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[Intervention Review]

Branched-chain amino acid supplementation for improving growth and development in term and preterm neonates

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ABSTRACT

Background

Branched-chain amino acids (BCAAs) play a vital role in neonatal nutrition. Optimal BCAA supplementation might improve neonatal nutrient storage, leading to better physical and neurological development and other outcomes.

Objectives

To determine the effect of BCAA supplementation on physical growth and neurological development in term and preterm neonates. We planned to make the following comparisons: parenteral nutrition with and without BCAA supplementation; enteral BCAA supplementation versus no supplementation; and any type of supplementation including enteral, parenteral and both ways versus no supplementation.

To investigate the supplementation effectiveness for different dosages assessed in the eligible trials.

Search methods

We conducted comprehensive searches using Cochrane Neonatal's standard search strategies: Cochrane Central Register of Controlled Trials (CENTRAL 2016, Issue 6), MEDLINE, Embase and CINAHL (up to July 2016). We updated the search with CENTRAL (2019, Issue 8), MEDLINE, Embase and CINAHL (up to August 2019). We also searched clinical trials registries and reference lists of retrieved articles.

Selection criteria

We planned to include individual and cluster-randomised and quasi-randomised controlled trials comparing BCAA supplementation versus placebo or no supplementation in term and preterm neonates. We excluded trials presented only as abstracts and cross-over trials.

Data collection and analysis

Two review authors independently assessed the eligibility of all potential studies identified from the search strategy. We planned to extract data using a pilot-tested standard data extraction form and assess risk of bias of the included studies following the methods described in the *Cochrane Handbook for Systematic Reviews of Interventions*. We planned to analyse treatment effects and report their effect estimates as per dichotomous or continuous data with 95% confidence intervals. We planned to conduct subgroup analysis to investigate heterogeneity, and perform sensitivity analysis where possible. We planned to use fixed-effect meta-analysis to combine data wherever appropriate. We planned to assess evidence quality using the GRADE approach.

Main results

We did not identify any potentially eligible studies that met the inclusion criteria in this review.

Authors' conclusions

We found no trial data to support or refute the idea that BCAA supplementation affects physical and neurological development and other outcomes in term and preterm neonates.

PLAIN LANGUAGE SUMMARY

Branched-chain amino acid supplementation for improving nutrition in term and preterm neonates

Review question

Does supplementation of branched-chain amino acids (BCAAs) improve physical and neurological development and other health outcomes in term and preterm neonates?

Background

Although leucine, isoleucine and valine—a group of essential amino acids—play an important role in neonatal nutrition, the optimal dosages are still unknown. Neonates usually consume BCAAs from breast milk and artificial formula, and those treated in hospitals due to various problems, including premature birth, asphyxia (lack of oxygen) and infections, may take them in from infusion solutions. Suboptimal intake of BCAAs can be caused by poor sucking and by inappropriate infusion therapy as well, which might lead to poor growth and neurological impairment. Thus, we attempted to reveal whether BCAA supplementation can improve health outcomes in term and preterm neonates.

Study characteristics

We did not find any eligible randomised controlled trials that assessed the effect of BCAA supplementation for term and preterm neonates. The evidence is up to date as of August 2019.

Key results and conclusions

This review is unable to suggest the effect of BCAA supplementation on physical growth and neurological development in term and preterm neonates. Future studies are required as this assessment is very important in the neonatal field.

BACKGROUND

Description of the condition

Three branched-chain amino acids (BCAAs)—leucine, isoleucine and valine—are part of a group of essential amino acids (EAAs) that play a pivotal role in neonatal nutrition (De Groof 2011; Kashyap 2011; Suryawan 2011), and a range of key body functions (Ferrier 2013; Harper 1984). A significant amount of essential proteins for the human body comes from BCAAs, including 40% of total amino acids and approximately 35% of muscle protein (Harper 1984). Leucine in particular is of immense importance because it builds body protein and stimulates protein synthesis in the skeletal muscles (Fujita 2007; Suryawan 2011). During the neonatal period, leucine can promote protein synthesis and contribute to increasing skeletal muscle volume in animals (Escobar 2005; Suryawan 2011). It is associated with metabolism of glucose and stimulates insulin secretion (Layman 2006; Matthews 1997). Isoleucine might stimulate the innate immune system in the intestine, and has been shown to protect infants from various harmful microbes (Alam 2011). Additionally, BCAAs play an indispensable role in the brain by constantly nourishing neurons with glutamate (Fernstrom 2005), which is an important excitatory neurotransmitter (Yudkoff 2005). Furthermore, BCAAs act as indirect modulators to stimulate the synthesis of aromatic amino-acid-based neurotransmitters like serotonin, dopamine and norepinephrine (Fernstrom 2005).

As the human body is unable to synthesise these amino acids, EAAs should be consumed directly from food sources, such as egg yolk, soy bean and maize (WHO 2007). Approximately 20% of amino acids in human breast milk are BCAAs, and are important for optimal neonatal health (Zhang 2013). The appropriate BCAA intake for neonates is unclear, however, and remains a very challenging area of study (De Groof 2011). In term infants, insufficient supply of BCAAs from breast milk or formula milk may be caused by poor sucking ability due to perinatal asphyxia, perinatal infection or the relatively small body weight at birth. Even in healthy term neonates, BCAAs from breast milk or formula milk may be inadequate during the first few days of life as they are only able to suck a small amount of milk from the mother's breast (Neville 1988). Similarly, preterm neonates are at higher risk of nutrition deficits due to insufficient nutrient reserves at birth, delayed introduction of enteral milk feeding, and increased energy expenditure based on various complications, including respiratory distress syndrome, patent ductus arteriosus, and infections (Shah 2009). Preterm neonates therefore warrant close investigation as a crucial subgroup. There is, moreover, a paucity of data on the short-term and long-term effect of BCAA supplementation for improving clinical outcomes in human neonates.

