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Protein supplementation of human milk for promoting growth in preterm infants (Review)

Amissah EA, Brown J, Harding JE

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[Intervention Review]

Protein supplementation of human milk for promoting growth in preterm infants

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ABSTRACT

Background

Preterm infants require high protein intake to achieve adequate growth and development. Although breast milk feeding has many benefits for this population, the protein content is highly variable, and inadequate to support rapid infant growth. This is a 2018 update of a Cochrane Review first published in 1999.

Objectives

To determine whether protein-supplemented human milk compared with unsupplemented human milk, fed to preterm infants, improves growth, body composition, cardio-metabolic, and neurodevelopmental outcomes, without significant adverse effects.

Search methods

We used the standard search strategy of Cochrane Neonatal to search CENTRAL, MEDLINE via PubMed, Embase, and CINAHL (February 2018). We also searched clinical trials databases, conference proceedings and the reference lists of retrieved articles for randomised controlled trials (RCT) and quasi-randomised trials.

Selection criteria

Published and unpublished RCTs were eligible if they used random or quasi-random methods to allocate hospitalised preterm infants who were being fed human milk, to additional protein supplementation or no supplementation.

Data collection and analysis

Two review authors independently abstracted data, assessed risk of bias and the quality of evidence at the outcome level, using GRADE methodology. We performed meta-analyses, using risk ratio (RR) for dichotomous data, and mean difference (MD) for continuous data, with their respective 95% confidence intervals (CIs). We used a fixed-effect model and had planned to explore potential causes of heterogeneity via subgroup or sensitivity analyses.

Main results

We included six RCTs, involving 204 preterm infants. Low-quality evidence showed that protein supplementation of human milk increased in-hospital rates of growth in weight (MD 3.82 g/kg/day, 95% CI 2.94 to 4.7; five RCTs, 101 infants; $I^2 = 73%$), length (MD 0.12 cm/wk, 95% CI 0.07 to 0.17; four RCTs, 68 infants; $I^2 = 89%$), and head circumference (MD 0.06 cm/wk, 95% CI 0.01 to 0.12; four RCTs, 68 infants; $I^2 = 84%$). There was no evidence of a clear difference in rate of growth of skin fold thickness between the supplemented and unsupplemented groups (triceps MD 0.06 mm/wk, 95% CI -0.09 to 0.21; one RCT, 20 infants; or subscapular MD 0.00 mm/wk, 95% CI -0.17 to 0.17; one RCT, 20 infants). Protein supplementation led to longer hospital stays (MD 18.5 days, 95% CI 4.39 to 32.61; one RCT, 20 infants; very low-

quality evidence), and higher blood urea nitrogen concentrations compared to the unsupplemented group (MD 0.95 mmol/L, 95% CI 0.81 to 1.09; four RCTs, 81 infants; $I^2 = 56\%$). Very low-quality evidence did not show that protein supplementation clearly increased the risk of feeding intolerance (RR 2.70, 95% CI 0.13 to 58.24; one RCT, 17 infants), or necrotizing enterocolitis (RR 1.11, 95% CI 0.07 to 17.12; one RCT, 76 infants), or clearly altered serum albumin concentrations (MD 2.5 g/L, 95% CI -5.66 to 10.66; one RCT, 11 infants), compared with the unsupplemented groups. No data were available about the effects of protein supplementation on long-term growth, body mass index, body composition, neurodevelopmental, or cardio-metabolic outcomes.

Authors' conclusions

Low-quality evidence showed that protein supplementation of human milk, fed to preterm infants, increased short-term growth. However, the small sample sizes, low precision, and very low-quality evidence regarding duration of hospital stay, feeding intolerance, and necrotising enterocolitis precluded any conclusions about these outcomes. There were no data on outcomes after hospital discharge. Our findings may not be generalisable to low-resource settings, as none of the included studies were conducted in these settings.

Since protein supplementation of human milk is now usually done as a component of multi-nutrient fortifiers, future studies should compare different amounts of protein in multi-component fortifiers, and be designed to determine the effects on duration of hospital stay and safety, as well as on long-term growth, body composition, cardio-metabolic, and neurodevelopmental outcomes.

PLAIN LANGUAGE SUMMARY

Protein supplementation of human milk for promoting growth in preterm infants

Review question

We reviewed the evidence to see whether the addition of extra protein to human milk, compared with no additional protein, fed to preterm infants, improved growth, body fat, obesity, heart problems, high blood sugar, and brain development, without significant side effects.

Background

Lack of adequate protein intake during the early stages of the preterm infant's life can result in poor growth and development. Preterm infants need more protein than full term babies. Breast milk has numerous benefits for babies born preterm (before 37 weeks), but its protein content is variable, and may not meet the nutritional needs of the rapidly growing preterm infant. Therefore, to meet their higher protein needs, and to promote optimum health and long-term development, additional protein, in the form of a fortifier, may be added to expressed breast milk for preterm babies.

Study characteristics

We found six randomised trials (trials in which each infant had an equal chance of being chosen to receive either treatment), involving 204 preterm infants. The search is up to date to February 2018.

Key results

Low-quality evidence showed that the addition of extra protein to breast milk increased short-term rates of weight gain (five trials), length gain (four trials), and head growth (four trials). Low-quality evidence from one trial did not show a clear difference in the rate of growth of skin fold thickness (measure of fat under the skin) between the supplemented and unsupplemented groups. Very low-quality evidence from one trial reported that infants who received additional protein stayed in hospital longer, while very low-quality evidence from four trials observed higher blood urea nitrogen concentrations (measure of kidney function and protein breakdown) in these infants, compared to those who received no additional protein. Very low-quality evidence from one trial suggested that adding extra protein to expressed breast milk did not clearly increase the risk of necrotising enterocolitis (inflammation of the intestine) or feeding intolerance, or clearly alter serum albumin concentrations (a measure of blood protein levels). No data were available on the effects of adding extra protein to human milk on long-term growth, body fat, obesity, high blood sugar, or brain development.

Conclusions

Adding extra protein to human milk for preterm infants may increase short-term growth. However, its effect on length of hospital stay, feeding intolerance, and necrotizing enterocolitis is uncertain, due to data limitations and very low-quality evidence. There were no data about effects on later health and development, or effects in low resource settings.

Since protein supplementation of human milk is now usually done as a component of multi-nutrient fortifiers, future studies should compare different amounts of protein in multi-component fortifiers, and be designed to determine the effects on length of hospital stay, safety, long-term growth, body fat, obesity, high blood sugar, and brain development.

SUMMARY OF FINDINGS

Summary of findings for the main comparison. Protein supplementation compared to control for promoting growth in preterm infants

Protein supplementation compared to no supplementation for promoting growth in preterm infants

Patient or population: preterm infants
Setting: hospital
Intervention: protein supplementation
Comparison: no protein supplementation

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	Nº of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with control	Risk with Protein supplementation				
Growth: weight (weight gain g/kg/day)	The mean ^d weight gain in the unsupplemented human milk group was 13.3 g/kg/day	Mean weight gain 3.82 g/kg/day higher (2.94 higher to 4.7 higher).	—	101 (5 RCTs)	⊕⊕⊕⊕ Low a b	mean difference (MD) 3.82, 95% CI 2.94 to 4.70
Growth: length (cm/week)	The mean ^d length gain in the unsupplemented human milk group was 0.41 cm/week	Mean length gain 0.12 cm/week higher (0.07 higher to 0.17 higher)	—	68 (4 RCTs)	⊕⊕⊕⊕ Low a b	MD 0.12, 95% CI 0.07 to 0.17
Growth: head circumference (cm/week)	The mean ^d head circumference gain in the unsupplemented human milk group was 0.68 cm/week	Mean head growth 0.06 cm/week higher (0.01 higher to 0.12 higher)	—	68 (4 RCTs)	⊕⊕⊕⊕ Low a b	MD 0.06, 95% CI 0.01 to 0.12
Neurodevelopmental outcomes	see comments	see comments	see comments	see comments	see comments	None of the included studies reported on neurodevelopmental outcomes.
Duration of hospital stay (days)	The mean duration of hospital stay in the unsupplemented human milk group was 48.7 days	Mean difference 18.5 days higher (4.39 higher to 32.61 higher)	—	20 (1 RCT)	⊕⊕⊕⊕ Very low a c	MD 18.5, 95% CI 4.39 to 32.61

Feeding intolerance	0 per 1000 ^e	0 per 1000 (0 to 0)	RR 6.61 (0.13 to 335.50)	17 (1 RCT)	⊕○○○ Very low ^{a c}	No events reported in the control group (0/8). One event reported in the fortified group (1/9).
Necrotising enterocolitis	25 per 1000 ^e	28 per 1000 (2 to 322)	RR 1.11 (0.07 to 18.49)	76 (1 RCT)	⊕○○○ Very low ^{a c}	One event reported in the control group (1/40). One event reported in the fortified group (1/36).

GRADE Working Group grades of evidence

High certainty: we are very confident that the true effect lies close to that of the estimate of the effect

Moderate certainty: we are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different

Low certainty: our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect

Very low certainty: we have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

^aMost of the studies lacked methodological details so that we were unable to judge risk of bias. This could have an impact on assessment of growth parameters and possibly the estimate of effect. We downgraded one level.

^bModerate-to-high heterogeneity among the included studies estimating the population mean difference. We downgraded one level.

^cFew patients, few events and very wide confidence intervals. We downgraded two levels.

^dThe base means were calculated as weighted mean, that is, the sum of (the mean from each study multiplied by the weight) divided by a summation of the weights for each study.

^eThe assumed base risks were calculated as the total number of events in the control group divided by the total number of participants in the control group.

BACKGROUND

Description of the condition

Optimum nutrition that meets the special needs of preterm infants remains a challenge. To match intrauterine growth ([AAP Committee on Nutrition 1985](#)), preterm infants require higher protein intake than full term infants, to accommodate their higher requirements for protein synthesis ([Agostoni 2010](#); [Hay 2010](#); [Underwood 2013](#)). Failing to consume sufficient amounts of protein, especially during the first few weeks, can result in compromised growth and organ development ([Embleton 2001](#); [Freitas 2016](#)), particularly of the brain and central nervous system ([Agostoni 2010](#); [Claas 2011](#); [Ghods 2011](#)).

Breast milk, fed to the preterm infant, is associated with several benefits, including: reduction in rates of late-onset sepsis ([Schanler 1999](#)), necrotizing enterocolitis (NEC; [Sisk 2007](#)), and retinopathy of prematurity ([Okamoto 2007](#)). Other benefits include: better feeding tolerance ([Boyd 2007](#)), improved neurodevelopmental outcomes ([Bertino 2012](#)), lower rates of metabolic syndrome ([AAP 2012](#)) and lower low-density lipoprotein levels in adolescence ([Bertino 2012](#)).

