (one physician aware of code for translation and did assessments of infants)</p>
Incomplete outcome data (attrition bias) All outcomes	High risk	<p>Complete follow-up: no</p> <p>Five infants (1.6%) were withdrawn from the study: two infants (0.7%) in the low protein intake group died as the result of apneic episodes, two infants (1.3%) in the high protein group and one infant (0.7%) in the low protein intake group were withdrawn from the study after they developed diarrhea</p>

Hillman 1994

Methods	<p>RCT: infants randomly assigned, by a previously generated random assignment table, to one of three formulas before initiation of feeding</p> <p>Infants assigned within three weight group strata: < 1000 g, 1000 to 1250 g, 1250 to 1500 g</p> <p>Blinding of randomization: yes</p> <p>Blinding of intervention: cannot tell</p> <p>Complete follow-up: no</p> <p>Blinding of outcome: cannot tell</p> <p>Five infants (19%) failed to complete at least four weeks of the study as a result of: transfer to other hospitals, NEC, sepsis, and respiratory deterioration. These five infants were equally distributed between groups and were replaced in their formula assignment by five additional infants</p> <p>High attrition at six weeks; only 13 of 27 infants remaining in the study</p>
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Hillman 1994 (Continued)

27 infants assessed at two and four weeks and 13 infants assessed at six weeks of age

Participants	27 infants < 1500 g Inclusion criteria: breathing room air, off total parenteral nutrition and diuretics
Interventions	High protein intake: 3.6 g/kg/d (n = 9); 3.2 g/kg/d (n = 9). Data for these two groups were combined in this review Low protein intake: 2.8 g/kg/d (n = 9) Formulas had identical nutrient content except for protein Infants enrolled when taking total fluid intake enterally. Infants assessed at two-week intervals while receiving study formula
Outcomes	Weight gain (birth to 30 days of age); growth of length and head circumference; time to discharge; serum calcium, phosphorus, and magnesium; bone mineral content; bone width; serum albumin; parathyroid hormone levels; urinary calcium/creatinine ratio; phosphorus/creatinine ratio; aminoaciduria; beta 2-microglobulins; and N-acetylglucosamine
Notes	Infants could not be on total parenteral nutrition at the time of enrollment Unclear when infants were switched from study to "regular" formula In several infants, aminoaciduria persisted after change to standard formula was made

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Infants randomly assigned, by a previously generated random assignment table, to one of three formulas before initiation of feeding Infants assigned within three weight group strata: < 1000 g, 1000 to 1250 g, 1250 to 1500 g
Allocation concealment (selection bias)	Low risk	Blinding of randomization: yes
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Blinding of intervention: cannot tell Blinding of outcome: cannot tell
Incomplete outcome data (attrition bias) All outcomes	High risk	Complete follow-up: no Five infants (19%) failed to complete at least four weeks of the study as a result of transfer to other hospitals, NEC, sepsis, and respiratory deterioration. These five infants were equally distributed between groups and were replaced in their formula assignment by five additional infants High attrition at six weeks; only 13 of 27 infants remaining in the study 27 infants assessed at two and four weeks and 13 infants assessed at six weeks of age

Kashyap 1986

Methods	RCT: state infants randomly assigned
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Higher versus lower protein intake in formula-fed low birth weight infants (Review)

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Kashyap 1986 (Continued)

Blinding of randomization: cannot tell

Blinding of intervention: yes (investigators and nurses)

Complete follow-up: no

Blinding of outcome: cannot tell

Seven infants (21%; two in group one, two in group two, and three in group three) were withdrawn for: medical conditions (eg, PDA) that limited intake (three infants), NEC (two infants), diarrhea (one infant), and stool or urine collection inadequate (one infant)

