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Cysteine, cystine or N-acetylcysteine supplementation in parenterally fed neonates (Review)

Soghier LM, Brion LP

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[Intervention Review]

Cysteine, cystine or N-acetylcysteine supplementation in parenterally fed neonates

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ABSTRACT

Background

Cysteine is a precursor of glutathione, an antioxidant that may reduce oxidation injury. The addition of cysteine to parenteral nutrition (PN) allows for the reduction of the amount of methionine in PN, thereby limiting hepatotoxicity and acidifies the solution, thereby increasing calcium and phosphate solubility and potentially improving bone mineralization.

Objectives

To determine the effects of supplementing PN with cysteine, cystine or its precursor N-acetylcysteine on neonatal growth and short and long-term outcomes.

Search methods

The standard search method of the Cochrane Neonatal Review Group was used. MEDLINE, EMBASE, the Cochrane Central Register of Controlled Trials (*The Cochrane Library*) and recent abstracts from the Society for Pediatric Research/ American Pediatric Society, Eastern Society for Pediatric Research, and Society for Parenteral and Enteral Nutrition were originally searched in 2005. In August 2009 updated searches were done of *The Cochrane Library,* MEDLINE (search via PubMed), CINAHL and EMBASE from 2006 to 2009.

Selection criteria

All randomized (RCTs) and quasi-randomized trials that examined the effects of cysteine, cystine or N-acetylcysteine supplementation of neonatal PN were reviewed.

Data collection and analysis

The standard methods of the Cochrane Collaboration and its Neonatal Review Group were used. Statistical analysis included relative risk, risk difference, and weighted mean difference (WMD).

Main results

Six trials fulfilled entry criteria. The majority of patients in these trials were preterm. Five small trials evaluated short-term cysteine supplementation of cysteine-free PN. One large multicenter RCT evaluated short-term N-acetylcysteine supplementation of cysteine-containing PN in extremely low birth weight infants (≤ 1000 grams).

Growth was not significantly affected by cysteine supplementation (1 trial) or by N-acetylcysteine supplementation (1 trial). Nitrogen retention was significantly increased by cysteine supplementation (4 trials) (WMD 31.8 mg/kg/day, 95% confidence interval +8.2, +55.4, n = 95, including 73 preterm infants).

Plasma levels of cysteine were significantly increased by cysteine supplementation but not by N-acetylcysteine supplementation. N-acetylcysteine supplementation did not significantly affect the risks of death by 36 postmenstrual weeks, bronchopulmonary dysplasia (BPD), death or BPD, retinopathy of prematurity (ROP), severe ROP, necrotizing enterocolitis requiring surgery, periventricular leukomalacia, intraventricular hemorrhage (IVH), or severe IVH.

Authors' conclusions

Available evidence from RCTs shows that routine short-term cysteine chloride supplementation of cysteine-free PN in preterm infants improves nitrogen balance. However, there is insufficient evidence to assess the risks of cysteine supplementation, especially regarding metabolic acidosis, which has been reported during the first two weeks of cysteine chloride administration. Available evidence from a large RCT trial does not support routine N-acetylcysteine supplementation of cysteine-containing PN in extremely low birth weight infants.

PLAIN LANGUAGE SUMMARY

Cysteine, cystine or N-acetylcysteine supplementation in parenterally fed neonates

Sick or preterm newborn infants may require intravenous nutrition, including intravenous administration of solutions containing amino acids. Newborn infants need cysteine (an amino acid) for growth under certain conditions. Cysteine may decrease the chance of liver disease and brittle bones. This systemic review was done to analyze whether adding cysteine (or related compounds) to intravenous nutrition affects growth and other outcomes in newborn infants. Five trials studied the effects of adding cysteine to intravenous nutrition that did not contain cysteine. Addition of cysteine significantly improved the babies' ability to build body proteins (analyzed in four studies); however, it did not improve growth (analyzed in one study); no other outcomes were available. One large randomized trial studied the effect of adding another chemical, N-acetyl-cysteine, to intravenous nutrition that already contained cysteine. This study showed no benefit and no toxicity of this intervention. We conclude that present data are insufficient to justify routine addition of cysteine to the intravenous nutrition of newborn infants that does not contain cysteine. Available evidence does not support routine addition of N-acetylcysteine to intravenous nutrition of newborn infants containing cysteine.