Women who give birth preterm initially produce breast milk with higher amounts of protein than are found in full term milk. However, the protein content is inconsistent ([Tudehope 2013](#)). It varies between mothers, decreases within a breastfeeding session, and decreases after the first two weeks postnatally, when it is particularly needed to support rapid infant growth ([Hay 2009](#); [Su 2014](#)). In addition, mothers of preterm infants face many difficulties that interfere with their establishment and maintenance of milk production. This limitation in breast milk supply may result in a reliance on donor human milk from mothers who gave birth at term, but this contains insufficient protein to support the high protein requirements of the preterm infant ([Schanler 2005](#); [Weber 2001](#)).

Further, feeding preterm infants unsupplemented breast milk during neonatal admissions has been associated with inadequate growth ([Brooke 1987](#); [Su 2014](#); [Tonkin 2014](#)), which in turn is associated with longer hospital stays, more infections, and adverse short and long-term developmental outcomes ([Ehrenkranz 2006](#); [Ehrenkranz 2010](#); [Lapillonne 2013](#)).

Thus, to meet the higher protein needs of rapidly growing preterm infants, and to promote their optimum health, additional protein in the form of a fortifier may be added to expressed breast milk.

Description of the intervention

Protein fortifiers are usually commercially available, and are produced in liquid or powder forms. They may also contain additional micronutrients and electrolytes, comprise hydrolyzed or intact protein, and can be bovine or human milk-based. They are mixed with human milk, and fed to the preterm infant once they begin to tolerate enteral feeds ([Di Natale 2011](#); [Ziegler 2011](#)).

Protein fortifiers increase the concentrations of protein, and potentially other micronutrients, in expressed breast milk. They are typically administered as a fixed dose per unit volume of breast milk, known as standardized fortification ([Di Natale 2013](#)). The amount also can be varied, depending on the measured or estimated protein content of the breast milk, to meet the infant's needs (targeted fortification).

How the intervention might work

Protein-fortified human milk is expected to improve postnatal growth and development, in part by providing essential amino acids and energy for tissue growth, and in part by interacting with endocrine systems, such as the insulin-like growth factor I (IGF-1) system. IGF-1 plays an important role in growth, body composition, and cognition of preterm infants ([Clemmons 2006](#); [Hansen-Pupp 2013](#); [Socha 2011](#)). At 30 weeks' postmenstrual age, there is a reciprocal relationship between IGF-1 and dietary protein in preterm infants ([Hansen-Pupp 2011](#)). Low protein levels are associated with low IGF-I concentrations ([Yeung 2003](#)), and lower lean mass in childhood ([Chiesa 2008](#); [Hellström 2016](#); [Lo 2002](#)). Therefore, the addition of protein to human milk is expected to raise IGF-1 concentrations, decrease fat mass accretion, and limit the initial growth failure of preterm infants ([Kim 2016](#); [Koletzko 2005](#)).

Complications from protein supplementation can occur. For example, fortifiers based on cow's milk (i.e. intact bovine protein) have been associated with the development of allergies in preterm infants from very early contact with heterologous proteins ([Srinivasan 2010](#)). In addition, powdered fortifiers are non-sterile products, and therefore, carry the risk of bacterial contamination, which could predispose the preterm infant to sepsis ([D'Netto 2000](#); [Reich 2010](#)). Furthermore, acidified, higher protein fortifiers have been shown to cause feeding intolerance and metabolic imbalances in preterm infants, possibly due to their immature metabolic processes and reduced kidney function ([Cibulskis 2015](#); [Thoene 2014](#)). Preterm infants are at increased risk of developing metabolic and renal tubular acidosis ([Koletzko 2005](#); [Manz 1997](#)). Thus, fortifiers which have been acidified as a form of sterilisation may have higher acid loads, and result in decreased growth ([Kalhoff 1993](#); [Kalhoff 2001](#)). Finally, the addition of liquid fortifiers to human milk may displace the volume of human milk, and cause the infant to receive an inadequate total volume of human milk ([Underwood 2013](#)).

Why it is important to do this review

Protein supplementation of human milk would help to increase protein intake in very preterm infants, while retaining the benefits of feeding human milk. However, fortifiers are often expensive, their long-term benefits, if any, are uncertain, and their use has been associated with some adverse effects ([Thoene 2014](#); [Tonkin 2014](#)). It is imperative to determine the benefits and harms of their use in both the short- and long-term.

OBJECTIVES

To determine whether protein-supplemented human milk, compared with unsupplemented human milk, fed to preterm infants, improves growth, body composition, cardio-metabolic, and neurodevelopmental outcomes, without significant adverse effects.

METHODS

Criteria for considering studies for this review

Types of studies

We considered published and unpublished randomised and quasi-randomised controlled trials for this review.

Types of participants

Preterm infants (less than 37 weeks' gestation) receiving enteral feeding of human milk, within a hospital setting.

Types of interventions

Human milk, with or without additional protein supplementation. Micronutrient supplements were allowed in both groups.

Types of outcome measures

The primary and secondary outcomes for this review were aligned with the outcomes of the Cochrane Review, Multi-nutrient fortification of human milk for preterm infants (Brown 2016).

Primary outcomes

- Growth: weight, length, head circumference, skinfold thickness (WHO 1995), body mass index and measures of body composition (lean, fat mass) and growth restriction (proportion of infants below the 10th percentile for the index population distribution of weight, length, or head circumference). Growth parameters were assessed from birth to hospital discharge, at or after two years' corrected age, during adolescence, and as adults.
- Neurodevelopmental outcomes after 12 months' post term: neurological evaluations, developmental scores, and classifications of disability, including auditory and visual disability. We defined neurodevelopmental impairment as the presence of one or more of the following: non-ambulant cerebral palsy, developmental quotient more than two standard deviations below the population mean, blindness (visual acuity less than 6/60), or deafness (any hearing impairment requiring or unimproved by amplification).

Secondary outcomes

- Duration of hospital admission
- Feeding intolerance that resulted in cessation of or reduction in enteral feeding
- Necrotising enterocolitis (NEC)
- Blood urea nitrogen (BUN) concentrations
- Serum albumin concentrations
- Metabolic acidosis, as defined by trialists
- Long-term measures of cardio-metabolic health, such as insulin resistance, obesity, diabetes, and hypertension

Search methods for identification of studies

We used the criteria and standard methods of Cochrane and Cochrane Neonatal (see [the Cochrane Neonatal search strategy for specialized register](#)).

Electronic searches

We conducted a comprehensive search of: the Cochrane Central Register of Controlled Trials (CENTRAL; 2018, Issue 1) in The Cochrane Library (accessed 2 February 2018), MEDLINE via PubMed (1966 to 2 February 2018), Embase (1980 to 2 February 2018), and CINAHL (1982 to 2 February 2018), using search terms for protein and breast milk, plus database-specific limiters for RCTs and neonates, which are detailed in [Appendix 1](#). We did not apply language restrictions.

Searching other resources

We also searched the reference lists of any articles selected for inclusion in this review, in order to identify additional relevant articles. We did not search any additional conference proceedings.

Data collection and analysis

We used the criteria and standard methods of Cochrane Neonatal to assess the methodological quality of the included trials.

Two review authors (EA, JB) independently extracted the data, compared data, and resolved differences by discussion, or by consulting with a third review author (JH).

We used the standard methods of Cochrane Neonatal to synthesise the data. We expressed results as relative risk and mean difference.

Selection of studies

Two review authors (EA and JB) independently screened the titles and abstracts of the records identified by the searches. We resolved conflicts by discussion, or by consulting with a third author (JH). We retrieved the full text of all potentially relevant articles, and linked reports of the same study. Two review authors (EA and JB) independently assessed the full-text articles for inclusion, using the eligibility criteria. We resolved conflicts by discussion, or by consulting with a third author (JH). We had planned to correspond with investigators to clarify study eligibility and obtain missing results if needed. We used Covidence for the study selection and data collection processes (Covidence).

Data extraction and management

We developed a data extraction form prior to data gathering, to enable two review authors to independently extract information from the studies. We extracted data such as source details, study eligibility, study design, participant characteristics, intervention and control details, and outcomes. We resolved conflicts in the data extraction and management process by discussion, or by consulting with a third review author. We then exported the data into Cochrane's review software, Review Manager 5 (Review Manager 2014).

Assessment of risk of bias in included studies

Two review authors (EA and JB) independently assessed the risk of bias (low, high, or unclear) of all included trials, using the Cochrane 'Risk of bias' tool, for the following domains (Higgins 2017):

- Sequence generation (selection bias)
- Allocation concealment (selection bias)
- Blinding of participants and personnel (performance bias)
- Blinding of outcome assessment (detection bias)
- Incomplete outcome data (attrition bias)
- Selective reporting (reporting bias)
- Any other bias

We resolved conflicts by discussion, or by consulting with a third review author. See [Appendix 2](#) for a more detailed description of risk of bias for each item.

Measures of treatment effect

For dichotomous data, we used the number of events in the control and intervention groups of each study to calculate risk ratios (RRs) with 95% confidence intervals (CIs). For continuous data, we calculated mean differences (MDs) between treatment groups with 95% CIs, where outcomes were measured in the same way. We did not need to use standardised mean differences (SMD) in this update, but they will be used in future updates where outcomes from studies are the same, but different methods have been used to collect the data. We did not calculate numbers needed to treat for an additional beneficial outcome (NNTB) or the numbers needed for an additional harmful outcome (NNTH), due to insufficient data.

Unit of analysis issues

We did not identify any unit of analysis issues. In future updates, if we identify cluster-randomised trials, we will undertake analysis at the individual level, taking clustering into account, as recommended in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2017).

Dealing with missing data

We noted levels of attrition. We carried out analyses using an intention-to-treat basis, where possible, for all of the outcomes. Where possible, we analysed all participants in the treatment group to which they were randomised, regardless of the actual treatment received. We did not contact any of the trial authors. In future updates, if data are missing, we will make an attempt to contact the trial authors. We were unable to conduct sensitivity analyses, and were unable to address the potential impact of missing data on the findings of the review, due to insufficient data.

Assessment of heterogeneity

We considered whether the clinical and methodological characteristics of the included studies were sufficiently similar for meta-analysis to provide a clinically meaningful summary. This was done by assessing statistical heterogeneity using the Chi² test and the I² statistic. We took an I² measurement greater than 50%, and P < 0.10 in the Chi² test for heterogeneity to indicate moderate-to-high heterogeneity. Where we detected moderate-to-high heterogeneity, we had planned to explore possible explanations for clinical heterogeneity via subgroup analyses or methodological heterogeneity via sensitivity analyses, or both. We had planned to take clinical and statistical heterogeneity into account when interpreting the results, especially if there was any variation in the direction of effect.

Assessment of reporting biases

Reporting biases arise when the dissemination of research findings is influenced by the nature and direction of results. Some types of reporting bias (e.g. publication bias, multiple publication bias, language bias) reduce the likelihood that all studies eligible for a review will be retrieved. If all eligible studies are not retrieved, the review may be biased. We aimed to conduct a comprehensive search for eligible studies, and were alert for duplication of data. We were unable to formally assess publication bias, as there were insufficient studies for any of the outcomes (10 or more studies required). In future updates, if we find 10 or more studies reporting an outcome, we will assess publication bias by visual inspection of a funnel plot.