Participants	<p>34 LBW infants weighing between 900 and 1750 g at birth, with gestational age between 27 and 37 weeks, met the following inclusion criteria: no gastrointestinal tract disease, or pulmonary disease severe enough to produce acidosis or to necessitate prolonged ventilatory assistance</p> <p>27 infants completed the study, nine in each group</p>
Interventions	<p>High protein intake: 3.6 g/kg/d (n = 9)</p> <p>Low protein intake: 2.2 g/kg/d (n = 9)</p> <p>Formulas had identical nutrient content except for protein</p> <p>As soon as enteral feedings were tolerated, the assigned formula was started. Formula increased until intake of 180 mL/kg/d was reached, and this was maintained throughout the study period (until infants reached 2200 g)</p>
Outcomes	<p>Weight, length, head circumference, triceps and subscapular skin-fold thickness, nutrient balance (N, Na, K, Cl, Ca, and P), blood urea nitrogen, albumin and transthyretin, acid-base status, alkaline phosphatase, and plasma amino acids levels</p> <p>Secondary analysis of this study was published by Schulze 1987, who reported the following outcomes: metabolizable energy, energy expenditure, and stored energy</p>
Notes	<p>Does not mention total parenteral nutrition. However, Schulze states that monitoring began when full feedings were tolerated. "Full feedings" was not defined</p> <p>Both carbohydrates and fats were altered to make the formulas isocaloric</p>

Risk of bias

Bias	Authors' judgement	Support for judgement
Allocation concealment (selection bias)	Unclear risk	Blinding of randomization: cannot tell, state infants randomly assigned
Blinding (performance bias and detection bias) All outcomes	Unclear risk	<p>Blinding of intervention: yes (investigators and nurses)</p> <p>Blinding of outcome: cannot tell</p>
Incomplete outcome data (attrition bias) All outcomes	High risk	<p>Complete follow-up: no</p> <p>Seven infants (21%; two in group one, two in group two, and three in group three) were withdrawn for medical conditions (eg, PDA) that limited intake (three infants), NEC (two infants), diarrhea (one infant), and stool or urine collection inadequate (one infant)</p>

Kashyap 1988

Methods	<p>RCT: assigned randomly</p> <p>Blinding of randomization: cannot tell</p> <p>Blinding of intervention: yes (investigators and nurses)</p> <p>Complete follow-up: no</p> <p>Blinding of outcome: cannot tell</p> <p>Six infants (12%) (two in group one, one in group two, and three in group three) were withdrawn for medical conditions (eg, PDA) that limited intake (two infants), NEC (two infants), failed to tolerate 180 mL/kg/d (one infant), and severe gastroesophageal reflux (one infant)</p>
Participants	<p>50 LBW infants weighing between 900 and 1750 g at birth</p> <p>Inclusion criteria: no gastrointestinal disease, renal disease, or pulmonary disease</p>
Interventions	<p>High protein intake: 3.8 g/kg/d (n = 15)</p> <p>Low protein intake: 2.8 g/kg/d (N = 14)</p> <p>Formulas varied, with K 14% higher in high protein formula, and Ca and Mg 15% and 20% higher, respectively, in low protein formula</p> <p>Assigned formula was started as soon as enteral feedings were tolerated. Formula increased until intake of 180 mL/kg/d was reached, and this was maintained throughout the study period (until infants reached 2200 g)</p>
Outcomes	Weight, length, head circumference, triceps and subscapular skin-fold thickness, nutrient balance (N, Na, K, Cl, Ca, and P), blood urea nitrogen, albumin and transthyretin, acid-base status and alkaline phosphatase, plasma amino acids, nutrient retention, energy balance, and composition of weight gain
Notes	Both carbohydrates and fats in the formulas were altered to make formulas isocaloric

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Assigned randomly
Allocation concealment (selection bias)	Unclear risk	Blinding of randomization: cannot tell
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Blinding of intervention: yes (investigators and nurses) Blinding of outcome: cannot tell
Incomplete outcome data (attrition bias) All outcomes	High risk	Complete follow-up: no Six infants (12%) (two in group one, one in group two, and three in group three) were withdrawn for medical conditions (eg, PDA) that limited intake (two infants), NEC (two infants), failed to tolerate 180 cc/kg/d (one infant), and severe gastroesophageal reflux (one infant)