Description of the intervention

Immediately after birth, administration of breast milk or formula milk is required to ensure an optimal supply of necessary nutrients in newborns. Based on the amino acid constituents of human breast milk, leucine, isoleucine and valine intake requirements for term neonates are defined as 165 mg/kg/day, 95 mg/kg/day and 95 mg/kg/day, respectively (WHO 2007; Zhang 2013). Estimates of amino acid intake requirements in term neonates based on a method involving stable isotopes led to optimum amounts of leucine, isoleucine and valine at 140 mg/kg/day, 105 mg/kg/day and 110 mg/kg/day, respectively (De Groof 2011), which differ from the dosage mentioned above. These recommendations are still

insufficient to ensure newborns optimal dosages of BCAAs because breast milk nutrients vary among mothers, depending on the maternal nutritional status, duration of lactation, and geographical locations (Neville 1988; Wurtman 1979; Zhang 2013). For example, some amino acids such as isoleucine are significantly higher in Asia, whereas in North America mothers' breast milk is rich in glutamate (Zhang 2013). Besides, for neonates with chronic disease or a critical condition, enteral feeding requirements may differ (Mager 2006). BCAAs are therefore distinct from other amino acids in that they play a wide variety of key roles in body function: as a result, BCAAs may be added to daily nutrients of neonates who are at risk of BCAA deficits. Nevertheless, as multiple nutrients are required for a balanced nutrition state and body function, past studies did not just assess BCAA supplementation in neonates but a combination of other nutrients. Cochrane Neonatal suggested that compared to no supplementation, protein-supplemented breast milk administered enterally resulted in statistically significant increases in short-term outcomes such as weight gain, linear growth and head growth in relatively well preterm infants (Amissah 2018). Higher amino acid administration in preterm infants was found to be associated with improved postnatal growth, a reduction in hyperglycaemia, and an increased risk of abnormal blood urea nitrogen (Osborn 2018).

How the intervention might work

Administration of BCAA supplementation can be performed both parenterally and enterally. The foremost choice, however, is enteral feeding with breast milk whenever possible, otherwise parenteral feeding should be sought. Parenteral administration of necessary nutrients to very preterm neonates (< 32 weeks of gestation) during the initial postnatal period is practised widely in most developed countries (Embleton 2014). In particular, this method is considered a relatively safe way to ensure nutritional intake, especially for neonates with enteral feeding intolerance and necrotising enterocolitis showing gastrointestinal malfunction (Koletzko 2008).

Enteral administration of additional BCAAs to all neonates may provide benefits to decrease associated negative health outcomes especially in those with inadequate intake of recommended dosages. Although evidence of BCAA supplementation effect in human neonates is very limited, the possible beneficial and adverse effects have been suggested in other populations. For example, a systematic review suggested that BCAA supplementation (range 5.5 g/day to 30 g/day) might have a positive effect on the improvement of muscle strength, ascites and oedema in adults with chronic liver disease (Ooi 2018). In contrast, limited evidence showed potential favourable effects of BCAA supplementation on body weight and serum albumin level in paediatric patients with liver dysfunction (Ooi 2018). Another systematic review showed that leucine administration (range 1.2 g/day to 6 g/day) increased lean muscle-mass content in sarcopaenic elderly individuals (Martinez-Arnau 2019).

Conversely, excessive BCAA intake may lead to short-term and long-term adverse outcomes. For instance, excess ingestion of BCAA can be associated with nausea and fatigue in manic patients (Scarna 2003). Previous research suggests that high plasma concentrations of BCAAs could possibly develop from formula feeding and that might have a negative effect on neonates' carbohydrate and insulin metabolism (De Groof 2011; Jarvenpaa 1982). Interestingly, one meta-analysis found that higher total BCAA intake could increase the risk of type 2 diabetes (odds ratio (OR) 1.32, 95% confidence

interval (CI) 1.14 to 1.53), and could decrease obesity risk (OR 0.62, 95% CI 0.47 to 0.82) in adults (Okeunle 2019). Since diabetes and obesity are associated with increased probability of developing cardiovascular diseases in the long term, including ischaemic heart disease and stroke in adulthood, which are the two leading causes of death worldwide (WHO 2014), it is of utmost importance to examine the long-term effects of BCAA supplementation on the progression of non-communicable diseases in newborns. Such risk may even be higher for formula-fed neonates, who have a higher likelihood of becoming overweight or obese (Koletzko 2009).

Why it is important to do this review

As BCAAs play an indispensable role in key body functions and growth, it is essential to gain a comprehensive understanding of the effect of BCAA supplementation in neonates including optimal dosages, duration of administration, and short- and long-term clinical outcomes. Existing Cochrane Reviews have assessed the impact of additional amino acids on neonatal outcomes, that is glutamine supplementation on preterm neonates' morbidity and mortality (Moe-Byrne 2016), as well as the effect of taurine supplementation on the growth and development of preterm or low birth weight (LBW) infants (Verner 2007). However, there is currently no synthesised evidence of the effectiveness of BCAA supplementation on positive or adverse outcomes for either term or preterm neonates. Although various nutrients are necessary for neonatal growth and development, BCAAs have distinctive importance considering their diverse body mechanisms beyond just nutritional metabolism (Nie 2018). Further research was therefore required to address this important literature gap in the field of neonatal health.

OBJECTIVES

To determine the effect of BCAA supplementation on physical growth and neurological development in term and preterm neonates. We planned to make the following comparisons: parenteral nutrition with and without BCAA supplementation; enteral BCAA supplementation versus no supplementation; and any type of supplementation including enteral, parenteral and both ways versus no supplementation.

To investigate the supplementation effectiveness for different dosages assessed in the eligible trials.

METHODS

Criteria for considering studies for this review

Types of studies

We considered all randomised (individual and cluster) or quasi-RCTs investigating BCAA supplementation effect in term and preterm neonates. We excluded trials presented only as abstracts and cross-over trials.

Types of participants

We aimed to include all preterm and term neonates randomised within 28 days after birth.

Types of interventions

Supplementation of any BCAAs (i.e. leucine, isoleucine or valine) versus placebo or no supplementation administered to the

neonates via enteral or parenteral route. We considered any dosages for inclusion. We considered comparisons of BCAAs to no BCAAs and additional BCAAs to basal BCAA levels contained in amino acid formulations and formula milk. We restricted neither the type of milk (i.e. breast milk, formula milk and both) nor the duration of the supplementation. Since the availability of BCAA supplementations' trial data from neonates are scarce, we also considered trials reporting biochemical changes observed following a single dose for any number of given days.

Types of outcome measures

We aimed to include all identified eligible trials regardless of their assessed outcome measures.