Data synthesis

We performed meta-analyses using Review Manager (Review Manager 2014). We used risk ratio (RR) for dichotomous data, and mean difference (MD) for continuous data, with their respective 95% confidence intervals (CIs). We used a fixed-effect model to combine data where similar interventions, populations and methods were employed by the trials. We planned to explore potential causes of heterogeneity via sub-group and sensitivity analyses and assessed the quality of evidence at the outcome level, using GRADE methodology.

Quality of evidence

We used the GRADE approach, as outlined in the *GRADE Handbook*, to assess the quality of evidence for the following (clinically relevant) outcomes: growth, neurodevelopment, duration of hospital admission, feeding intolerance that resulted in cessation or reduction in enteral feeding, and necrotizing enterocolitis (Schünemann 2013).

Two authors (EA and JB) independently assessed the quality of the evidence for each of the outcomes above. We considered evidence from randomised controlled trials as high quality, but downgraded the evidence one level for serious (or two levels for very serious) limitations, based upon the following: design (risk of bias), consistency across studies, directness of the evidence, precision of estimates, and presence of publication bias. We used GRADEpro GDT software to create a 'Summary of findings' table, to summarise results and report the quality of the evidence (GRADEpro GDT).

The GRADE approach leads to an assessment of the quality of a body of evidence at one of four levels:

- High: we are very confident that the true effect lies close to that of the estimate of the effect.
- Moderate: we are moderately confident in the effect estimate; the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
- Low: our confidence in the effect estimate is limited; the true effect may be substantially different from the estimate of the effect.
- Very low: we have very little confidence in the effect estimate; the true effect is likely to be substantially different from the estimate of effect.

Subgroup analysis and investigation of heterogeneity

We considered whether an overall summary was meaningful by assessing clinical and methodological heterogeneity among trials (see section above on 'Assessment of Heterogeneity'). If we had found moderate-to-high heterogeneity, we had planned to perform subgroup and sensitivity analyses. We had planned to carry out the following subgroup analyses to evaluate differences in outcome between: gestational age subgroups (less than 30, versus 30 up to 34, versus 34 up to 37 completed weeks), birth weight subgroups (less than 1 kg versus 1 kg or above), male versus female, and types of protein supplements (bovine versus human, and with versus without micronutrients or minerals). However, there were insufficient data to allow us to conduct any subgroup analyses.

Sensitivity analysis

We had planned to conduct sensitivity analyses by examining only those trials considered to have a low risk of bias for allocation concealment and randomisation. We were unable to do this as all the included studies were judged to be of unclear risk of bias for both allocation concealment and randomisation.

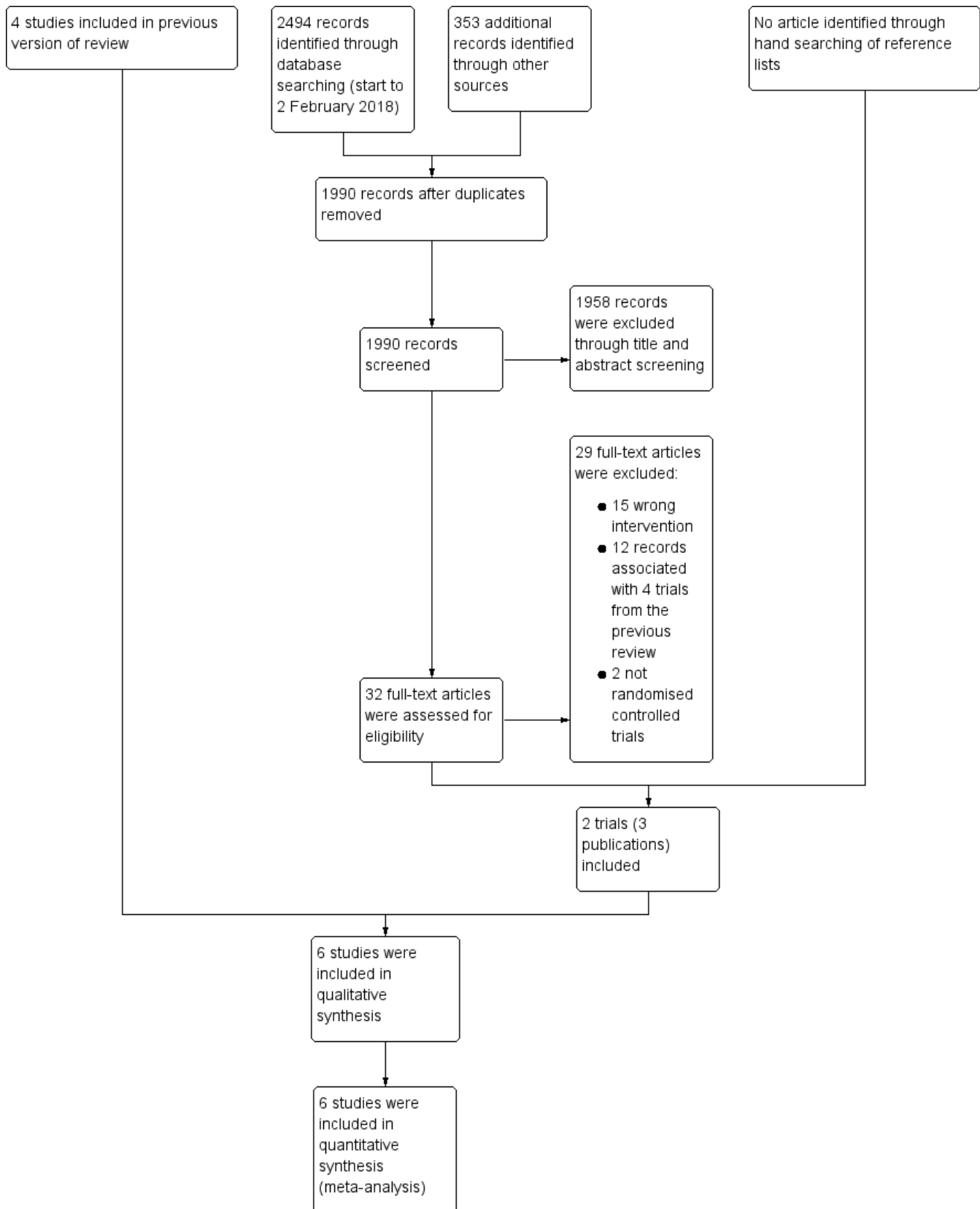
RESULTS

Description of studies

Results of the search

From initial search results of 1990 citations, we identified two additional studies (three publications) for inclusion in this update of the review ([Faerk 2001](#); [Greer 1986](#)). For a full description of our selection process, please see our 'Study flow diagram' ([Figure 1](#)).

Figure 1. Study flow diagram: review update



Included studies

We included six studies in this review, and extracted data from full-text publications for all six studies (Boehm 1988a; Faerk 2001; Greer 1986; Polberger 1989; Putet 1987; Rönnholm 1982). All studies were published in English between 1982 and 2001, and included a total of 204 preterm infants who fulfilled our predefined criteria. All the studies were reported to be randomised controlled trials. Four were single centre studies, while two were conducted at two centres each (Faerk 2001; Polberger 1989). Three studies were two-armed randomised controlled studies (Boehm 1988a; Putet 1987; Rönnholm 1982), Faerk 2001 was three-armed, and Greer 1986 and Polberger 1989 were four-armed studies. Sample sizes ranged from 14 (Polberger 1989), to 103 preterm infants (Faerk 2001). Three studies were carried out in Europe (Boehm 1988a; Faerk 2001; Polberger 1989), and one in the USA (Greer 1986), but the locations of the other two were unclear. None of our included studies was conducted in a developing country. We summarised the details of the included studies in the 'Characteristics of included studies' table.

Participants

All the studies examined preterm infants less than 32 gestational weeks, except Boehm 1988a (less than 33 gestational weeks) and Rönnholm 1982 (up to and including 36 gestational weeks). It was not clear what gestational age threshold Putet 1987 studied, but they studied only male infants. Boehm 1988a was the only study that studied the effects of protein supplementation of human milk in standard birth weight groups (very low birth weight (VLBW) and low birth weight (LBW) infants). Greer 1986, Polberger 1989, Putet 1987, and Rönnholm 1982 studied infants with birth weight of less than 1600 g, Faerk 2001 studied infants less than 1200 g and more than 1200 g, and Boehm 1988a studied infants between 1000 g and 1990 g. All studies included infants with no medical problems or major congenital malformations except Faerk 2001, who included infants with medical illnesses, such as bronchopulmonary dysplasia, septicaemia, and intraventricular haemorrhage.

Interventions

Lyophilized human milk protein supplements were used in two studies (Boehm 1988a; Polberger 1989), and bovine casein hydrolysate was used in one study (Putet 1987). One study used bovine whey with mineral supplements (Greer 1986), Faerk 2001 used Eoprotin, and Rönnholm 1982 used human milk protein concentrate. Protein intakes ranged from 0.6 g/kg/day to 4.5 g/kg/day in the intervention groups. All infants in the intervention group of four studies received vitamin and mineral supplements (Faerk 2001; Greer 1986; Polberger 1989; Rönnholm 1982). Four studies

used both maternal and donor breast milk (Boehm 1988a; Faerk 2001; Polberger 1989; Rönnholm 1982). Greer 1986 used maternal milk exclusively for infants in both the intervention and control groups, while Putet 1987 did not specify maternal or donor milk. All the studies used standardised rather than targeted fortification.

Comparators

Two studies used unsupplemented human milk alone (Boehm 1988a; Putet 1987), while Faerk 2001, Polberger 1989, and Rönnholm 1982 used human milk supplemented with vitamins and minerals. Greer 1986 used human milk with added vitamin supplements. Faerk 2001 included an unsupplemented arm, in which infants received additional formula if the mother's breast milk was insufficient. Data from this arm of the study were not included in our analysis.

Outcomes

All the studies included at least one of our outcomes of interest. Greer 1986, Polberger 1989, Putet 1987, and Rönnholm 1982 contributed data about the rate of growth, as weight, length, and head circumference. Faerk 2001 contributed weight, length, and head circumference data at term, and Polberger 1989 also provided data on weight at the end of the study. Only one study contributed data on skin fold thickness and duration of hospital stay (Greer 1986), while another contributed data on feeding intolerance and serum albumin concentrations (Polberger 1989). Faerk 2001 provided data on necrotising enterocolitis, and four studies contributed data on blood urea concentrations (Boehm 1988a; Greer 1986; Polberger 1989; Putet 1987). No trial reported data on long-term growth, body mass index (BMI), body composition, neurodevelopmental, and cardio-metabolic outcomes.