BACKGROUND

Description of the condition

The inability of sick term and preterm infants to feed enterally frequently leads to the use of total parenteral nutrition (TPN) in order to provide adequate calories and nutrients to promote growth. Most commercial parenteral solutions contain a mixture of both essential and non-essential amino acids. Low plasma levels of any essential amino acid indicate a relative deficiency state and this may be detrimental to nitrogen balance and growth (Rose 1955).

Description of the intervention

Several facts suggest that the addition of L-cysteine, cystine or N-acetylcysteine to TPN may be beneficial in neonates, especially very low birth weight (VLBW) infants (infants with birth weight < 1500 g) .

Several publications have suggested that L-cysteine could be a conditionally essential (i.e., essential under certain conditions) amino acid for very preterm but not for borderline preterm or full-term neonates (Uauy 1993; Brunton 2000; Heird 1998; Riedijk 2006). In contrast, one randomized trial comparing various intakes of cyst(e)ine in enterally fed very low birth weight infants did not support the hypothesis that cyst(e)ine is a conditionally essential amino acid in these infants. (Riedijk 2007; Riedijk 2008).

Cysteine is incorporated into proteins and is a precursor of taurine and glutathione. In adults, cysteine can be synthesized from ingested methionine via the trans-sulfuration pathway and hence is considered one of the non-essential amino acids (Rose 1955). In the fetus, hepatic cystathionase, the rate-limiting enzyme that converts cystathionine to cysteine, is either absent or barely detectable, resulting in decreased synthesis of cysteine from methionine and high serum concentration of cystathionine (Sturman 1970; Pascal 1972; Gaull 1972; Heinonen 1974). Preterm infants have higher plasma cystathionine levels than term infants (Viña 1995). Hepatic cystathionase activity increases with gestational age (Sturman 1970; Heinonen 1974; Zlotkin 1982) and increases rapidly after birth (Zlotkin 1982) reaching mature levels at about three months in term infants. *In vivo* studies have shown that cysteine synthesized in preterm liver may be present in apolipoprotein B but not in plasma, suggesting that endogenous cysteine is used preferentially for hepatic protein synthesis (Miller 1996). In contrast, a recent study showed that cysteine synthesized in the liver was found in plasma of VLBW infants in amounts that increased with maturation (Shew 2005). Extrahepatic cystathionase, which matures earlier than the hepatic enzyme, might account for some conversion of methionine into cysteine even in preterm infants (Uauy 1993).

How the intervention might work

Parenterally fed infants or adults who do not receive cysteine supplements in TPN have low plasma levels of cystine (Zlotkin 1981; Uauy 1993), the oxidized, disulfide form of cysteine. Regression analysis suggests that an intravenous intake of cysteine of 500 micromoles per kg per day is required in preterm infants to reach plasma cysteine levels comparable to those in full-term, breast-fed infants (Van Goudoever 1994). Snyderman found that enteral supplementation of cysteine improved growth in preterm infants at two to four months of age (Snyderman 1971). These data support the possibility that cysteine may be a conditionally essential amino acid in preterm infants; supplementation of cysteine could improve growth and nitrogen retention under certain conditions. Liver toxicity may be induced by high methionine content of commercial parenteral nutritional solutions that do not contain cysteine (Moss 1999; Brunton 2000). Because of this, some manufacturers have added cysteine to commercial TPN and reduced methionine content accordingly. Supplementation of an essential amino acid (in this case cysteine) may not result in any improvement in growth if caloric intake or intake of other essential or conditionally essential nutrients (e.g., tyrosine) is limiting (Helms 1987; Heird 1993).

Studies have suggested that preterm infants have a poorly developed antioxidant system, making various organs more susceptible to oxidation injury, thereby increasing the risk for intracranial hemorrhage, periventricular leukomalacia, retinopathy of prematurity, chronic lung disease, and necrotizing enterocolitis (Thibeault 2000). Cysteine deficiency may be an etiological factor in the limited ability of preterm babies to produce glutathione, a natural antioxidant. The first step of synthesis of glutathione involves gammma-glutamylcysteine synthetase, which mediates a peptide linkage between glutamate and cysteine. Studies have demonstrated a decrease in glutathione synthesis in erythrocytes of neonates when methionine was used as a cysteine precursor (Viña 1995). The effect was more pronounced in preterm infants less than 32 weeks of age. Supplementation of parenteral nutrition with cysteine increases endogenous synthesis of taurine and glutathione (Shew 2000a).