Primary outcomes

1. Physical development
 - a. Weight gain during the 28 days after birth (grams)
 - b. Increase in height during the 28 days after birth (centimetres)
 - c. Increase in head circumference during the 28 days after birth (centimetres)
2. Neurological development
 - a. Major neurodevelopmental disability after 18 months' post-term age
 - i. Cerebral palsy (yes/no)
 - ii. Developmental delay (more than two standard deviations (SD) below the mean in a validated mental development test) or intellectual impairment (more than two SD below the mean in a validated intelligence test) (yes/no)
 - iii. Blindness (vision < 6/60 in both eyes) (yes/no)
 - iv. Sensorineural deafness (requiring amplification) (yes/no)

We planned to analyse children aged 12 to 24 months and 25 months to 5 years separately.

Secondary outcomes

1. All-cause mortality within the first year of life
2. Systemic infection assessed during the trial period diagnosed by positive blood culture or clinical diagnosis by an attending physician (yes/no)
3. Feeding tolerance assessed by the number of days since birth until full establishment of enteral feeding, e.g. at least 150 mL/kg/day, independent of parenteral fluids or nutrition (days)
4. Cognitive and educational outcomes at five years or more diagnosed by a physician, e.g. autism spectrum disorder, attention deficit/hyperactivity disorder, learning disorder (yes/no)
5. Adverse events during the trial period, e.g. nausea, vomiting and fatigue reported by healthcare staff and abnormal blood tests such as urea nitrogen (yes/no). We planned to report other adverse outcomes if reported in individual trials

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 specialised register).

Electronic searches

We conducted comprehensive searches in two different time points and merged the results. We pulled the first search from the Cochrane Central Register of Controlled Trials (CENTRAL 2016, Issue 6) in the Cochrane Library, MEDLINE via PubMed, EMBASE and CINAHL, from the inception to 14 July 2016. The second search included CENTRAL (2019, Issue 8), OVID Medline Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) (1946 to 2 August 2019), MEDLINE via PubMed (to 2 August 2019) for the previous year and CINAHL (1981 to 2 August 2019). We have included the search strategies for each database in [Appendix 1](#). We did not apply language restrictions.

We also searched clinical trial registries for ongoing or recently completed trials. We searched the World Health Organization's International Clinical Trials Registry Platform (ICTRP) (www.who.int/ictpr/search/en), and the US National Library of Medicine's ClinicalTrials.gov, via Cochrane CENTRAL. Additionally, we searched the [ISRCTN Registry](#) for any unique trials that might not have been retrieved by the Cochrane CENTRAL database search.

Searching other resources

We also searched the reference lists of potentially eligible articles retrieved for this review.

Data collection and analysis

We used the standardised method of Cochrane and the Cochrane Neonatal Review Group (www.neonatal.cochrane.org/resources-review-authors).

Selection of studies

Two review authors, Shoichiro Amari (SA) and Fumihiko Namba (FN), independently assessed the eligibility of all potential studies that we identified as a result of the search strategies. We resolved any disagreement through discussion or, if required, consultation with a third author—Sadequa Shahrook (SS), Erika Ota (EO) or Rintaro Mori (RM).

Data extraction and management

We planned to use a pilot-tested standardised form for data extraction designed by the Cochrane Effective Practice and Organisation of Care Group. When we identify eligible studies in future updates of this review, two review authors will extract data using this form. We will resolve disagreements through discussion or, if required, consultation with a third person (EO or RM). We will enter data into Review Manager 5 software to check for data authenticity ([Review Manager 2014](#)). When information regarding any of the above is unclear, we will attempt to contact authors of the original reports to ask them to provide further details. Moreover, owing to the paucity of available trial data in this area, we will consider including abstracts from eligible trial design for our future review update.

Assessment of risk of bias in included studies

Two review authors (SA and FN) planned to independently assess the risk of bias (low, high, or unclear) of the included trials using the following domain criteria ([Higgins 2011](#)).

1. Sequence generation (selection bias)

2. Allocation concealment (selection bias)
3. Blinding of participants and personnel (performance bias)
4. Blinding of outcome assessment (detection bias)
5. Incomplete outcome data (attrition bias)
6. Selective reporting (reporting bias)
7. Any other bias

We planned to resolve any disagreements by discussion or by involving a third assessor. See [Appendix 2](#) for a detailed risk of bias description for each of the domains.

Measures of treatment effect

Dichotomous data

For dichotomous data, we planned to report risk ratio (RR) and risk difference (RD) with 95% CIs. If the RD was statistically significant (P value < 0.05), we planned to use the number needed to treat for an additional beneficial outcome (NNTB) and the number needed to treat for an additional harmful outcome (NNTH).

Continuous data

For continuous data, we planned to report the mean difference (MD) with 95% CIs.

Unit of analysis issues

Cluster-randomised trials

We planned to include cluster-randomised trials in the analyses along with individually randomised trials. To take into account the design effect, we planned to adjust sample sizes using the methods described in the *Cochrane Handbook for Systematic Reviews of Interventions* ([Higgins 2011](#)); and an estimate of the intracluster correlation coefficient (ICC) derived from the trial, if possible, or from a similar trial or from a study of a similar population. Had the ICCs been used from other sources, we would have reported it and would have conducted sensitivity analyses to investigate the variation effect in the ICCs. If we could identify both cluster-randomised trials and individually randomised trials, we would have synthesised the most relevant information from each. We planned to combine the study results if there had been a little heterogeneity between their designs, and if we considered the interaction between the intervention effect and the randomisation unit to be unlikely. We would have acknowledged heterogeneity in the randomisation unit prior to performing a sensitivity analysis for investigating the effects of the randomisation unit.

Trials with more than two treatment groups

If we had identified trials with more than two intervention groups (multi-arm studies), we planned to include only directly relevant arms. If we had identified studies with various relevant arms, we would have combined groups to generate a single pair-wise comparison ([Higgins 2011](#)), and we would have included the disaggregated data in the corresponding subgroup category. If the control group was shared by two or more study arms, we would have divided the control group over the number of relevant subgroup categories to avoid double counting the participants. For dichotomous data, we planned to divide the events and the total population; and for continuous data, we planned to assume the same mean and standard deviation but to divide the total population. We aimed to provide the detailed approach in the 'Characteristics of included studies' tables.

Dealing with missing data

We planned to note levels of attrition if we found any studies eligible for inclusion. We planned to explore the impact of including studies with high levels of missing data in the overall assessment of treatment effect by using sensitivity analysis.

For all outcomes, we planned to carry out analyses, as far as possible, on an intention-to-treat basis: that is, we would have attempted to include all participants randomised to each group in the analyses, and we would have analysed all participants in the group to which they had been allocated, regardless of whether or not they had received the allocated intervention. The denominator for each outcome in each trial would have been the number randomised minus any participants whose outcomes were known to be missing.