Excluded studies

We excluded 17 full-text articles from this 2018 update. Thirteen studies (15 publications) used interventions that did not meet our criteria (Abrams 2014; Barrington 2016; Berseth 2012; Bhat 2001; Ditzenberger 2013; Gathwala 2012; Hair 2014; Hayashi 1994; Hill 1997; Modanlou 1986; Moltu 2013; Polberger 1997; Valman 1971), and two studies were not randomised (Bishara 2017; Boehm 1988b). See the 'Characteristics of excluded studies' table and Figure 1 for details of exclusions.

Risk of bias in included studies

Overall, we scored most items as unclear risk of bias, as there was insufficient methodological detail to make a judgement. We summarised our evaluations for individual studies in the 'Risk of bias' graph and summary (Figure 2; Figure 3).

Figure 2. Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies

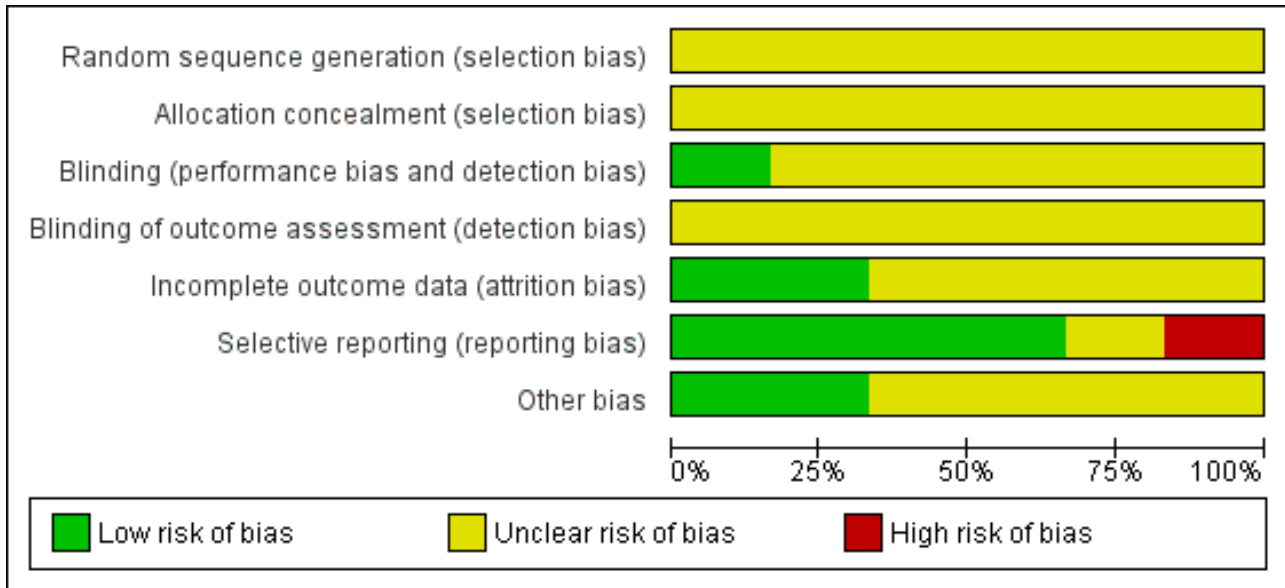


Figure 3. Risk of bias summary: review authors' judgements about each risk of bias item for each included study

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding (performance bias and detection bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
Boehm 1988a	?	?	?	?	+	+	?
Faerk 2001	?	?	+	?	+	+	?
Greer 1986	?	?	?	?	?	+	+
Polberger 1989	?	?	?	?	?	-	?
Putet 1987	?	?	?	?	?	+	+
Rönholm 1982	?	?	?	?	?	?	?

Allocation

We judged all six included studies as unclear risk of bias for random sequence generation and allocation concealment as they did not provide sufficient methodological detail to make a judgement (Boehm 1988a; Faerk 2001; Greer 1986; Polberger 1989; Putet 1987; Rönholm 1982).

Blinding

Of all the studies included, only one had a low risk of performance bias, as they blinded study participants and personnel (Faerk 2001). We judged the remaining five studies as unclear risk of performance bias. We judged detection bias at unclear risk of bias for all six studies.

Incomplete outcome data

We judged two studies to have a low risk of attrition bias (Boehm 1988a; Faerk 2001), but all other studies as unclear risk.

Selective reporting

We judged two studies to have low risk of selective reporting bias (Greer 1986; Putet 1987). We judged Polberger 1989 as high risk of selective reporting bias because the authors did not report head circumference results at all time points, due to an initial finding of a minimal relation to protein intake in their trial. The remaining studies had unclear risk of reporting bias.

Other potential sources of bias

We judged other other potential sources of bias as unclear risk, except for [Greer 1986](#) and [Putet 1987](#), which we judged had low risk of bias, as no extreme baseline imbalances were identified.

Effects of interventions

See: [Summary of findings for the main comparison Protein supplementation compared to control for promoting growth in preterm infants](#)

1.0 Protein supplementation versus control

1.1 Growth: weight

1.1.1 Weight gain

Five randomised controlled trials, including 101 infants, contributed data ([Boehm 1988a](#); [Greer 1986](#); [Polberger 1989](#); [Putet 1987](#); [Rönnholm 1982](#)). Protein supplementation of human milk was associated with more weight gain compared with unsupplemented human milk (mean difference (MD) 3.82 g/kg/day, 95% confidence interval (CI) 2.94 to 4.7; five RCTs, 101 infants; $I^2 = 73%$; low-quality evidence; [Analysis 1.1](#)). We downgraded the evidence for risk of bias, as there was insufficient methodological information, and moderate heterogeneity among the studies estimating the population mean difference.

1.1.2 Weight at term-equivalent age

Only [Faerk 2001](#) reported the weight at term. There was no evidence of a clear difference between the protein supplemented and the unsupplemented groups (MD 61.0 g, 95% CI -160.23 to 282.23; one RCT, 76 infants; [Analysis 1.1](#)).

1.1.3 Weight at the end of the study

[Polberger 1989](#) reported weight at the end of the study, which was when the infant weighed 2200 g or when breastfeeding was initiated. There was no evidence of a clear difference in weight between the protein supplemented and the unsupplemented groups (MD 250.0 g, 95% CI -41.56 to 541.56; one RCT, 14 infants; [Analysis 1.1](#)).

1.2 Growth: length

1.2.1 Length gain

Four randomised controlled trials, including 68 infants, contributed data ([Greer 1986](#); [Polberger 1989](#); [Putet 1987](#); [Rönnholm 1982](#)). Protein supplementation of human milk was associated with more linear growth compared with unsupplemented human milk (MD 0.12 cm/week, 95% CI 0.07 to 0.17; four RCTs, 68 infants, $I^2 = 89%$; low-quality evidence; [Analysis 1.2](#)). The evidence was downgraded for risk of bias, as there was insufficient methodological information, and high heterogeneity among the trials estimating the population mean difference.

1.2.2 Length at term-equivalent age

[Faerk 2001](#) reported length at term. There was no evidence of a clear difference between the protein supplemented and unsupplemented groups (MD -0.5 cm, 95% CI -1.65 to 0.65; one RCT, 76 infants; [Analysis 1.2](#)).

1.3 Growth: head circumference

1.3.1 Head circumference gain

Four randomised controlled trials, including 68 infants, contributed data ([Greer 1986](#); [Polberger 1989](#); [Putet 1987](#); [Rönnholm 1982](#)). Protein supplementation of human milk was associated with a greater increase in head circumference compared with unsupplemented human milk (MD 0.06 cm/week, 95% CI 0.01 to 0.12; four RCTs, 68 infants, $I^2 = 84%$; low-quality evidence; [Analysis 1.3](#)). We downgraded the evidence for risk of bias, as there was insufficient methodological information and high heterogeneity among the trials estimating the population mean difference.

1.3.2 Head circumference at term-equivalent age

[Faerk 2001](#) reported the head circumference at term. There was no evidence of a clear difference between the protein supplemented and the unsupplemented groups (MD 0.3 cm, 95% CI 0.-24 to 0.84; one RCT, 76 infants; [Analysis 1.3](#)).

1.4 Growth: skin fold thickness

One trial including 20 children reported data on the rate of growth of skin fold thickness ([Greer 1986](#)). Neither the triceps nor the subscapular measurements showed a clear difference between the protein supplemented and unsupplemented groups (triceps MD 0.06 mm/week, 95% CI -0.09 to 0.21; one RCT, 20 infants; subscapular MD 0.0 mm/week, 95% CI -0.17 to 0.17; one RCT, 20 infants; [Analysis 1.4](#)).

1.5 Duration of hospital stay

One trial reported on duration of hospital stay ([Greer 1986](#)). The protein supplemented group had a longer hospital stay than the unsupplemented group (MD 18.5 days, 95% CI 4.39 to 32.61; one RCT, 20 infants; very low-quality evidence; [Analysis 1.5](#)). We downgraded the evidence for risk of bias, as there was insufficient methodological information to judge the risk of bias, few patients, few events, and very wide confidence intervals.

1.6 Feeding intolerance

Only [Polberger 1989](#) reported data on feeding intolerance. There was one event reported in the supplemented group and no events reported in the unsupplemented group (risk ratio (RR) 2.70, 95% CI 0.13 to 58.24; one RCT, 17 infants; very low-quality evidence; [Analysis 1.6](#)). We downgraded the evidence for risk of bias, as there was insufficient methodological information to judge the risk of bias, few patients, few events, and very wide confidence intervals.

1.7 Necrotising enterocolitis

One trial reported data on the incidence of necrotising enterocolitis ([Faerk 2001](#)). There was one infant in each of the protein supplemented and unsupplemented groups who developed necrotising enterocolitis (RR 1.11, 95% CI 0.07 to 17.12; one RCT, 76 infants; very low-quality evidence; [Analysis 1.7](#)). We downgraded the evidence for risk of bias as there was insufficient methodological information to judge the risk of bias, few patients, few events, and very wide confidence intervals.

1.8 Blood urea nitrogen (BUN)

Four randomised controlled trials contributed data ([Boehm 1988a](#); [Greer 1986](#); [Polberger 1989](#); [Putet 1987](#)). BUN concentrations were higher in the protein supplemented group than in the

unsupplemented group (MD 0.95 mmol/L, 95% CI 0.81 to 1.09; four RCTs, 81 infants; $I^2 = 56\%$; [Analysis 1.8](#)).

1.9 Serum albumin concentrations

Only [Polberger 1989](#) reported data on serum albumin concentrations. There was no evidence of a clear difference in serum albumin concentrations between the protein supplemented and unsupplemented groups (MD 2.5 g/L, 95% CI -5.66 to 10.66; one RCT, 11 infants; [Analysis 1.9](#)).

No data were reported for the following outcomes: long-term growth, body mass index (BMI), body composition, neurodevelopmental, and cardio-metabolic outcomes.

The heterogeneity test indicated moderate-to-high variation among studies reporting growth outcomes, but there were insufficient data to allow us to conduct our prespecified subgroup analyses.