Raiha 1976

Methods	<p>RCT: assigned randomly</p> <p>Blinding of randomization: cannot tell</p> <p>Blinding of intervention: yes</p> <p>Complete follow-up: no</p> <p>Blinding of outcome: yes</p> <p>Three infants (3%) were dropped from the study during the first three days because of respiratory problems. All other infants were in the study for at least three weeks. Two infants were withdrawn at three and three and one-half weeks because of progressive metabolic acidosis and "progressive nitrogen retention"</p>
Participants	<p>106 infants</p> <p>Inclusion criteria: free of physical abnormality or obvious disease, gestational age between 28 and 36 weeks, birth weight < 2100 g, and size appropriate for gestational age</p>
Interventions	<p>Very high protein intake: 4.5 g/kg/d (N = 41)</p> <p>Low protein intake: 2.3 g/kg/d (n = 43)</p> <p>Whey:casein ratios were 40:60 or 82:18. Whey-based formula: K (17%), Ca (15%), and P (12%) were higher in the high protein intake formula</p> <p>Casein-based formula: Na (28%) and Mg (12%) were higher in the low protein intake formula</p> <p>Feedings began before 24 hours of age, and volume increased gradually until infant reached 150 mL/kg/d, providing 2.3 or 4.5 g/kg/d of protein. This intake was maintained until infants reached 2400 g</p>
Outcomes	<p>Weight (reported as initial weight loss, rate of gain from regained birth weight to 2400 g, time from birth to regained BW, time from regained BW to 2400 g, time from birth to 2400 g), vomiting, edema, hypoglycemia, acid-base studies, mean temperature, linear and head circumference growth, BUN, serum ammonia, urine osmolality, albumin</p> <p>Four publications from the same study</p> <p>Second publication by Rassin 1977 reported selected aliphatic amino acids in plasma and urine</p> <p>Third publication by Gaul 1977 reported levels of sulfur amino acids in plasma and urine</p> <p>Fourth publication by Rassin 1977 reported levels of tyrosine and phenylalanine in plasma and urine</p>
Notes	<p>Study took place before routine use of parenteral nutrition</p> <p>Lactose content varied to keep formulas isocaloric</p>

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Assigned randomly
Allocation concealment (selection bias)	Unclear risk	Blinding of randomization: cannot tell
Blinding (performance bias and detection bias) All outcomes	Low risk	<p>Blinding of intervention: yes</p> <p>Blinding of outcome: yes</p>

Raiha 1976 (Continued)

Incomplete outcome data (attrition bias) All outcomes	High risk	Complete follow-up: no Three infants (3%) were dropped from the study during the first three days because of respiratory problems. All other infants were in the study for at least three weeks. Two infants were withdrawn at three and three and one-half weeks because of progressive metabolic acidosis and "progressive nitrogen retention"
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Svenningsen 1982

Methods	RCT: no details Blinding of randomization: cannot tell Blinding of intervention: cannot tell Complete follow-up: no Blinding of outcome: cannot tell One death in the human milk-fed group. One infant in the high protein group was lost to follow-up
Participants	48 VLBW and preterm infants (n = 18 in human milk group) No inclusion/exclusion criteria
Interventions	High protein intake: 3.2 g/kg/d (n = 16) Low protein intake: 2.6 g/kg/d (n = 14) Formulas had identical nutrient content except for protein TFI = 170 mL/kg/d
Outcomes	Mean weight, body length, head circumference, albumin, urea-N, and metabolic acidosis Second study (n = 46 by Svenningsen 1982) reported long-term follow-up growth parameters until two years of age and neurodevelopmental outcomes at six months, one year and two years of age. Also measured B-haemoglobin and B-hematocrit on capillary samples
Notes	Birth to second week: IV glucose, electrolytes, and some on parenteral nutrition (note: some infants given pooled human milk) Infants not randomly assigned until the third week of life Formulas made isocaloric; however, energy source not specified End of second week: all fed per orally with human milk Definition of "septicemia" unclear

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	RCT: no details
Allocation concealment (selection bias)	Unclear risk	Blinding of randomization: cannot tell

Higher versus lower protein intake in formula-fed low birth weight infants (Review)

Svenningsen 1982 (Continued)

Blinding (performance bias and detection bias) All outcomes	Unclear risk	Blinding of intervention: cannot tell Blinding of outcome: cannot tell
Incomplete outcome data (attrition bias) All outcomes	High risk	Complete follow-up: no One death in the human milk-fed group. One infant in the high protein group was lost to follow-up

Wauben 1995

Methods	RCT: infants randomly allocated by use of a computer-created randomization table Blinding of randomization: cannot tell Blinding of intervention: no Complete follow-up: yes Blinding of outcome: no (personal communication July 10, 2003)	
Participants	16 appropriate for gestational age "healthy" infants between 28 and 35 weeks Personal communication July 10, 2003: eligible infants 1000 to 2500 g, AGA, and no history of NEC	
Interventions	High protein intake: 3.1 g/kg/d (n = 8) Low protein intake: 2.7 g/kg/d (n = 8) Nutrient intakes other than protein and energy were not reported Study started when infants were receiving full enteral feeding (TFI = 160 mL/kg/d) Study period 10 days: two days for adaptation to the study formula, eight days for observation	
Outcomes	Protein accretion, weight gain, energy, and protein balance	
Notes		