Assessment of heterogeneity

We planned to assess statistical heterogeneity in each meta-analysis using the I^2 and χ^2 statistics. We planned to interpret I^2 statistics as follows.

1. Less than 25% — no heterogeneity
2. 25% to 49% — low heterogeneity
3. 50% to 74% — moderate heterogeneity
4. 75% and above — high heterogeneity

We planned to consider the meta-analysis inappropriate when I^2 was 75% or more (high heterogeneity). In addition, we planned to employ the χ^2 test of homogeneity. We planned to explore clinical variation across studies by comparing the distribution of important participant factors among trials and trial factors (randomisation concealment, blinding of outcome assessment, lacking follow-up, treatment type and co-interventions). We planned to interpret heterogeneity as present when the P value was less than 0.1.

Assessment of reporting biases

If there were 10 or more studies included in the meta-analysis, we would have investigated reporting biases (such as publication bias) by visually examining the degree of asymmetry in funnel plots. If asymmetry was suggested by a visual assessment, we would have performed exploratory analyses.

Data synthesis

We planned to carry out statistical analysis using the Review Manager 5 software (Review Manager 2014). We planned to use the standard methods of the Cochrane Neonatal Review Group to synthesise data using RRs, RDs, NNTB/NNTH, MDs, standardised MDs and 95% CIs. We planned to use fixed-effect meta-analysis to combine the included trial data.

Certainty of evidence

We planned to use the GRADE approach, as outlined in the GRADE Handbook (Schünemann 2013), to assess the certainty of evidence of the following (clinically relevant) outcomes: physical development including weight gain; increase in height and head circumference; neurological development; all-cause mortality; and systemic infection.

Two review authors (SA and FN) planned to independently assess the certainty of the evidence for each of the above outcomes. We

planned to embrace evidence from RCTs as high certainty but to downgrade 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 planned to use the GRADEpro GDT Guideline Development Tool to create nine 'Summary of findings' tables to report the certainty of the evidence.

The GRADE approach results in an assessment of the certainty of a body of evidence as one of four grades.

1. High certainty: further research is very unlikely to change our confidence in the estimate of effect.
2. Moderate certainty: further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
3. Low certainty: further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
4. Very low certainty: we are very uncertain about the estimate.

Subgroup analysis and investigation of heterogeneity

If we identified substantial heterogeneity in meta-analyses ($I^2 > 50\%$), we would have investigated it using subgroup analyses and sensitivity analyses. We planned to consider whether an overall summary was meaningful; and if it was, to use random-effects analysis.

We planned to carry out the following subgroup analyses on the primary outcomes, wherever possible.

1. Term neonates (37 weeks' gestation or more) versus preterm neonates (36 weeks' gestation or less)
2. Exclusively breast-fed neonates versus mostly breast-fed neonates versus mostly formula-fed neonates and inconsistently lactated neonates versus exclusively formula-fed neonates
3. Body weight at birth, e.g. less than 2500 grams versus 2500 grams and above
4. Male versus female neonates
5. Developed versus developing country settings: we will consider high-income and upper-middle-income economies classified by the World Bank as developed and lower-middle-income and low-income economies as developing (World Bank 2015).

We would have assessed subgroup differences using interaction tests available within Review Manager software (Review Manager 2014). We planned to report the results of subgroup analyses quoting the χ^2 statistics and P values and the interaction test I^2 values.

Sensitivity analysis

We planned sensitivity analyses to determine whether findings were affected (and heterogeneity reduced) by including only studies at low overall risk of bias, defined as adequate randomisation and allocation concealment, masking of intervention and measurement, and less than 10% loss to follow-up for outcome assessment.

RESULTS

Description of studies

Results of the search

The literature search conducted in July 2016 and August 2019 identified 643 references after removing duplicates. After screening we assessed 76 studies. (See [Figure 1](#)).

Figure 1. Study flow diagram.

Included studies

We identified no trials that matched our inclusion criteria.

Excluded studies

We excluded 76 studies that included one duplicate title (Socha 2011). We found five titles presented as abstracts and none met our review inclusion criteria for eligible study design (Coloso 1997; De Groof 2011; Protheroe 1995; Rigo 1985; Sáenz 2001), two of which were reviewed by two review authors (SA and FN) based on the corresponding full-text report (De Groof 2011; Sáenz 2001). Seventeen studies from 19 reports did not apply randomised or quasi-randomised trial design (Antony 1967; Berry 1982; Chin 1990; De Boo 2005; De Groof 2011; Helms 1987; Mager 2006; NCT01304394; NCT01813526; NCT01820494; NCT02536482; NL1539; Poindexter 1997; Rigo 1985; Sáenz 2001; Schober 1989; van Toledo-Eppinga 1996). We identified 11 studies as cross-over trials (Chin 1992; Denne 1994; Kadrofske 2006; NCT00196482; NCT01062815; NCT01569776; NCT02414243; NL3889; Parimi 2005; Protheroe 1995; Verbruggen 2011). Three studies were narrative reviews (Chuang 2006; Haschke 2016; Koletzko 2013); while one was a case report (Sperl 1994). The remaining 44 studies did not compare BCAA supplementation against placebo or no supplementation. Nine of the 44 studies did not assess dose differences in BCAA intake (Darmaun 1997; Liet 1999; NCT00005775; NCT00005889; NCT00254176; NCT01470768; NCT01599286; NCT02719405; Vlaardingerbroek 2014). Although 35 studies assessed different BCAA dosages administered to varying study arms, we did not include these studies because of their group variation with other nutrients including amino acids and lipids. Parenteral administration of amino acids was the focus in 15 out of 35 studies (Adamkin 1995; Battista 1996; Camelo 1995; ChiCTR-IPR-15006106; Coloso 1997; Lai 1999; Maldonado 1988; NCT00120926; NCT01062724; NCT01860573; Rivera 1993; Thureen 2003; van den Akker 2006; van den Akker 2007; Van Goudoever 1995), of which 13 administered commercially available amino acid solutions (Adamkin 1995; Battista 1996; Camelo 1995; Lai 1999; Maldonado 1988; NCT00120926; NCT01062724; NCT01860573; Rivera 1993; Thureen 2003; van den Akker 2006; van den Akker 2007; Van Goudoever 1995). Enteral feeding route was applied in 20 studies (Fleddermann 2015; Geukers 2015; Giovannini 1994; Hagelberg 1990; Hanning 1992; Kirchberg 2015; Lonnerdal 1990; Lonnerdal 2016; Manary 2004; NCT00664768; NCT01109966; NCT01583673; NCT01699386; NCT01940068; NCT02410057; NCT02500563; NL4677; Sáenz 2003; Socha 2011; Yogman 1982); and commercially available formulas were examined in eight studies (Giovannini 1994; Lonnerdal 2016; NCT00664768; NCT01109966; NCT01583673; NCT01699386; NCT01940068; Sáenz 2003).