DISCUSSION

Summary of main results

The evidence from six randomised controlled trials, involving 204 preterm infants, showed that protein supplementation of human milk increased in-hospital rates of weight gain, length gain, and head growth in preterm infants. There was no evidence of a clear difference in the rate of growth of skin fold thickness between the supplemented and unsupplemented groups. Protein supplementation was associated with longer hospital stays in one study, and higher blood urea nitrogen (BUN) concentrations. The evidence did not show that protein supplementation clearly altered the risk of necrotising enterocolitis (NEC) or feeding intolerance, or that it altered serum albumin concentrations. No data were available for the assessment of the effects of protein supplementation on long-term growth outcomes, body mass index (BMI), body composition, neurodevelopmental, or cardio-metabolic outcomes. We observed moderate-to-high heterogeneity among studies reporting growth outcomes.

Overall completeness and applicability of evidence

The lack of data on long-term growth, BMI, body composition, neurodevelopmental, and cardio-metabolic outcomes made it impossible to determine long-term health and developmental effects of protein supplementation of human milk fed to preterm infants. Few trials reported our secondary outcomes (duration of hospital stay, feeding intolerance, NEC, and serum albumin concentrations), and therefore these outcomes had small sample sizes and very low-quality evidence supported their estimates of effect, making it difficult to make an evidence-based statement on these effects. In addition, due to incomplete data reporting, we were unable to identify reasons for the moderate-to-high heterogeneity among the sample population. We were also unable to conduct any of our planned subgroup analyses. Finally, the included studies were all performed in developed countries, and so our findings may not be generalisable to preterm infants in less developed countries.

Quality of the evidence

We graded the overall quality of evidence for the primary outcomes as low-quality, because of lack of reported methodological details and moderate-to-high heterogeneity among the studies estimating

the population mean difference. Without details such as blinding of study personnel and outcome assessors, it was difficult to adequately judge the risk of bias and quality of evidence, as blinding could have an impact on the assessment of growth parameters, and hence, possibly the estimate of effect size. All secondary outcomes were graded as very low-quality evidence due to small sample sizes, few events, and small number of studies.

Potential biases in the review process

Due to small numbers of included studies, we were unable to create funnel plots to assess the potential risk of publication or reporting bias. We minimised bias by conducting a systematic search of the literature, and data extraction was undertaken independent by two review authors.

Agreements and disagreements with other studies or reviews

We are not aware of any previous systematic reviews conducted on this topic, except for our previous review, which included four single centre randomised and quasi-randomised controlled trials published between 1982 and 1989, and involving 90 very low birth weight infants ([Kuschel 2000a](#)). The results of this updated systematic review are similar to those of the previous review, including increases in weight gain, length gain, head circumference, and BUN levels in the protein supplemented groups, but no clear effect on albumin concentrations or the risk of NEC. To our knowledge, this review is the first to include body composition and cardio-metabolic outcomes, for which we found no available data.

AUTHORS' CONCLUSIONS

Implications for practice

Protein supplementation of expressed breast milk fed to preterm infants increased short-term rates of weight gain, length gain, and head growth, without evidence of a clearly increased risk of necrotising enterocolitis or feeding intolerance. Long-term benefits and harms are unknown. Further, preterm infants fed solely breast milk have other nutritional deficiencies, including energy and minerals, so protein supplementation of human milk is now usually done as a component of multi-nutrient fortifiers. We conclude that protein fortification of human milk in preterm infants could be considered in settings where the risk of poor postnatal growth is high and multi-nutrient fortification is not available or feasible.

Implications for research

Although there was evidence that protein supplementation increased short-term growth, there were few data, and overall, very low-quality evidence regarding potential short-term risks and long-term benefits and harms. Therefore, future studies should compare different amounts of protein in multi-component fortifiers, and be designed to determine the effects on duration of hospital stay and safety, as well as on long-term growth, body composition, cardio-metabolic, and neurodevelopmental outcomes. These studies should also be conducted in resource-poor settings.

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The methods section of this review is based on a standard template used by Cochrane Neonatal.

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* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES
Characteristics of included studies [ordered by study ID]

Boehm 1988a

Methods	Parallel randomised controlled trial, single centre
Participants	Inclusion criteria: infants without major clinical problems, VLBW or LBW infants. Exclusion criteria: not specified Setting: neonatal unit of the Karl Marx University Children's Hospital in Leipzig, Germany Timing: not specified
Interventions	Lyophilised human milk protein (6 g/100mL) supplementation of maternal or donor preterm human milk (17 infants) versus unsupplemented human milk (16 infants) Not stated when intervention ceased
Outcomes	Primary outcomes: outcomes not specified into primary and secondary, but protein intake on 8th and 21st days of age, weight gain, daily serum urea, and alpha-amino-nitrogen concentrations taken 60 minutes after a feed were measured Secondary outcomes: not specified
Notes	Conflicts of interest: no details Source of funding: no details Both the VLBW and LBW arms of the study were included in this review.

Boehm 1988a (Continued)

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No details
Allocation concealment (selection bias)	Unclear risk	No details
Blinding (performance bias and detection bias) All outcomes	Unclear risk	No details
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	No details
Incomplete outcome data (attrition bias) All outcomes	Low risk	All infants randomised appear to have been analysed
Selective reporting (reporting bias)	Low risk	The study protocol was not available, but it is clear by comparison of the methods and results sections that all the results of pre-specified outcomes were stated.
Other bias	Unclear risk	No other sources of bias noted.

Faerk 2001

Methods	Parallel randomised controlled trial, multiple centres
Participants	<p>Inclusion criteria: preterm infants with gestational age of < 32 weeks and no major congenital malformations</p> <p>Exclusion criteria: not stated</p> <p>Setting: NICU at Rigshospitalet and Hvidovre Hospital in Copenhagen. Some infants were discharged to local neonatal units at Glostrup and Hilleroed Hospitals, but continued in the study.</p> <p>Timing: not stated</p>
Interventions	<p>Human milk (mother + donor) supplemented with protein, calcium, and phosphate (Eoprotin, 0.4 g protein, 35 mg Ca and 17 mg P/100 mL; N = 51) versus human milk + phosphate (10 mg P/100 mL; N = 52)</p> <p>All infants received 800 IU of vitamin D daily.</p> <p>The intervention ceased at the end of the 36th gestational week or when infants were fully breast fed, whichever came first.</p>
Outcomes	<p>Primary: total body mineral content at term by DEXA scan</p> <p>Secondary: body weight, crown-heel length, head circumference, body composition, and bone area at term.</p>
Notes	Conflicts of interest: not stated

Protein supplementation of human milk for promoting growth in preterm infants (Review)

Faerk 2001 (Continued)

Source of funding: not stated

This study had three arms - unsupplemented human milk or formula versus human milk supplemented with phosphate versus human milk supplemented with protein, calcium and phosphate. Infants in the unsupplemented arm were fed only their own mother's milk. However, if the mother's breast milk was insufficient, her infant was fed formula as well. Data on the unsupplemented human milk or formula arm were not included in this review.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No mention of how the random sequence was generated.
Allocation concealment (selection bias)	Unclear risk	The randomisation was done in an independent centre in Copenhagen (a human milk bank). They used sealed envelopes which gave some assurance. However, it was not stated if the envelopes were opaque.
Blinding (performance bias and detection bias) All outcomes	Low risk	"The study was kept blinded for the PI, the parents, all the attending physicians, and at Hvidovre Hospital, Rigshospitalet, all the attending staff".
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	No details
Incomplete outcome data (attrition bias) All outcomes	Low risk	A total of 217 infants fulfilled the inclusion criteria, parents of 52 infants refused to participate. 165 were randomised, 15 withdrew from the fortifier group due to unacceptable or no DEXA scans and other reasons, while 12 withdrew from the unfortified group also for unacceptable or no DEXA scans and other reasons. Other reasons included: abdominal discomfort in three cases, non-acceptance of blinding principle in one case, on request of the attending physician in one case, and in two cases no reasons were stated. The remaining 11 withdrawals were from the formula group, which is not part of this review.
Selective reporting (reporting bias)	Low risk	The study protocol was available and all of the study's prespecified (primary and secondary) outcomes were reported in the prespecified way.
Other bias	Unclear risk	No demographic tables were provided to make the assessment. The sample included sick preterm infants

Greer 1986

Methods	Parallel randomised controlled trial, single centre
Participants	<p>Inclusion criteria: "all infants with birth weight < 1600 grams, gestational age < 32 weeks who were admitted to the neonatal intensive care unit at Madison General Hospital were potentially eligible for enrolment into the study". Included infants received less than 3 weeks of mechanical ventilation and were receiving full enteral feedings by 21 days of age. No infants had chronic lung disease (supplemental oxygen \geq 28 days) or received furosemide therapy.</p> <p>Exclusion criteria: major congenital anomalies, chronic intrauterine infection, significant gastrointestinal disease (hepatitis, cholestatic jaundice, malabsorption), seizure disorders requiring anticonvulsant therapy, or if there was more than a 2-week discrepancy between the two determinations of gestational age (history of last menstrual period and Ballard examination for infants > 27 gestational weeks or fetal ultrasound dating for those \leq 27 weeks' gestational age)</p>

Greer 1986 (Continued)

Setting: neonatal units at Madison General hospital, Wisconsin

Timing: 28 February 1983 to 1 April 1985

Interventions	<p>Exclusively mother's own milk supplemented with 0.85 gm/dL bovine whey, 90 mg/dL calcium, and 45 mg/dL phosphorus (N = 10) versus mother's own milk with no fortification (N = 10)</p> <p>All infants in these groups, except those fed formula, received a standard daily multivitamin supplement containing 400 IU of vitamin D.</p> <p>Not clear when the intervention ceased.</p>
Outcomes	<p>Primary outcomes: outcomes were not specified as primary or secondary, but short-term growth parameters assessed were weight, length, head circumference, and skin fold thickness. Bone mineral content, bone width, serum calcium, phosphorus, creatinine, blood urea nitrogen, total protein, methionine, taurine and cysteine concentrations, alkaline phosphatase, urinary calcium, phosphate, inorganic sulphate, and creatinine concentrations, Breast milk composition and length of stay were also assessed.</p>
Notes	<p>Conflicts of interest: no details</p> <p>Source of funding: Ross Laboratories, Columbus, Ohio</p> <p>This study had four arms - unsupplemented human milk versus human milk supplemented with protein, calcium, and phosphorus, and Similac Special Care (special formula) versus standard formula. Data from the formula arms of this study were not used in this review.</p>

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No details
Allocation concealment (selection bias)	Unclear risk	No details
Blinding (performance bias and detection bias) All outcomes	Unclear risk	No details
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	No details
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	The authors stated that the 38 infants who completed the study represented "22% (38/176) of the total number of admitted infants who met the criteria for birth weight and gestational age". However, no flow chart or details were provided concerning attrition.
Selective reporting (reporting bias)	Low risk	There was no protocol available, but all pre-specified outcomes in the methods section were reported.
Other bias	Low risk	No baseline imbalances identified and no other flaws of study design noted