Taurine is present in all cells and is conjugated to biliary pigments, which are excreted into bile as soluble biliary salts. In adults, taurine can be synthesized from cysteine and methionine. However, in preterm infants, taurine is also considered a conditionally essential amino acid. Taurine needs to be included in parenteral solutions to prevent cholestasis (Howard 1992).

Adding cysteine-hydrochloride (cysteine-HCl) to TPN solutions increases calcium and phosphate solubility. Cysteine-HCl reduces the pH of TPN significantly (Laine 1991; Parikh 2005), thereby increasing calcium and phosphate solubility and allowing increased mineral intake, a limiting factor in mineral accretion in preterm infants receiving TPN. Increased mineral accretion could reduce the incidence of osteopenia of prematurity and fractures in VLBW infants. However, acidification of the TPN by cysteine-chloride in the smallest infants (especially those < 1250 g) may be associated with metabolic acidosis for the first two weeks (Uauy 1993; Laine 1991; Heird 1988). Such metabolic acidosis can be prevented by the administration of base (acetate or lactate) at a 2:1 molar (base:cysteine hydrochloride) ratio (Uauy 1993; Laine 1991).

Stability of cysteine in TPN

The poor stability of cysteine in commercial amino acid solutions has led to the development of cysteine substitutes with greater stability. Cysteine is easily oxidized to cystine, which precipitates rapidly and is virtually insoluble in TPN solutions. In solutions containing both cysteine and glucose, the cysteine content of the fluid decreases by 40% within the first 10 hours from both oxidization and formation of D-glucocysteine (Bjelton 1990). N-acetyl-cysteine, a soluble precursor, improves stability in solution, thereby allowing higher total concentrations of cysteine, cystine and its precursor in TPN. However, plasma cystine levels have been shown to be less than one third the lower limit of the reference range when cyst(e)ine intake consisted of small doses and no correlation was found between plasma cystine levels and N-acetyl-cysteine intake at these doses (Van Goudoever 1994).

Review Authors' note:
In this review, international units (micromoles) were used. The molecular weights of cysteine-hydrochloride and N-acetyl-cysteine are, respectively, 157.7 and 163.2. Therefore, to convert micromole to mg, multiply by 0.1577 and 0.1632, respectively.

Why it is important to do this review

This updates the review "Cysteine, cystine or N-acetylcysteine supplementation in parenterally fed neonates (Soghier 2006a)".

OBJECTIVES

The primary aim of this review was to determine the effect of supplementation of parenterally fed neonates with cysteine, cystine or its precursor N-acetylcysteine on growth assessed by weight gain, length, head circumference and nitrogen retention.

The secondary aims were to determine the effect of cysteine, cystine or its precursor N-acetylcysteine supplementation on mortality, morbidity secondary to oxidation injury (neurological, respiratory, ophthalmologic, gastrointestinal), bone accretion, acidosis or cholestasis.

Primary comparisons:

1. Cysteine or cystine vs. placebo supplementation to cysteine-free PN
2. N-acetylcysteine vs. placebo
3. Cysteine or cystine vs. N-acetylcysteine

Planned subgroup analyses included the following:

1. Enteral versus parenteral (cysteine, cystine or N-acetylcysteine) route
2. Gestational age: preterm (< 37 weeks of gestation) versus full-term infants.
3. Adequacy of intake of calories and other amino acids, or lack of adequate ((Kleinman 2004; Roberts 2001) intake of at least one other element
4. TPN versus partial parenteral nutrition (PN)
5. RCTs versus quasi-randomized trials

METHODS

Criteria for considering studies for this review

Types of studies

All randomized and quasi-randomized controlled trials in which cysteine, cystine or N-acetyl cysteine was used for supplementation in comparison to placebo, other interventions or no supplementation in neonates. Cross-over were eligible if they assessed short-term outcomes. Cluster trials were eligible.

Types of participants

Infants 28 days postnatal age or less receiving no more than minimal enteral nutrition (maximum 15% of the daily calorie intake) were included.