We identified a comparative study assessing BCAA supplementation but without any changes in its nutrition composition except for the BCAAs (Berry 1982). Valine, isoleucine and leucine in crystalline form were orally administered to participants aged 11 days to 22 years with hyperphenylalaninaemia, either with a free natural protein diet or with a low-phenylalanine diet. We excluded this intervention as it did not satisfy our review study design criteria.

With regard to the reported outcomes in the excluded interventions in this review, anthropometric indices were the end points in 11 studies (ChiCTR-IPR-15006106; Giovannini 1994;

Hagelberg 1990; Hanning 1992; Lonnerdal 2016; NCT00664768; NCT01583673; NCT01699386; NCT01860573; NL4677; Socha 2011); and neurological development was measured in only one study identified on the Chinese clinical trial registry (ChiCTR-IPR-15006106). The rest of the identified studies primarily reported biochemical components of amino acids using neonatal blood and urine samples.

Risk of bias in included studies

We were not able to assess the risk of bias of the studies, as we identified no trials that met the inclusion criteria of our review.

Effects of interventions

We were not able to measure the effects of BCAA supplementation as we found no eligible trials to include in this review.

DISCUSSION

Summary of main results

We found no randomised or quasi-randomised trials assessing the effect of BCAA supplementation versus placebo or no supplementation administered to neonates. As a result, we are unable to suggest whether administration of BCAA supplementation provides benefits for neonatal physical growth and neurological development.

One probable reason for this data paucity could originate from researchers' interests primarily directed either to protein or total amino acids rather than a specific group of amino acids such as BCAAs.

Secondly, as multiple nutrients are required for optimal nutrition, it is probable that past studies refrained from administering only BCAAs as that could have done more harm than benefit to the newborns.

Thirdly, the availability of the nutritional compounds used in the eligible studies or their presence in the market might also have an impact. We identified studies using commercial amino acid solutions and neonatal formula as intervention components (Adamkin 1995; Battista 1996; Camelo 1995; Giovannini 1994; Lai 1999; Lonnerdal 2016; Maldonado 1988; NCT00120926; NCT00664768; NCT01062724; NCT01109966; NCT01583673; NCT01699386; NCT01860573; NCT01940068; Rivera 1993; Sáenz 2003; Thureen 2003; van den Akker 2006; van den Akker 2007; Van Goudoever 1995). The authors of such intervention studies attempted to capture the effect difference in either total amount of the amino acids or composite amino acid mixtures. The nutrient mix in such compounds given through the enteral route typically includes carbohydrates, lipids and amino acids, which differed by study arms (Giovannini 1994; Lonnerdal 2016; NCT00664768; NCT01109966; NCT01583673; NCT01699386; NCT01940068; Sáenz 2003). Therefore, we did not consider them eligible to provide evidence for the effect of BCAA supplementation in our review (please see the [Excluded studies](#) list). This review finding suggests the need for future studies to fill the knowledge gap which Nie 2018 highlighted: the authors suggested that BCAAs and their derivatives might act as potential biomarkers of cardiovascular diseases, type 2 diabetes mellitus, cancer and other non-communicable diseases. Adequate intake of BCAAs during infancy may therefore avert the

progression of these diseases and, as a result, allow greater health benefits in the long-term.

Overall completeness and applicability of evidence

We identified no eligible studies for inclusion.

Quality of the evidence

We identified no eligible studies for inclusion.

Potential biases in the review process

We implemented all possible countermeasures to overcome potential biases in the review process. Two review authors independently assessed all titles identified from the combined searches including the full texts of the final eligible study list. We screened the reference lists of the full-text papers. However, we cannot rule out the possibility that our searches, albeit comprehensive, failed to isolate some potentially eligible trials.

Agreements and disagreements with other studies or reviews

We did not find any trial data to support, refute or remain neutral on the effect of BCAA supplementation in neonates with comparison to other available studies.

AUTHORS' CONCLUSIONS

Implications for practice

Our review is unable to suggest the benefits or harms of BCAA supplementation on neonatal physical and neurological development and other important outcomes.

Implications for research

BCAAs play a critical role in newborns, and they can be found in breast milk and commercially available nutritional products. While newborns who are adequately fed are usually not prone to BCAA deficiency, the optimal doses for healthy full-term, sick, and preterm infants have yet to be determined. Further studies of BCAA-deficient groups (e.g. sick and preterm infants) to determine the optimal dose, based on further pharmacokinetic or observational studies (or both), may be considered. Because commercially available nutritional formulations typically contain other amino acids and nutrients, it may be difficult to estimate the effects of BCAAs solely. It is therefore preferable to perform comparative studies using pure BCAA formulations in addition to standardised nutrition, if possible. Although short-term weight gain and biochemical changes are often adopted as the study outcomes, it will be desirable to also assess growth, development, and other physical changes over a period of at least one year.

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CHARACTERISTICS OF STUDIES

Characteristics of excluded studies [ordered by study ID]