Polberger 1989

Methods	Parallel randomised controlled trial, in two neonatal units
Participants	<p>Inclusion criteria: birth weight < 1500 grams, appropriate-for-gestational-age, tolerance of complete enteral feeding (170 mL/kg/day), no obvious disease or major malformations, no oxygen therapy, informed parental consent, and acceptance of a blind trial</p> <p>Exclusion criteria: not stated</p> <p>Setting: two neonatal units of the University of Lund in Malmo and Lund</p> <p>Timing: not stated</p>
Interventions	<p>1.0 grams lyophilised human milk protein per 100 mL unpasteurised human milk (maternal or term banked donor; N = 7) versus unsupplemented milk (N = 7)</p> <p>Intervention ceased when the infant reached approximately 2200 g or was breast fed.</p> <p>All infants were supplemented with additional vitamins, calcium lactate (30 mg/kg/day) and sodium phosphate (20 mg/kg/day). From 4 weeks, 2 mg/kg/day elemental iron was given to all infants.</p>
Outcomes	The outcomes were not specified as primary or secondary, but the following were assessed: short-term growth parameters (weight, crown-heel length, occipitofrontal head circumference), intake of protein, fat, carbohydrates, energy, and electrolytes (sodium, potassium, calcium).
Notes	<p>Conflicts of interest: no details</p> <p>Source of Funding: supported in part by the Swedish Medical Research Council, Grant No. B85-I'IX-06259, and Stiftelsen Saniarite</p> <p>This study had four arms: unsupplemented versus supplemented with protein versus supplemented with fat versus supplemented with fat and protein. The analyses of the fat and combined fat and protein arms are discussed in other reviews on multi-component and fat supplementation respectively (Brown 2016; Kuschel 2000b).</p> <p>Of the 34 infants enrolled in the study: 6 were withdrawn following randomisation for apnoea (N = 2), intolerance to accepting to the fixed volume (N = 3) and need for intravenous therapy (N = 1).</p>

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No details
Allocation concealment (selection bias)	Unclear risk	The study used closed envelopes without specifying if they were opaque or not.
Blinding (performance bias and detection bias) All outcomes	Unclear risk	The study was stated to be double blinded, but who was blinded was not specified.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	No details provided as to who was blinded.
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	The missing data i.e. 6 infants, < 20%, were excluded for the following reasons: 2 had apnoea, 3 developed feeding intolerance, and 1 needed IV therapy. However, the authors did not report whether there were any differences between infants excluded and included in the study.
Selective reporting (reporting bias)	High risk	The authors stated "head circumference showed so little relation to protein intake that it was not included in further statistical evaluation (Table 3)". This

Polberger 1989 (Continued)

could suggest possible selective reporting and analytical bias, as not all outcomes were reported.

Other bias	Unclear risk	The authors stated "there was a difference in sex distribution between the groups, but later analyses confirmed that this difference had no implications on the results". However, no further details were provided.
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Putet 1987

Methods	Parallel randomised controlled trial, single centre	
Participants	Inclusion criteria: VLBW boys of appropriate weight for gestational age and no infant had medical problems at the time of the study Exclusion criteria: not stated Setting: not stated Timing: not stated	
Interventions	Cow's milk casein hydrolysate (1 g per 100 mL) added to pooled human milk (8 infants) versus un-supplemented pooled human milk (8 infants). Duration of intervention was not clear.	
Outcomes	Primary: not specified as primary and secondary outcomes, but short-term growth parameters were assessed, including weight, length, head circumference. Total serum protein, BUN, acid-base status, free amino acids, nitrogen retention, bicarbonate level, oxygen consumption, and energy expenditures were also assessed.	
Notes	Conflicts of interest: no details Source of funding: supported by a grant from University Claude Bernard (Lyon) and by an INSERM Contract (PRC 123.0228)	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No details
Allocation concealment (selection bias)	Unclear risk	No details
Blinding (performance bias and detection bias) All outcomes	Unclear risk	No details
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	No details
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	No details
Selective reporting (reporting bias)	Low risk	The protocol was not available, but it appeared that all pre-specified outcomes were measured and reported.

Putet 1987 (Continued)

Other bias	Low risk	No other apparent sources of bias related to the study design noted. No extreme baseline imbalances observed.
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Rönholm 1982

Methods	Parallel randomised controlled trial, single centre
Participants	<p>Inclusion criteria: infants with birth weight less than 1500 grams were enrolled at 2 days of age, if free of major illness and major malformation</p> <p>Exclusion criteria: infants were excluded after randomisation if they developed major disease, died, or were lost to follow up</p> <p>Setting: not stated</p> <p>Timing: not stated</p> <p>Source of funding: supported by a grant from University Claude Bernard (Lyon) and by an INSERM Contract (PRC 123.0228).</p>
Interventions	<p>0.8 g human milk protein per 100 mL human milk (pasteurised maternal or term donor milk) (N = 10) versus unsupplemented human milk (N = 8). Target fluid volume 200 mL/kg/day.</p> <p>All infants received supplemental iron and vitamins. Control infants received calcium supplements (10 mg/kg/day).</p> <p>It is not clear when the intervention was ceased.</p>
Outcomes	Not specified into primary and secondary, but short-term growth parameters, such as weight, length, head circumference were measured. Serum protein concentrations, BUN, acid-base balance, and plasma concentrations of tyrosine and phenylalanine were measured at two weeks of age.
Notes	<p>Conflicts of interest: no details</p> <p>Source of funding: grants from the Foundation for Pediatric Research in Finland</p> <p>Data were extracted from the 1982 publication (Rönholm 1982). In the 1986 report (Rönholm 1986), half the infants in each group received supplementation with MCT oil, 1.0 g/100 mL and it was not possible to extract data for protein supplementation alone versus unsupplemented milk alone.</p>

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No details
Allocation concealment (selection bias)	Unclear risk	No details
Blinding (performance bias and detection bias) All outcomes	Unclear risk	No details
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	No details
Incomplete outcome data (attrition bias)	Unclear risk	Of the 23 preterm infants included in the study, 5 (21.7%) were excluded from the study because they subsequently developed major disease, died, or were

Protein supplementation of human milk for promoting growth in preterm infants (Review)

Rönnholm 1982 *(Continued)*

All outcomes		lost to follow-up. The authors did not report whether there were any differences between infants excluded and included in the study.
Selective reporting (reporting bias)	Unclear risk	Comparing the methods to the results section, it appeared all outcomes measured were reported, except for the acid-base status, which did not appear to be prespecified.
Other bias	Unclear risk	No baseline characteristics were provided to assess for baseline imbalances.

Abbreviations

VLBW: very low birth weight

LBW: low birth weight

DEXA: dual-energy X-ray absorptiometry

NICU: neonatal intensive care unit

PI: principal investigator

BUN: blood urea nitrogen

MCT: medium chain triglyceride

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Abrams 2014	Comparison of human milk-based versus cow's milk-based protein supplementation
Barrington 2016	Comparison of human milk or formula versus milk with 100 mg/day of bovine lactoferrin powder
Berseth 2012	Unable to locate the full article
Bhat 2001	Supplementation was with a multi-nutrient fortifier
Bishara 2017	Not a randomised trial
Boehm 1988b	No evidence of randomisation
Ditzenberger 2013	Composition of the fortifier was not clear, infants received formula when mothers milk was insufficient, and a Microlipid was added to increase caloric density.
Gathwala 2012	Supplementation was with a multi-nutrient fortifier.
Hair 2014	The control group received supplementation.
Hayashi 1994	The fortifier contained extra carbohydrate.
Hill 1997	Studies in the abstract of podium presentations were not related to our review.
Modanlou 1986	Supplementation was with a multi-nutrient fortifier.
Moltu 2013	The control group received supplementation.
Polberger 1997	No unsupplemented arm
Valman 1971	The intervention was either expressed human milk and breast milk substitute or evaporated milk formula.