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	RCT: infants randomly allocated by use of a computer-created randomization table
Allocation concealment (selection bias)	Unclear risk	Blinding of randomization: cannot tell
Blinding (performance bias and detection bias) All outcomes	High risk	Blinding of intervention: no Blinding of outcome: no (personal communication July 10, 2003)
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete follow-up: yes

Status as of May 2005 of additional data requested from authors.

Bhatia 1991

Provided data on length, head circumference, and blood urea nitrogen levels. Data on length and head circumference were not reported in a manner that permit inclusion in this systematic review. Last correspondence August 14, 2003.

Hillman 1994

Awaiting information on head circumference, length, and incidence of stage II NEC or greater. Last correspondence September 3, 2003.

Kashyap 1986,

Provided data on age when level of plasma amino acids was assessed, criteria for diagnosis of NEC, and clarification of units of measurement for blood urea nitrogen. Last correspondence June 30, 2004.

Raiha 1976

Provided data on phenylalanine levels. Last correspondence October 27, 2004.

Svenningsen 1982

Awaiting information on length, head circumference, pH, base deficit, and neurodevelopmental outcomes.

Awaiting clarification regarding definition of "septicemia." Last correspondence January 24, 2004.

Wauben 1995

Provided data on NEC and clarified that all infants enrolled in the study weighed < 2.5 kg and that there was no blinding of investigators. Last correspondence July 9, 2003.

Abbreviations

AGA = appropriate for gestational age.

BW = body weight.

DEXA = dual-energy x-ray absorptiometry.

IQ = intelligence quotient.

IV = intravenous.

LBW = low birth weight.

NEC = necrotizing enterocolitis.

PDA = patent ductus arteriosus.

RCT = randomized controlled trial.

SGA = small for gestational age.

TFI = total fluid intake.

VLBW = very low birth weight.

Characteristics of excluded studies [ordered by study ID]

Study	Reason for exclusion
Bell 1986	Compared intakes of protein whereby groups fell within the same predesignated high protein intake group (3.4 and 3.9 g/kg/d)
Brumberg 2010	Not eligible because infants were receiving parenteral nutrition and breast milk as well
Clark 2009	Not the intervention of interest, as higher doses of amino acid supplementation in parenteral nutrition were examined
Costa-Orvay 2011	Not eligible because the energy difference (16%) is greater than the protein difference (A vs B 13%) and the comparison B versus C does not fit the predefined protein categories (both are in the high group)
Darling 1985	Not the intervention of interest, as formulas differed in quality of protein (different ratio of whey-casein and casein hydrolysate)
Davidson 1967	Experimental protocol was modified during the study period (see page 700). Selective exclusion of infants based on review of clinical course (done before deciphering of feeding code)
Fairey 1997	Compared intakes of protein whereby groups fell within the same predesignated high protein intake group (3.1 and 3.7 g/kg/d)
Fanaro 2010	Not eligible because it was not random and infants were receiving breast milk as well
Fewtrell 1997	Compared intakes of protein whereby groups fell within the same predesignated high protein intake group (3.6 and 3.9 g/kg/d)

Higher versus lower protein intake in formula-fed low birth weight infants (Review)

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Study	Reason for exclusion
Greer 1988	Both protein intakes fell in the same predesignated criterion of low protein intake (2.8 and 2.9 g/kg/d)
Lucas 1990	Infants received parenteral nutrition during the study period
Mihatsch 2001	Not the intervention of interest, as comparing high lactose versus negligible lactose. Did not meet all criteria of relevance—infants receiving study formula and total parenteral nutrition
Moro 1984	Not the intervention of interest, as protein intake was the same in comparison groups
Picaud 2001	Not the intervention of interest, as compared quality of protein (partially hydrolyzed vs standard formula)
Singhal 2010	Not eligible because participants were term infants
Siripoonya 1989	Not the intervention of interest, as compared quality of protein (special care formula vs standard whey-predominant formula)
Spencer 1992	Compared intakes of protein whereby groups fell within the same predesignated low protein intake group (2.9 and 2.95 g/kg/d)
Szajewska 2001	Compared intakes of protein whereby groups fell within the same predesignated high protein intake group (3.3 and 3.8 g/kg/d)
Tan 2008	Not the intervention of interest, as comparing hyperalimented or standard parenteral and enteral nutrition
van Goudoever 2000	Not intervention of interest, as compared normal energy versus low energy formula. Compared intakes of protein whereby groups fell within the same predesignated high protein intake group (3.3 and 3.3 g/kg/d)