Study	Reason for exclusion
Adamkin 1995	This study was an RCT, but it compared two types of amino acid solution that differed not only in BCAAs but also in other amino acids.
Antony 1967	This was a case study of 18 infants and children with hypoglycemia.
Battista 1996	This study was an RCT, but it compared two types of amino acid solution that differed not only in BCAAs but also in other amino acids.
Berry 1982	This was a case study of 11 patients with phenylketonuria, who received oral BCAA supplementation.
Camelo 1995	This study was an RCT, but it compared two types of parenteral nutrition solution that differed not only in amino acids but also in glucose, lipids and electrolytes.
ChiCTR-IPR-15006106	This study was an RCT, but it compared two doses of amino acid solution that differed not only in BCAAs but also in other amino acids. Although this study was planned to be completed in 2016, the actual completion date was not provided.
Chin 1990	This was a case study of 28 children with chronic end-stage liver disease.
Chin 1992	This study was a crossover trial comparing a BCAA-enriched formula to a standard formula. There was a difference in not only BCAAs but also aromatic amino acids between the two groups.
Chuang 2006	This article was a narrative review of genetic disorders of BCAA metabolism.
Coloso 1997	This study was an RCT, but it compared two types of parenteral nutrition that differed in the percentages of BCAAs with the same amount of total amino acids with nutrition without amino acids, resulting in different levels of not only BCAAs but also other amino acids.
Darmaun 1997	This study was an RCT, but it compared two groups with or without glutamine supplementation.
De Boo 2005	This study analysed protein kinetics in 10 preterm infants fed with fortified breast milk or commercially available preterm formula with the use of non-randomised allocation.
De Groof 2011	This study was an experimental study which quantified the requirements of BCAAs for term neonates using the indicator amino acid oxidation method without a control group.
Denne 1994	This study was a crossover trial comparing enteral nutrition to parenteral nutrition.
Fleddermann 2015	This study was an RCT, but it compared the effect of a protein-reduced infant formula to an isocaloric standard formula.
Geukers 2015	This study was an RCT, but it compared two regimens of enteral nutrition that differed not only in proteins but also in carbohydrates and fats.

Study	Reason for exclusion
Giovannini 1994	This study was an RCT, but it compared four types of formula that differed not only in proteins but also in carbohydrates and fats.
Hagelberg 1990	This study was an RCT, but it compared a human milk fortifier and a cow's milk fortifier that differed not only in amino acid composition but also in fats and electrolytes.
Hanning 1992	This study was an RCT, but it compared two types of formula that differed not only in BCAAs but also in other amino acids.
Haschke 2016	This article was a narrative review of metabolic programming in newborn infants.
Helms 1987	This study compared two types of amino acid solutions with the use of historical control groups.
Kadrofske 2006	This study was a crossover trial comparing two doses of amino acid solution.
Kirchberg 2015	This study was an RCT, but it compared two doses of amino acid solution that differed not only in BCAAs but also in other amino acids.
Koletzko 2013	This study was a narrative review of the nutritional effects on postnatal growth.
Lai 1999	This study was an RCT, but it compared two types of amino acid solution that differed not only in BCAAs but also in other amino acids.
Liet 1999	This study was an RCT, but it compared a mixed lipid emulsion of medium-chain triacylglycerols and long-chain triacylglycerols (LCT) to a pure LCT emulsion.
Lonnerdal 1990	This study was an RCT, but it compared five different types of formula that differed in protein levels and the ratio of whey to casein.
Lonnerdal 2016	This study was an RCT, but it compared three different doses of osteopontin supplementation.
Mager 2006	This study was an experimental study which measured BCAA needs in five children with mild-to-moderate chronic liver cholestasis using the indicator amino acid oxidation method.
Maldonado 1988	This study was an RCT, but it compared two types of amino acid solution that differed not only in BCAAs but also in other amino acids.
Manary 2004	This study was an RCT, but the participants of this study were aged 12 months or more. This study aimed at assessing the effect of different components and amounts of proteins.
NCT00005775	This study was an RCT, but it compared the effect of glutamine supplementation to placebo. This study was completed in 2001.
NCT00005889	This study consisted of five patterns of controlled trial: Pattern I: standard total parenteral nutrition versus glucose alone; Pattern II: an amino acid solution versus a lipid emulsion; Pattern III: two different dosages of glycerol; Pattern IV: glutamine versus alanine; and Pattern V: glucagon plus glucose versus glucagon plus an amino acid solution and a lipid emulsion. Although this study started in 1999, the completion date was not provided.
NCT00120926	This study was an RCT, but it compared two different regimens of an amino acid solution that differed not only in BCAAs but also in other amino acids. This study was completed in 2006.
NCT00196482	This study was a crossover trial comparing two types of human milk fortifier. This study was completed in 2006.

Study	Reason for exclusion
NCT00254176	This study was an RCT, but it compared the effect of cysteine supplementation to that of the placebo. Although the estimated study completion was in 2011, the actual completion date was not provided.
NCT00664768	This study was an RCT, but it assessed the effect of synbiotics in infants with cow's milk allergy. This study was completed in 2012.
NCT01062724	This study was an RCT, but it compared two types of amino acid solution that differed not only in BCAAs but also in other amino acids. This study was completed in 2016.
NCT01062815	This study was a crossover trial comparing cyclic parenteral nutrition to continuous parenteral nutrition. This study was completed in 2010.
NCT01109966	This study was an RCT, but it compared two types of amino acid formula that differed not only in BCAAs but also in other amino acids. This study was completed in 2012.
NCT01304394	This study was an interventional study assessing the safety of a formulation for parenteral nutrition with the use of single group assignment. This study was completed in 2009.
NCT01470768	This study was an RCT, but it compared two doses of docosahexanoic acids and arachidonic acids. Although the estimated study completion was in 2013, the actual completion date was not provided.
NCT01569776	This study was a crossover trial assessing the effect of a new amino acid-based formula in infants at the age of 2 months or more compared to a commercially available hypoallergenic formula. This study was completed in 2013.
NCT01583673	This study was an RCT, but it compared a new amino acid formula to a commercially available amino acid formula. This study was completed in 2013.
NCT01599286	This study was an RCT, but compared the effect of supplementation of N-carbamoyl-L-glutamic acid to placebo. Although the estimated study completion was in 2020, the actual study completion date was not provided.
NCT01699386	This study was an RCT, but it compared two types of formula that differed not only in BCAAs but also in other amino acids. This study was completed in 2000.
NCT01813526	This study was an interventional study with the use of single group assignment evaluating infants with protein sensitive colitis fed with a nutritionally complete free amino acid-based medical food. This study was completed in 2001.
NCT01820494	This study was an interventional study with the use of single group assignment which assessed the growth of infants fed an experimental formula for the nutritional management of chronic diarrhea. This study was completed in 2002.
NCT01860573	This study was an RCT, but it compared two different regimens of an amino acid solution that differed not only in BCAAs but also in other amino acids. This study was completed in 2016.
NCT01940068	This study was an RCT, but it compared two types of formula that differed not only in BCAAs but also in other amino acids. This study was completed in 2013.
NCT02410057	This study was an RCT, but it compared formulas that differed in alpha-lactalbumin, not in BCAAs. The completion of this study was estimated to be in 2024.
NCT02414243	This study was a crossover trial comparing a new amino acid formula to a commercially available amino acid formula. This study was completed in 2016.