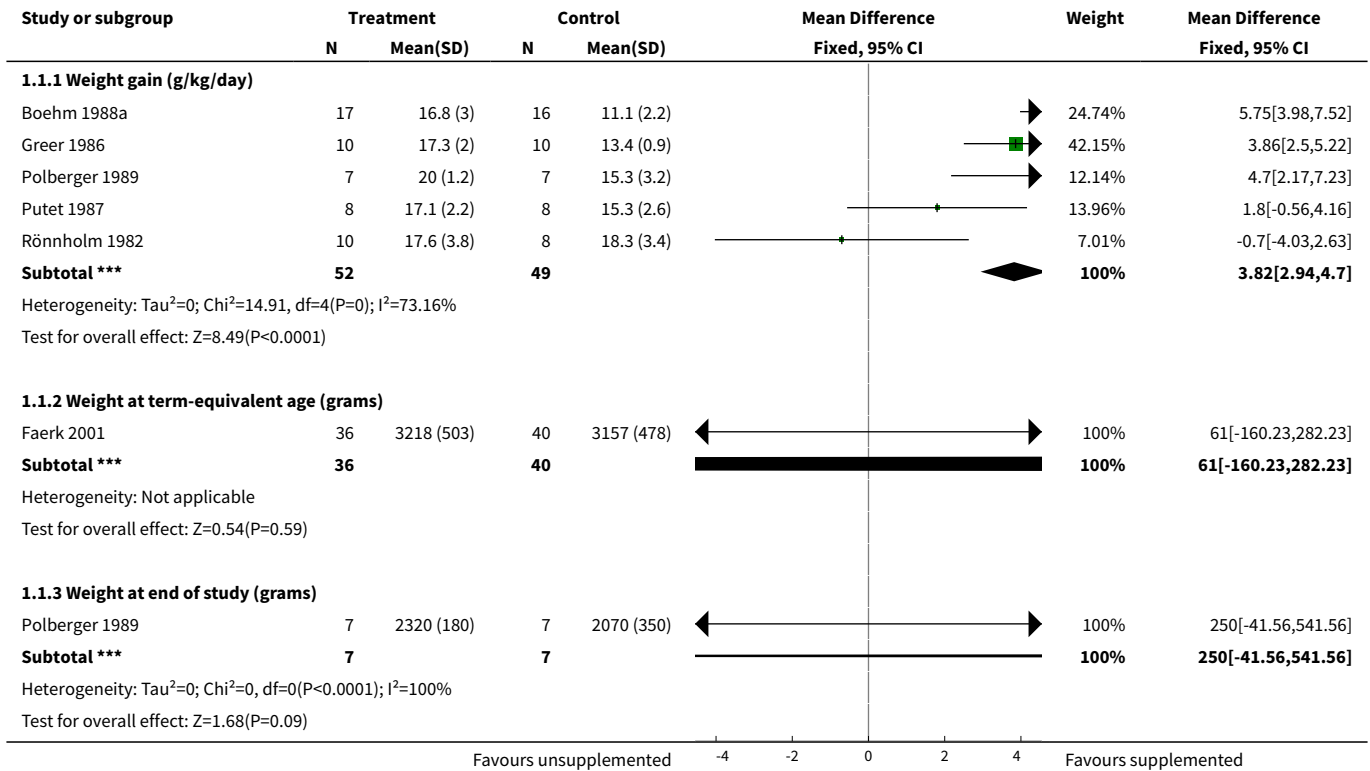
DATA AND ANALYSES

Comparison 1. Protein supplementation versus no supplementation

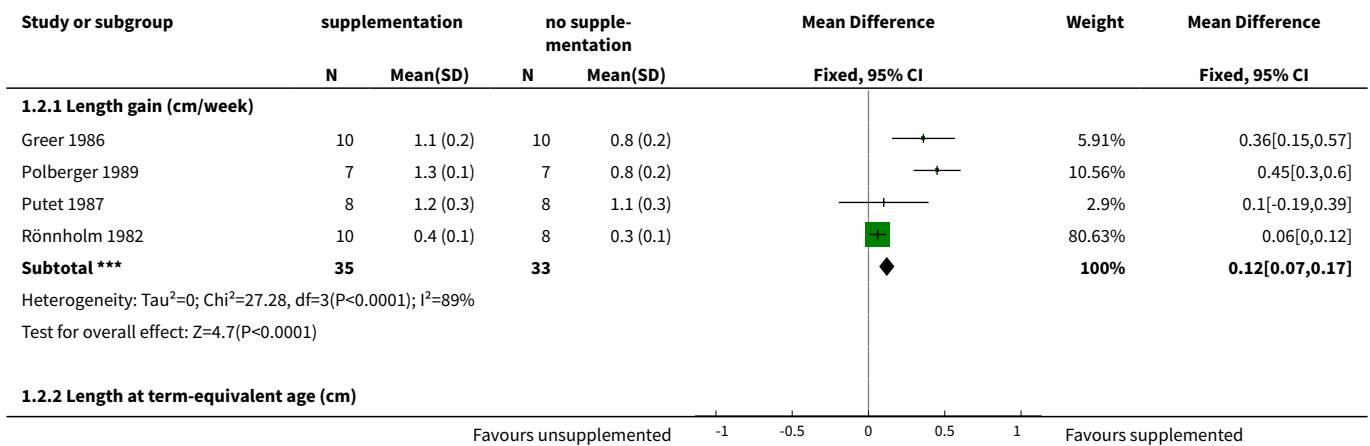
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Growth: weight	6		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
1.1 Weight gain (g/kg/day)	5	101	Mean Difference (IV, Fixed, 95% CI)	3.82 [2.94, 4.70]
1.2 Weight at term-equivalent age (grams)	1	76	Mean Difference (IV, Fixed, 95% CI)	61.0 [-160.23, 282.23]
1.3 Weight at end of study (grams)	1	14	Mean Difference (IV, Fixed, 95% CI)	250.00 [-41.56, 541.56]
2 Growth: length	5		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
2.1 Length gain (cm/week)	4	68	Mean Difference (IV, Fixed, 95% CI)	0.12 [0.07, 0.17]
2.2 Length at term-equivalent age (cm)	1	76	Mean Difference (IV, Fixed, 95% CI)	-0.5 [-1.65, 0.65]
3 Growth: head circumference	5		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
3.1 Head growth (cm/week)	4	68	Mean Difference (IV, Fixed, 95% CI)	0.06 [0.01, 0.12]
3.2 Head circumference at term-equivalent age (cm)	1	76	Mean Difference (IV, Fixed, 95% CI)	0.30 [-0.24, 0.84]
4 Growth: skin fold thickness	1		Mean Difference (IV, Fixed, 95% CI)	Totals not selected
4.1 Skinfold thickness (mm/week): triceps	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
4.2 Skinfold thickness (mm/week): subscapular	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
5 Duration of hospital stay (days)	1		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
6 Feeding intolerance	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
7 Necrotising enterocolitis	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
8 Blood urea (mmol/L)	4	81	Mean Difference (IV, Fixed, 95% CI)	0.95 [0.81, 1.09]

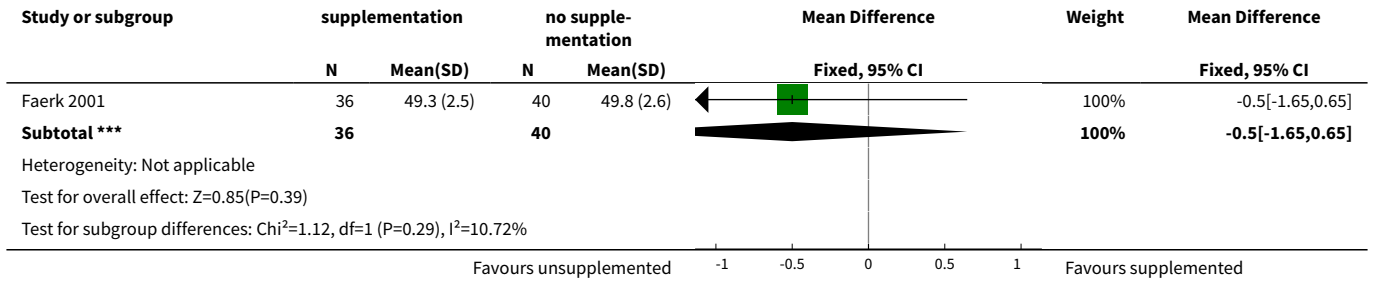
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
9 Serum albumin (g/L)	1		Mean Difference (IV, Fixed, 95% CI)	Subtotals only

Analysis 1.1. Comparison 1 Protein supplementation versus no supplementation, Outcome 1 Growth: weight.

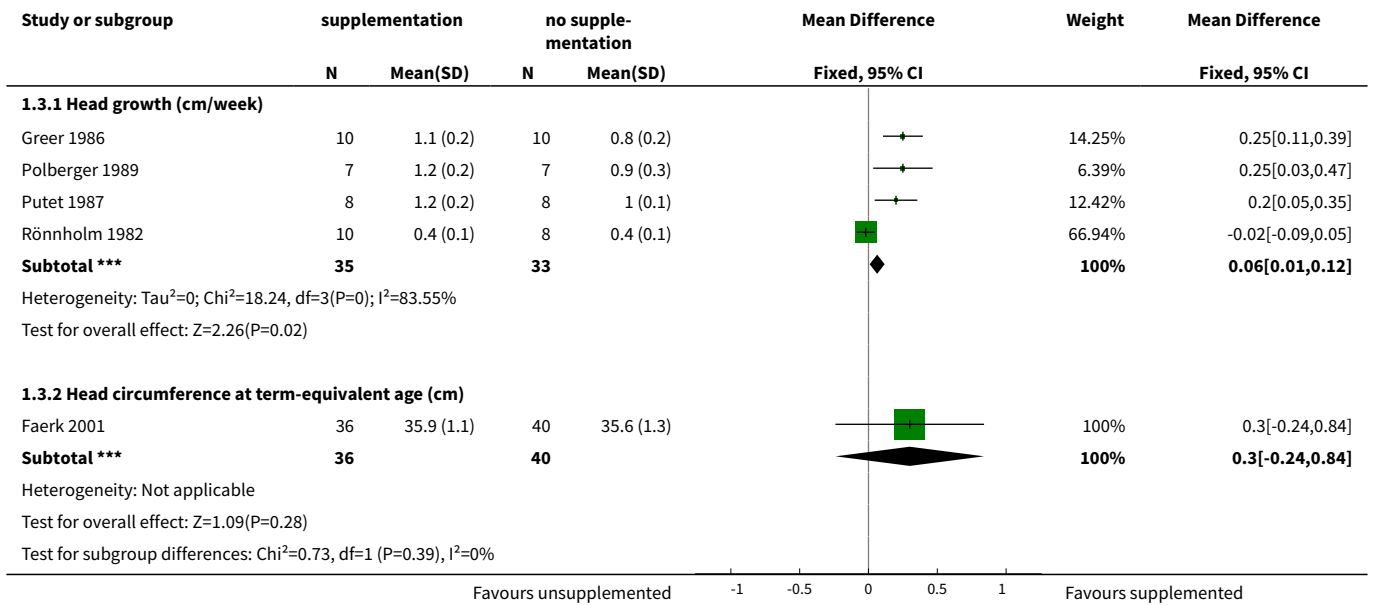


Analysis 1.2. Comparison 1 Protein supplementation versus no supplementation, Outcome 2 Growth: length.

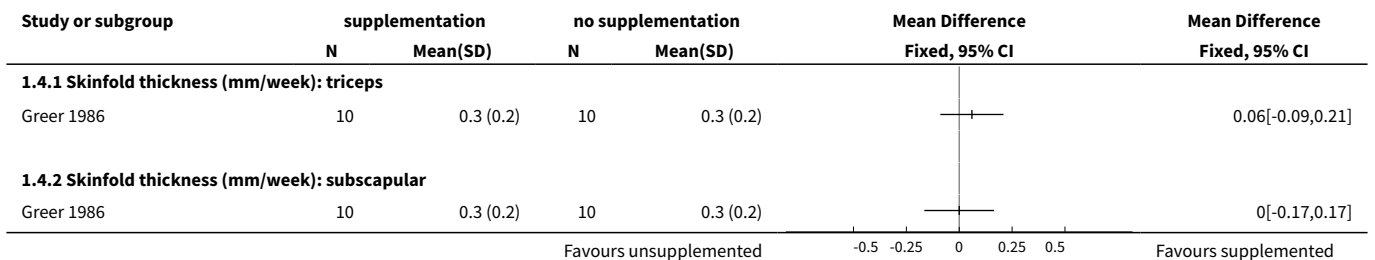




Analysis 1.3. Comparison 1 Protein supplementation versus no supplementation, Outcome 3 Growth: head circumference.



Analysis 1.4. Comparison 1 Protein supplementation versus no supplementation, Outcome 4 Growth: skin fold thickness.



Analysis 1.5. Comparison 1 Protein supplementation versus no supplementation, Outcome 5 Duration of hospital stay (days).

Study or subgroup	supplementation		no supplementation		Mean Difference Fixed, 95% CI	Weight	Mean Difference Fixed, 95% CI
	N	Mean(SD)	N	Mean(SD)			
Greer 1986	10	67.2 (16.3)	10	48.7 (15.9)		0%	18.5[4.39,32.61]

Analysis 1.6. Comparison 1 Protein supplementation versus no supplementation, Outcome 6 Feeding intolerance.

Study or subgroup	supple- mentation	no supple- mentation	Risk Ratio M-H, Fixed, 95% CI	Weight	Risk Ratio M-H, Fixed, 95% CI
	n/N	n/N			
Polberger 1989	1/9	0/8		0%	2.7[0.13,58.24]

Analysis 1.7. Comparison 1 Protein supplementation versus no supplementation, Outcome 7 Necrotising enterocolitis.

Study or subgroup	supple- mentation	no supple- mentation	Risk Ratio M-H, Fixed, 95% CI	Weight	Risk Ratio M-H, Fixed, 95% CI
	n/N	n/N			
Faerk 2001	1/36	1/40		0%	1.11[0.07,17.12]

Analysis 1.8. Comparison 1 Protein supplementation versus no supplementation, Outcome 8 Blood urea (mmol/L).