Characteristics of studies awaiting assessment *[ordered by study ID]*

[Mimouni 1989](#)

Methods	Not known
Participants	Not known
Interventions	Not known
Outcomes	Not known
Notes	

[Nichols 1966](#)

Methods	Not known
Participants	Not known
Interventions	Not known

Higher versus lower protein intake in formula-fed low birth weight infants (Review)

Nichols 1966 (Continued)

Outcomes	Not known
Notes	

Thom 1984

Methods	Not known
Participants	Not known
Interventions	Not known
Outcomes	Not known
Notes	

DATA AND ANALYSES

Comparison 1. High versus low protein intake (restricted to studies meeting all a priori inclusion criteria)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Growth parameters	5		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
1.1 Weight gain (g/kg/d)	5	114	Mean Difference (IV, Fixed, 95% CI)	2.36 [1.31, 3.40]
1.2 Linear growth (cm/wk)	2	48	Mean Difference (IV, Fixed, 95% CI)	0.16 [-0.02, 0.34]
1.3 Head growth (cm/wk)	1	18	Mean Difference (IV, Fixed, 95% CI)	0.37 [0.16, 0.58]
2 Nitrogen utilization	2	41	Mean Difference (IV, Fixed, 95% CI)	1.92 [1.00, 2.84]
2.1 Blood urea nitrogen (mg/dL)	2	41	Mean Difference (IV, Fixed, 95% CI)	1.92 [1.00, 2.84]
3 Nitrogen balance	2	34	Mean Difference (IV, Fixed, 95% CI)	143.73 [128.70, 158.77]
3.1 Nitrogen accretion (mg/kg/d)	2	34	Mean Difference (IV, Fixed, 95% CI)	143.73 [128.70, 158.77]
4 Phenylalanine levels	2	41	Mean Difference (IV, Fixed, 95% CI)	0.34 [-0.27, 0.96]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
4.1 Plasma phenylalanine concentration (μmol/dL)	2	41	Mean Difference (IV, Fixed, 95% CI)	0.34 [-0.27, 0.96]
5 Necrotizing enterocolitis	2	46	Risk Difference (M-H, Fixed, 95% CI)	0.0 [-0.12, 0.12]
5.1 NEC (Bell's stage II or greater) comparing high and low protein intakes (same micronutrient content)	2	46	Risk Difference (M-H, Fixed, 95% CI)	0.0 [-0.12, 0.12]
6 Metabolic acidosis (pH, base excess)	1		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
6.1 pH	1	18	Mean Difference (IV, Fixed, 95% CI)	0.01 [-0.02, 0.04]
6.2 Base excess (mEq/L)	1	18	Mean Difference (IV, Fixed, 95% CI)	-0.20 [-2.43, 2.03]
7 Serum albumin (g/L)	1	18	Mean Difference (IV, Fixed, 95% CI)	44.0 [23.59, 64.41]
7.1 Serum prealbumin (g/L)	1	18	Mean Difference (IV, Fixed, 95% CI)	44.0 [23.59, 64.41]
8 Sepsis	1	30	Risk Ratio (M-H, Fixed, 95% CI)	0.44 [0.04, 4.32]
8.1 Septicemia	1	30	Risk Ratio (M-H, Fixed, 95% CI)	0.44 [0.04, 4.32]
9 Diarrhea	1	18	Risk Difference (M-H, Fixed, 95% CI)	0.0 [-0.19, 0.19]
9.1 Diarrhea episodes (babies with one or more episodes of diarrhea)	1	18	Risk Difference (M-H, Fixed, 95% CI)	0.0 [-0.19, 0.19]

Analysis 1.1. Comparison 1 High versus low protein intake (restricted to studies meeting all a priori inclusion criteria), Outcome 1 Growth parameters.