Study	Reason for exclusion
NCT02500563	This study was an RCT, but it compared the effect of an amino acid-based formula compared to an extensively hydrolysed casein infant formula. This study was completed in 2017.
NCT02536482	This study was an interventional study with the use of single group assignment which assessed tolerability to a new amino acid-based formula in children with cow's milk protein allergy. This study was completed in 2015.
NCT02719405	This study was an RCT, but it compared three different types of enteral nutrition that differed not only in BCAAs but also in other amino acids: amino acid formula, extensively hydrolysed casein formula (EHCF) and EHCF with lactobacillus. This study was completed in 2018.
NL1539	This study was an interventional study with a single group which assessed the requirement of nine essential amino acids in preterm and term neonates. This study was completed in 2014.
NL3889	This study was a crossover trial comparing a new hydrolysed formula to amino acid-based formula. This study was completed in 2016.
NL4677	This study was an RCT, but it compared a modified low protein formula to a standard formula that differed not only in BCAAs but also in other amino acids. This study was completed in 2017.
Parimi 2005	This study was a crossover trial comparing three different regimens of amino acid solution that differed not only in BCAAs but also in other amino acids.
Poindexter 1997	This study was a case study of comparing nutritional metabolism in 3-day-old and 3-week-old infants.
Protheroe 1995	This study was a crossover trial assessing the effect of BCAA-enriched feeding in infants with compensated liver diseases.
Rigo 1985	This was a case study of low birth weight infants comparing three types of amino acid solution with different phenylalanine/leucine ratios.
Rivera 1993	This study was an RCT, but it compared glucose plus amino acids to glucose alone, resulting to a difference not only in BCAAs but also in other amino acids.
Schober 1989	This study was a case study of 20 postoperative neonates assessing the effect of two different regimens of total parenteral nutrition.
Socha 2011	This study was an RCT, but it compared two doses of amino acid formula that differed not only in BCAAs but also in other amino acids.
Sperl 1994	This article was a report of two patients with disorders of BCAA metabolism.
Sáenz 2001	This study was a case study of 37 infants who received two types of amino acid solution that differed not only in BCAAs but also in other amino acids.
Sáenz 2003	This study was an RCT, but it compared parenteral nutrition with enteral feeding of breast milk or formula to only parenteral nutrition.
Thureen 2003	This study was an RCT, but it compared two doses of an amino acid solution that differed not only in BCAAs but also in other amino acids.
van den Akker 2006	This study was an RCT, but it compared glucose plus amino acids to glucose alone that differed not only in BCAAs but also in other amino acids.

Study	Reason for exclusion
van den Akker 2007	This study was an RCT, but it compared glucose plus amino acids to glucose alone that differed not only in BCAAs but also in other amino acids.
Van Goudoever 1995	This study was an RCT, but it compared glucose plus amino acids to glucose alone that differed not only in BCAAs but also in other amino acids.
van Toledo-Eppinga 1996	This study was a case study of 30 infants who received either of three different regimens of nutrition including premature formula, fortified human milk and parenteral nutrition that differed not only in BCAAs but also in other amino acids.
Verbruggen 2011	This study was a crossover trial comparing two doses of amino acid solution that differed not only in BCAAs but also in other amino acids.
Vlaardingerbroek 2014	This study was an RCT, but it compared amino acids plus lipids to only amino acids.
Yogman 1982	This study was an RCT, but it compared the effect of tryptophan to that of valine.

BCAAs: branched-chain amino acids

APPENDICES

Appendix 1. Search methods

The 1st electronic search (14 July 2016)

Search Terms: branched chain amino acid OR BCAA

Plus the following database-specific terms:

PubMed: ((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: (infant, newborn or newborn or neonate or neonatal or premature or very low birth weight or low birth weight or VLBW or LBW or Newborn or infan* or neonat*) AND (human not animal) AND (randomized controlled trial or controlled clinical trial or randomized or placebo or clinical trials as topic or randomly or trial or clinical trial)

CINAHL: (infant, newborn OR newborn OR neonate OR neonatal OR premature OR low birth weight OR VLBW OR LBW or Newborn or infan* or neonat*) 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)

Cochrane Library: (infant or newborn or neonate or neonatal or premature or very low birth weight or low birth weight or VLBW or LBW)

The 2nd electronic search (2 August 2019)

The RCT filters have been created using Cochrane's highly sensitive search strategies for identifying randomised trials ([Higgins 2011](#)). The neonatal filters were created and tested by the Cochrane Neonatal Information Specialist.

Cochrane CENTRAL via CRS Web

Terms:

1. MESH DESCRIPTOR Amino Acids, Branched-Chain EXPLODE ALL AND CENTRAL:TARGET
2. "branched chain" ADJ2 ("amino acid" or "amino acids") AND CENTRAL:TARGET
3. BCAA or BCAAs AND CENTRAL:TARGET
4. isoleucine or "L isoleucine" or alloisoleucine AND CENTRAL:TARGET398
5. leucine or "L leucine" AND CENTRAL:TARGET
6. valine or "L valine" AND CENTRAL:TARGET
7. #1 OR #2 OR #3 OR #4 OR #5 OR #6
8. MESH DESCRIPTOR Infant, Newborn EXPLODE ALL AND CENTRAL:TARGET

9. infant or infants or infantile or infancy or newborn* or "new born" or "new borns" or "newly born" or neonat* or baby* or babies or premature or prematures or prematurity or preterm or preterms or "pre term" or premies or "low birth weight" or "low birthweight" or VLBW or LBW or ELBW or NICU AND CENTRAL:TARGET
10. #9 OR #8 AND CENTRAL:TARGET
11. #7 AND #10

OVID Medline

Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) 1946 to Present:
Terms:

1. exp Amino Acids, Branched-Chain/
2. ("branched chain" adj2 ("amino acid" or "amino acids")).mp.
3. (BCAA or BCAAs).mp.
4. (isoleucine or "L isoleucine" or alloisoleucine).mp.
5. (leucine or "L leucine").mp.
6. (valine or "L valine").mp.
7. 1 or 2 or 3 or 4 or 5 or 6
8. exp infant, newborn/
9. (newborn* or new born or new borns or newly born or baby* or babies or premature or prematurity or preterm or pre term or low birth weight or low birthweight or VLBW or LBW or infant or infants or infantile or infancy or neonat*).ti,ab.
10. 8 or 9
11. randomized controlled trial.pt.
12. controlled clinical trial.pt.
13. randomized.ab.
14. placebo.ab.
15. drug therapy.fs.
16. randomly.ab.
17. trial.ab.
18. groups.ab.
19. or/11-18
20. exp animals/ not humans.sh.
21. 19 not 20
22. 10 and 21
23. 7 and 22