Study or subgroup	supplementation		no supplementation		Mean Difference Fixed, 95% CI	Weight	Mean Difference Fixed, 95% CI
	N	Mean(SD)	N	Mean(SD)			
Boehm 1988a	17	4.1 (0.2)	16	3.2 (0.2)		87.62%	0.88[0.73,1.03]
Greer 1986	10	3.4 (1.6)	10	1.8 (1)		1.5%	1.58[0.41,2.75]
Polberger 1989	5	2.4 (0.5)	7	0.9 (0.5)		6.09%	1.53[0.95,2.11]
Putet 1987	8	2.8 (0.9)	8	1.5 (0.3)		4.78%	1.3[0.64,1.96]
Total ***	40		41			100%	0.95[0.81,1.09]

Heterogeneity: Tau²=0; Chi²=6.8, df=3(P=0.08); I²=55.89%
Test for overall effect: Z=12.95(P<0.0001)

Analysis 1.9. Comparison 1 Protein supplementation versus no supplementation, Outcome 9 Serum albumin (g/L).

Study or subgroup	supplementation		no supplementation		Mean Difference Fixed, 95% CI	Weight	Mean Difference Fixed, 95% CI
	N	Mean(SD)	N	Mean(SD)			
Polberger 1989	5	29.5 (6.3)	6	27 (7.5)		0%	2.5[-5.66,10.66]

APPENDICES

Appendix 1. Search methodology

PubMed

((((((((((Dietary Protein[MeSH] OR protein[tiab])) OR (((hydrolysate[tiab] OR hydrolys*[tiab] OR hydrolyz*[tiab])) NOT formula*))) AND (((Milk, Human[MeSH] OR breastmilk*[tiab])) OR (((human[tiab] OR breast[tiab] OR expressed[tiab] OR mother*[tiab] OR maternal[tiab] OR donor*[tiab])) AND milk*[tiab]))) AND (((infant, newborn[MeSH] OR newborn OR neonate OR neonatal OR premature OR low birth weight OR VLBW OR LBW or infan* or neonat*) AND (randomized controlled trial [pt] OR controlled clinical trial [pt] OR randomized [tiab] OR placebo [tiab] OR drug therapy [sh] OR randomly [tiab] OR trial [tiab] OR groups [tiab]) NOT (animals [mh] NOT humans [mh]))))))))

Embase

1	exp protein intake/
2	((protein or hydrolysate or hydrolys* or hydrolyz*) not formula\$.ti,ab.
3	1 or 2
4	exp breast milk/
5	((human or breast or expressed) adj2 milk\$.ti,ab.
6	((mother\$ or maternal or donor\$) adj2 milk\$.ti,ab.
7	4 or 5 or 6
8	(infan* or newborn or neonat* or premature or very low birth weight or low birth weight or VLBW or LBW).mp.
9	exp infant/
10	8 or 9
11	(human not animal).mp.
12	(randomized controlled trial or controlled clinical trial or randomized or placebo or clinical trials as topic or randomly or trial or clinical trial).mp.
13	10 and 11 and 12
14	3 and 7 and 13

CINAHL

S1	MH "Dietary Proteins+" OR ((TI (protein OR hydrolysate OR hydrolys* OR hydrolyz*)) NOT formula*) OR ((AB (protein OR hydrolysate OR hydrolys* OR hydrolyz*)) NOT formula*)
S2	MH "Milk, Human+" OR TI (((human or breast or expressed) N2 milk*)) OR AB (((human or breast or expressed) N2 milk*)) OR TI (((mother* or maternal or donor*) N2 milk*)) OR AB (((mother* or maternal or donor*) N2 milk*)) OR TI "breastmilk" OR AB "breastmilk"
S3	((infan* OR newborn OR neonat* OR premature OR low birth weight OR VLBW OR LBW)) AND ((randomized controlled trial OR controlled clinical trial OR randomized OR placebo OR clinical trials as topic OR randomly OR trial OR PT clinical trial))
S4	S1 AND S2 AND S3

Cochrane Central Register of Controlled Trials

1	MESH DESCRIPTOR Milk, Human EXPLODE ALL AND CENTRAL:TARGET
2	((human OR breast OR expressed) NEAR2 milk*):TI,AB,KW AND CENTRAL:TARGET
3	((mother* or maternal or donor*) NEAR2 milk*): TI,AB,KW AND CENTRAL:TARGET
4	#1 OR #2 OR #3
5	(infan* or newborn or neonat* or premature or preterm or very low birth weight or low birth weight or VLBW or LBW) AND CENTRAL:TARGET
6	MESH DESCRIPTOR Dietary Proteins EXPLODE ALL AND CENTRAL:TARGET
7	((protein or hydrolysate or hydrolys* or hydrolyz*) NOT formula*):TI,AB,KW AND CENTRAL:TARGET
8	#6 OR #7
9	#4 AND #5 AND #8

Appendix 2. Risk of bias tool
1. Sequence generation (checking for possible selection bias). Was the allocation sequence adequately generated?

For each included study, we categorized the method used to generate the allocation sequence as:

- low risk (any truly random process e.g. random number table, computer random number generator);
- high risk (any non-random process e.g. odd or even date of birth, hospital or clinic record number); or
- unclear risk.

2. Allocation concealment (checking for possible selection bias). Was allocation adequately concealed?

For each included study, we categorized the method used to conceal the allocation sequence as:

- low risk (e.g. telephone or central randomisation, consecutively numbered sealed opaque envelopes);
- high risk (open random allocation, unsealed or non-opaque envelopes, alternation, date of birth); or
- unclear risk

3. Blinding of participants and personnel (checking for possible performance bias). Was knowledge of the allocated intervention adequately prevented during the study?

For each included study, we categorized the methods used to blind study participants and personnel from knowledge of which intervention a participant received. Blinding was assessed separately for different outcomes or class of outcomes. We categorized the methods as:

- low risk, high risk, or unclear risk for participants; and
- low risk, high risk, or unclear risk for personnel.

4. Blinding of outcome assessment (checking for possible detection bias). Was knowledge of the allocated intervention adequately prevented at the time of outcome assessment?

For each included study, we categorized the methods used to blind outcome assessment. Blinding was assessed separately for different outcomes or class of outcomes. We categorized the methods as:

- low risk for outcome assessors;
- high risk for outcome assessors; or
- unclear risk for outcome assessors.

5. Incomplete outcome data (checking for possible attrition bias through withdrawals, dropouts, protocol deviations). Were incomplete outcome data adequately addressed?

For each included study and for each outcome, we described the completeness of data, including attrition and exclusions from the analysis. We noted whether attrition and exclusions were reported, the numbers included in the analysis at each stage (compared with the total randomised participants), reasons for attrition or exclusion where reported, and whether missing data were balanced across groups, or were related to outcomes. Where sufficient information was reported or supplied by the trial authors, we re-included missing data in the analyses. We categorized the methods as:

- low risk (< 20% missing data);
- high risk (\geq 20% missing data); or
- unclear risk.

6. Selective reporting bias. Are reports of the study free of suggestion of selective outcome reporting?

For each included study, we described how we investigated the possibility of selective outcome reporting bias and what we found. For studies in which study protocols were published in advance, we compared prespecified outcomes versus outcomes eventually reported in the published results. If the study protocol was not published in advance, we contacted study authors to gain access to the study protocol. We assessed the methods as:

- low risk (where it is clear that all of the study's prespecified outcomes and all expected outcomes of interest to the review have been reported);
- high risk (where not all the study's prespecified outcomes have been reported; one or more reported primary outcomes were not prespecified outcomes of interest and are reported incompletely and so cannot be used; study fails to include results of a key outcome that would have been expected to have been reported); or
- unclear risk.

7. Other sources of bias. Was the study apparently free of other problems that could put it at a high risk of bias?

For each included study, we described any important concerns we had about other possible sources of bias (for example, whether there was a potential source of bias related to the specific study design or whether the trial was stopped early due to some data-dependent process). We assessed whether each study was free of other problems that could put it at risk of bias as:

- low risk;
- high risk;
- unclear risk

If needed, we explored the impact of the level of bias through undertaking sensitivity analyses.

WHAT'S NEW

Date	Event	Description
2 February 2018	New search has been performed	<ul style="list-style-type: none"> We updated the search for eligible studies to February 2018. We identified 2 new randomised controlled trials including 123 children and included them in the review (Faerk 2001; Greer 1986). We included body mass index and measures of body composition in the growth parameters of the primary outcome. We also included new secondary outcome measures: long-term measures of cardio-metabolic health (such as insulin resistance, obesity, diabetes and hypertension). These outcomes were aligned with those of the Cochrane Review, Multi-Nutrient Fortification of Human Milk for Preterm Infants (Brown 2016). We incorporated the GRADE approach to assess the quality of evidence.
23 January 2018	New citation required but conclusions have not changed	The main conclusions of the original review remained unchanged. The updated review identified that protein supplementation led to longer hospital stays compared with no supplementation.

HISTORY

Protocol first published: Issue 3, 1997

Review first published: Issue 3, 1999

Date	Event	Description
3 November 2008	Amended	Converted to new review format.
13 December 1999	New citation required and conclusions have changed	Substantive amendment

CONTRIBUTIONS OF AUTHORS

Emma Amissah searched for published, unpublished, and ongoing studies, assessed study eligibility, performed data extraction and 'Risk of bias' assessment of included studies, analysed data, interpreted results of the analysis, and updated the review. She wrote all drafts and addressed comments from co-authors.

Julie Brown assessed study eligibility, performed data extraction and 'Risk of bias' assessment of included studies, assisted in the interpretation of analyses, and provided comments on drafts.

Jane Harding answered queries on trial eligibility, assisted in the interpretation of analyses, and provided comments on all drafts of the review.

All authors read and approved the final version of the review.

DECLARATIONS OF INTEREST

Emma Amissah: None known.

Julie Brown: None known.

Jane Harding: None known.

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External sources

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- National Institute for Health Research, UK.

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DIFFERENCES BETWEEN PROTOCOL AND REVIEW

The original protocol was published in 1997, and was the basis of the last version of this review, written in 1999. We aligned the review outcomes in this update with those of the Cochrane Review, Multi-nutrient fortification of human milk for preterm infants (Brown 2016). We added body mass index and measures of body composition as part of growth parameters of the primary outcome. We also included new secondary outcome measures: long-term measures of cardio-metabolic health (such as insulin resistance, obesity, diabetes, and hypertension) We also added the 'Summary of findings' table and GRADE recommendations, which were not included in the original protocol. We expanded the search terms to find relevant studies for this update; therefore, we ran the search without any date limits to ensure that no eligible studies were missed.

INDEX TERMS

Medical Subject Headings (MeSH)

*Dietary Proteins; *Dietary Supplements; *Infant Nutritional Physiological Phenomena; *Milk, Human; Infant, Premature [*growth & development]; Randomized Controlled Trials as Topic

MeSH check words

Humans; Infant, Newborn