Analysis 1.2. Comparison 1 High versus low protein intake (restricted to studies meeting all a priori inclusion criteria), Outcome 2 Nitrogen utilization.

Analysis 1.3. Comparison 1 High versus low protein intake (restricted to studies meeting all a priori inclusion criteria), Outcome 3 Nitrogen balance.

Analysis 1.4. Comparison 1 High versus low protein intake (restricted to studies meeting all a priori inclusion criteria), Outcome 4 Phenylalanine levels.

Analysis 1.5. Comparison 1 High versus low protein intake (restricted to studies meeting all a priori inclusion criteria), Outcome 5 Necrotizing enterocolitis.

Analysis 1.6. Comparison 1 High versus low protein intake (restricted to studies meeting all a priori inclusion criteria), Outcome 6 Metabolic acidosis (pH, base excess).

Analysis 1.7. Comparison 1 High versus low protein intake (restricted to studies meeting all a priori inclusion criteria), Outcome 7 Serum albumin (g/L).

Analysis 1.8. Comparison 1 High versus low protein intake (restricted to studies meeting all a priori inclusion criteria), Outcome 8 Sepsis.

Analysis 1.9. Comparison 1 High versus low protein intake (restricted to studies meeting all a priori inclusion criteria), Outcome 9 Diarrhea.

Comparison 3. Very high versus high protein intake (restricted to studies meeting all a priori inclusion criteria)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Growth parameters at discharge	1	154	Mean Difference (IV, Fixed, 95% CI)	0.01 [-0.14, 0.15]
1.1 Weight gain at discharge (g/d)	1	77	Mean Difference (IV, Fixed, 95% CI)	3.10 [-0.04, 6.24]
1.2 Linear growth at discharge (cm/wk)	1	77	Mean Difference (IV, Fixed, 95% CI)	0.0 [-0.14, 0.14]
2 Growth parameters at term	1	148	Mean Difference (IV, Fixed, 95% CI)	0.10 [0.00, 0.20]
2.1 Weight gain at term (g/d)	1	74	Mean Difference (IV, Fixed, 95% CI)	2.20 [-1.15, 5.55]
2.2 Linear growth at term (cm/wk)	1	74	Mean Difference (IV, Fixed, 95% CI)	0.10 [0.00, 0.20]
3 Growth parameters at 12 weeks corrected age	1	146	Std. Mean Difference (IV, Fixed, 95% CI)	-0.02 [-0.36, 0.33]
3.1 Weight gain at 12 weeks corrected age (g/d)	1	73	Std. Mean Difference (IV, Fixed, 95% CI)	-0.04 [-0.53, 0.45]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
3.2 Linear growth at 12 weeks corrected age (g/d)	1	73	Std. Mean Difference (IV, Fixed, 95% CI)	0.0 [-0.49, 0.49]

Analysis 3.1. Comparison 3 Very high versus high protein intake (restricted to studies meeting all a priori inclusion criteria), Outcome 1 Growth parameters at discharge.

Analysis 3.2. Comparison 3 Very high versus high protein intake (restricted to studies meeting all a priori inclusion criteria), Outcome 2 Growth parameters at term.

Analysis 3.3. Comparison 3 Very high versus high protein intake (restricted to studies meeting all a priori inclusion criteria), Outcome 3 Growth parameters at 12 weeks corrected age.

Comparison 4. High versus low protein intake (adding studies comparing formulas with differences in other nutrients)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Growth parameters	6		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
1.1 Weight gain (g/kg/d)	6	143	Mean Difference (IV, Fixed, 95% CI)	2.53 [1.62, 3.45]
1.2 Linear growth (cm/wk)	3	77	Mean Difference (IV, Fixed, 95% CI)	0.16 [0.03, 0.30]
1.3 Head growth (cm/wk)	2	47	Mean Difference (IV, Fixed, 95% CI)	0.23 [0.12, 0.35]
2 Nitrogen utilization	2	47	Mean Difference (IV, Fixed, 95% CI)	3.22 [2.48, 3.96]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
2.1 Blood urea nitrogen (mg/dL)	2	47	Mean Difference (IV, Fixed, 95% CI)	3.22 [2.48, 3.96]
3 Nitrogen balance	3	63	Mean Difference (IV, Fixed, 95% CI)	112.57 [101.37, 123.77]
3.1 Nitrogen accretion (mg/kg/d)	3	63	Mean Difference (IV, Fixed, 95% CI)	112.57 [101.37, 123.77]
4 Phenylalanine levels	2	47	Mean Difference (IV, Fixed, 95% CI)	0.25 [-0.20, 0.70]
4.1 Plasma phenylalanine concentration (μmol/dL)	2	47	Mean Difference (IV, Fixed, 95% CI)	0.25 [-0.20, 0.70]

Analysis 4.1. Comparison 4 High versus low protein intake (adding studies comparing formulas with differences in other nutrients), Outcome 1 Growth parameters.