MEDLINE via PubMed

Terms:

("Amino Acids, Branched-Chain"[Mesh] OR ("branched chain" AND ("amino acid" OR "amino acids"))) OR BCAA OR BCAAs OR isoleucine OR "L isoleucine" OR alloisoleucine OR leucine OR "L leucine" OR valine OR "L valine") AND (((infant, newborn[MeSH] OR newborn*[TIAB] OR "new born"[TIAB] OR "new borns"[TIAB] OR "newly born"[TIAB] OR baby*[TIAB] OR babies[TIAB] OR premature[TIAB] OR prematurity[TIAB] OR preterm[TIAB] OR "pre term"[TIAB] OR "low birth weight"[TIAB] OR "low birthweight"[TIAB] OR VLBW[TIAB] OR LBW[TIAB] OR infant[TIAB] OR infants[TIAB] OR infantile[TIAB] OR infancy[TIAB] OR neonat*[TIAB]) 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]))) Filters activated: Publication date from 2018/07/01

CINAHL via EBSCOhost

Terms:

("branched chain" AND ("amino acid" OR "amino acids"))) OR BCAA OR BCAAs OR isoleucine OR "L isoleucine" OR alloisoleucine OR leucine OR "L leucine" OR valine OR "L valine")
AND
(infant or infants or infantile or infancy or newborn* or "new born" or "new borns" or "newly born" or neonat* or baby* or babies or premature or prematures or prematurity or preterm or preterms or "pre term" or premies or "low birth weight" or "low birthweight" 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)

ISRCTN.com

Search terms:

"branched chain amino acids" AND neonates
"branched chain amino acids" AND infants

"branched chain amino acids" AND newborns
isoleucine AND neonates
isoleucine AND infants
isoleucine AND newborns
leucine AND neonates
leucine AND infants
leucine AND newborns
valine AND neonates
valine AND infants
valine AND newborns

Appendix 2. Risk of bias

We planned to use the standard methods of Cochrane and Cochrane Neonatal to assess the methodological certainty of the trials. For each trial, we planned to seek information regarding the method of randomisation, blinding, and reporting of all outcomes of all the infants enrolled in the trial. We planned to assess each criterion as being at either low, high, or unclear risk of bias. Two review authors separately planned to assess each study and resolve any disagreements through discussion. We planned to add this information to the 'Characteristics of included studies' table. We planned to evaluate the following issues and enter the findings into the 'Risk of bias' table.

1. Sequence generation (checking for possible selection bias). Was the allocation sequence adequately generated?

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

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

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

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

1. low risk (e.g. telephone or central randomisation; consecutively numbered sealed opaque envelopes);
2. high risk (open random allocation; unsealed or non-opaque envelopes, alternation; date of birth); or
3. 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 planned to categorise the methods used to blind study participants and personnel from knowledge of which intervention a participant received. Blinding was to be assessed separately for different outcomes or class of outcomes. We planned to categorise the methods as:

1. low risk, high risk or unclear risk for participants; and
2. 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 planned to categorise the methods used to blind outcome assessment. Blinding was to be assessed separately for different outcomes or class of outcomes. We planned to categorise the methods as:

1. low risk for outcome assessors;
2. high risk for outcome assessors; or
3. 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 planned to describe the completeness of data including attrition and exclusions from the analysis. We planned to note 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 planned to re-include missing data in the analyses. We planned to categorise the methods as:

1. low risk (< 20% missing data);
2. high risk ($\geq$ 20% missing data); or
3. unclear risk.

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

For each included study, we planned to describe 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 planned to compare prespecified outcomes versus outcomes eventually reported in the published results. If the study protocol was not published in advance, we planned to contact study authors to gain access to the study protocol. We planned to assess the methods as:

1. 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);
2. 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
3. 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 planned to describe 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 planned to assess whether each study was free of other problems that could put it at risk of bias as:

1. low risk;
2. high risk; or
3. unclear risk.

If needed, we planned to explore the impact of the level of bias through undertaking sensitivity analyses.

HISTORY

Protocol first published: Issue 7, 2016

Review first published: Issue 10, 2020

CONTRIBUTIONS OF AUTHORS

SA drafted the protocol and all review versions with close supervision from SS. SA and FN contributed to the screening of the titles and full-text screening to identify potentially eligible studies. Final review draft and revised versions were written by SS, assisted by SA. All authors contributed in finalising this review version.

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

DECLARATIONS OF INTEREST

SA has no interest to declare.

SS has no interest to declare.

FN has no interest to declare.

EO has no interest to declare.

RM has no interest to declare.

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DIFFERENCES BETWEEN PROTOCOL AND REVIEW

We made the following changes to the published protocol ([Amari 2016](#)).

- As of July 2019, Cochrane Neonatal no longer searches Embase for its reviews. RCTs and controlled clinical trials (CCTs) from Embase are added to the Cochrane Central Register of Controlled Trials (CENTRAL) via a robust process (see [How CENTRAL is created](#)). Cochrane Neonatal has validated their searches to ensure that relevant Embase records are found while searching CENTRAL.
- Also starting in July 2019, Cochrane Neonatal no longer searches for RCTs and CCTs from [ClinicalTrials.gov](#) or from the World Health Organization's International Clinical Trials Registry Platform [International Clinical Trials Registry Platform](#) (ICTRP), as records from both platforms are added to CENTRAL on a monthly basis (see [How CENTRAL is created](#)). Comprehensive search strategies are executed in CENTRAL to retrieve relevant records. The ISRCTN (at [www.isrctn.com](#), formerly Controlled-trials.com), is searched separately.
- For the 2019 update, we developed a new search strategy, which we ran without date limits ([Appendix 1](#)).
- We changed the title from 'Branched-chain amino acid supplementation for improving nutrition in term and preterm neonates' to 'Branched-chain amino acid supplementation for improving growth and development in term and preterm neonates.' We also changed the objective statement to reflect this: "To determine the effect of BCAA supplementation on physical growth and neurological development in term and preterm neonates."
- We updated the Background section to better suit the objectives of the review.

INDEX TERMS

Medical Subject Headings (MeSH)

Amino Acids, Branched-Chain [*administration & dosage]; Child Development [*drug effects]; Infant, Newborn [*growth & development]; Infant, Premature [growth & development]

MeSH check words

Humans