Analysis 4.2. Comparison 4 High versus low protein intake (adding studies comparing formulas with differences in other nutrients), Outcome 2 Nitrogen utilization.

Analysis 4.3. Comparison 4 High versus low protein intake (adding studies comparing formulas with differences in other nutrients), Outcome 3 Nitrogen balance.

Analysis 4.4. Comparison 4 High versus low protein intake (adding studies comparing formulas with differences in other nutrients), Outcome 4 Phenylalanine levels.

Comparison 5. Very high versus low protein intake (adding studies comparing formulas with differences in other nutrients)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Weight gain (g/wk)	1	84	Mean Difference (IV, Fixed, 95% CI)	-6.47 [-19.05, 6.11]
1.1 28 to 30 weeks gestation	1	27	Mean Difference (IV, Fixed, 95% CI)	-10.0 [-38.86, 18.86]
1.2 31 to 33 weeks gestation	1	29	Mean Difference (IV, Fixed, 95% CI)	-12.0 [-30.93, 6.93]
1.3 34 to 36 weeks gestation	1	28	Mean Difference (IV, Fixed, 95% CI)	2.0 [-18.74, 22.74]
2 Linear growth (cm/wk)	1	84	Mean Difference (IV, Fixed, 95% CI)	-0.03 [-0.10, 0.04]
2.1 28 to 30 weeks gestation	1	27	Mean Difference (IV, Fixed, 95% CI)	0.0 [-0.08, 0.08]
2.2 31 to 33 weeks gestation	1	29	Mean Difference (IV, Fixed, 95% CI)	-0.17 [-0.33, -0.01]
2.3 34 to 36 weeks gestation	1	28	Mean Difference (IV, Fixed, 95% CI)	-0.01 [-0.17, 0.15]
3 Phenylalanine levels	1	84	Mean Difference (IV, Fixed, 95% CI)	3.15 [1.31, 4.99]
3.1 Plasma phenylalanine concentration ($\mu\text{mol/dL}$)	1	84	Mean Difference (IV, Fixed, 95% CI)	3.15 [1.31, 4.99]

Analysis 5.1. Comparison 5 Very high versus low protein intake (adding studies comparing formulas with differences in other nutrients), Outcome 1 Weight gain (g/wk).

Analysis 5.2. Comparison 5 Very high versus low protein intake (adding studies comparing formulas with differences in other nutrients), Outcome 2 Linear growth (cm/wk).

Analysis 5.3. Comparison 5 Very high versus low protein intake (adding studies comparing formulas with differences in other nutrients), Outcome 3 Phenylalanine levels.

Comparison 6. Very high versus high protein intake (adding studies comparing formulas with differences in other nutrients)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Growth parameters	2	113	Mean Difference (IV, Fixed, 95% CI)	4.36 [1.78, 6.95]
1.1 Weight gain (g/kg/d)	1	18	Mean Difference (IV, Fixed, 95% CI)	6.40 [0.38, 12.42]
1.2 Weight gain (g/d)	2	95	Mean Difference (IV, Fixed, 95% CI)	3.90 [1.04, 6.77]
2 Nitrogen utilization	1	18	Mean Difference (IV, Fixed, 95% CI)	1.4 [0.40, 2.40]
2.1 Blood urea nitrogen (mg/dL)	1	18	Mean Difference (IV, Fixed, 95% CI)	1.4 [0.40, 2.40]
3 Nitrogen balance	1	18	Mean Difference (IV, Fixed, 95% CI)	88.00 [25.17, 150.83]
3.1 Nitrogen accretion (mg/kg/d)	1	18	Mean Difference (IV, Fixed, 95% CI)	88.00 [25.17, 150.83]
4 Low IQ or Bayley score at 18 months and/or later (all infants)	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
4.1 IQ score < 90 at three years of age based on corrected age	1	216	Risk Ratio (M-H, Fixed, 95% CI)	0.70 [0.46, 1.08]
5 Low IQ or Bayley score at 18 months and/or later (in infants < 1300 g)	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
5.1 IQ score < 90 at three years of age based on chronological age	1	47	Risk Ratio (M-H, Fixed, 95% CI)	0.30 [0.14, 0.64]
5.2 IQ score < 90 at five years of age	1	49	Risk Ratio (M-H, Fixed, 95% CI)	0.31 [0.15, 0.66]
6 Phenylalanine levels	1	18	Mean Difference (IV, Fixed, 95% CI)	0.30 [-0.67, 1.27]
6.1 Plasma phenylalanine concentration (μmol/dL)	1	18	Mean Difference (IV, Fixed, 95% CI)	0.30 [-0.67, 1.27]
7 Metabolic acidosis (base excess)	1	18	Mean Difference (IV, Fixed, 95% CI)	0.5 [-1.19, 2.19]
7.1 Base excess (mEq/L)	1	18	Mean Difference (IV, Fixed, 95% CI)	0.5 [-1.19, 2.19]
8 Serum albumin (g/L)	1	18	Mean Difference (IV, Fixed, 95% CI)	0.0 [-2.77, 2.77]

Analysis 6.1. Comparison 6 Very high versus high protein intake (adding studies comparing formulas with differences in other nutrients), Outcome 1 Growth parameters.

Analysis 6.2. Comparison 6 Very high versus high protein intake (adding studies comparing formulas with differences in other nutrients), Outcome 2 Nitrogen utilization.

Analysis 6.3. Comparison 6 Very high versus high protein intake (adding studies comparing formulas with differences in other nutrients), Outcome 3 Nitrogen balance.

Analysis 6.4. Comparison 6 Very high versus high protein intake (adding studies comparing formulas with differences in other nutrients), Outcome 4 Low IQ or Bayley score at 18 months and/or later (all infants).

Analysis 6.5. Comparison 6 Very high versus high protein intake (adding studies comparing formulas with differences in other nutrients), Outcome 5 Low IQ or Bayley score at 18 months and/or later (in infants < 1300 g).

Analysis 6.6. Comparison 6 Very high versus high protein intake (adding studies comparing formulas with differences in other nutrients), Outcome 6 Phenylalanine levels.

Analysis 6.7. Comparison 6 Very high versus high protein intake (adding studies comparing formulas with differences in other nutrients), Outcome 7 Metabolic acidosis (base excess).

Analysis 6.8. Comparison 6 Very high versus high protein intake (adding studies comparing formulas with differences in other nutrients), Outcome 8 Serum albumin (g/L).

WHAT'S NEW

Date	Event	Description
21 May 2013	New search has been performed	This updates the review "Higher versus lower protein intake in formula-fed low birth weight infants" (Fenton 2009)
21 May 2013	New citation required but conclusions have not changed	One new study included. No change to conclusions

HISTORY

Protocol first published: Issue 1, 2003

Review first published: Issue 1, 2006

Date	Event	Description
20 July 2009	New search has been performed	This updates the review "Higher versus lower protein intake in formula-fed low birth weight infants" (Premji 2006). One new study included. No change to conclusions.
27 October 2008	Amended	Converted to new review format.
11 October 2005	New citation required and conclusions have changed	Substantive amendment

CONTRIBUTIONS OF AUTHORS

S. Premji (SSP) and T. Fenton (TRF): wrote protocol, searched for trials, selected studies, extracted data, wrote review.

SSP: input data into RevMan, performed data analyses.

H. Al-Wassia (HA): searched for trials, selected studies, extracted data, revised the review.

TRF: corresponded with study authors, double-checked data entry, performed data analyses.

R. Sauve (RSS): principal investigator on the intramural support, facilitated consensus decision making, revised review.

DECLARATIONS OF INTEREST

None.

SOURCES OF SUPPORT

Internal sources

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External sources

- Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Department of Health and Human Services, USA.

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INDEX TERMS

Medical Subject Headings (MeSH)

Child Development [*physiology]; Dietary Proteins [*administration & dosage]; Infant Formula [*chemistry]; Infant, Low Birth Weight [*growth & development]; Randomized Controlled Trials as Topic

MeSH check words

Humans; Infant, Newborn