Types of interventions

Intravenous or enteral supplementation of cysteine, cystine or N-acetylcysteine versus no supplementation, placebo or other intervention. This included any dose, formulation, or duration, but excluded studies in which the intervention included not only supplementation of cysteine, cystine or N-acetylcysteine but also supplementation of other nutrients. Starting age needed to be within 28 days of birth.

Types of outcome measures

Primary outcomes

1. Growth: weight gain (g), head circumference gain (cm) and length gain (cm), both short-term (during the intervention period after one week and at the end of the intervention) and long-term (after the intervention period at the time of discharge or at 36 weeks of postmenstrual age [obtained by adding gestational age and postnatal age]) and at one year of age
2. Nitrogen retention (mg/kg/day).

Secondary outcomes

1. Mortality before discharge
2. Chronic lung disease defined as continuous positive airway pressure or oxygen requirement (with or without characteristic radiographic findings) persisting beyond 36 weeks postmenstrual age
3. Retinopathy of prematurity of any stage or severe (stage 3 or more) (ICROP 2005)
4. Necrotizing enterocolitis defined as clinical evidence of abdominal distension and feeding intolerance plus radiological evidence of pneumatosis intestinalis with or without portal venous gas and pneumoperitoneum (greater than stage 1, using Bell's modified criteria) (Kanto 1994)
5. Cystic periventricular leukomalacia defined as cysts in the periventricular area on ultrasonogram, computerized tomography or magnetic resonance imaging scan
6. Intracranial hemorrhage of any grade or severe (grade 3 or 4) (Papile 1978)
7. Incidence of osteopenia of prematurity (defined as decrease bone mineral content and biochemical abnormalities), fractures
8. Incidence of metabolic acidosis (defined based on normal values [mean minus 2 standard deviations] of pH and base deficit for gestational age and postnatal age)
9. Incidence of cholestasis (serum level of direct bilirubin > 20% of total serum bilirubin, or serum level of direct bilirubin > 1 mg/dl if total level of bilirubin is < 5 mg/dl) (AAP 2004)
10. Calcium and phosphate retention (mmol/kg/day)
11. Plasma cyst(e)ine levels (µmol/100 ml), single measurement on the last day of the intervention period. This must be obtained before completion of the intervention because of rapid decrease in plasma level afterwards (Gomez 1993). Unless indicated, levels were assumed to correspond to free cyst(e)ine, because the bound fraction is not measured by conventional methods (Uauy 1993). Plasma levels of cyst(e)ine were calculated by adding the levels of cysteine and hemicystine.

In the protocol there was a typographical error. There was a mismatch between aim, objectives and outcomes: nitrogen retention was listed as primary in aim and objectives and as secondary in outcome measures. This mismatch was corrected in the review.

Search methods for identification of studies

Electronic searches

Searches were conducted using MEDLINE (1966 to December 2005), EMBASE (1974 to December 2005), the Cochrane Central Register of Controlled Trials (CENTRAL, The Cochrane Library, Issue 1, 2006) and personal files. The MeSH headings used were cysteine, cysteine, cystine, N-acetyl cysteine and parenteral nutrition, limited to infant, newborn. Reports in any language were examined.

In August 2009, the search was updated as follows:
The Cochrane Library, MEDLINE (search via PubMed), CINAHL and EMBASE were searched from 2006 to 2009.
Clinicaltrials.gov was searched with no date restriction.
Search terms: *cysteine* OR *cystine* OR *n acetyl cysteine* AND *parenteral nutrition*. Limits: human, newborn infant and clinical trial. No language restrictions were applied.

Searching other resources

The proceedings of Society for Pediatric Research/ American Pediatric Society, Eastern Society for Pediatric Research, and Society for Parenteral and Enteral Nutrition (1991 to December 2005) were hand searched for abstracts using the headings: cysteine and N-acetylcysteine. For the 1992 and 1993 editions of the Society for Pediatric Research/ American Pediatric Society (no subject headings) the sections on developmental biology, developmental pharmacology, gastroenterology and nutrition, and neonatal nutrition and metabolism were searched.

Data collection and analysis

The criteria and standard methods of the Cochrane Collaboration and its Neonatal Review Group were used.

Selection of studies

Studies identified in the search were included if they met the inclusion criteria. All randomized and quasi-randomized controlled trials fulfilling the selection criteria described in the previous section were included. All investigators reviewed the results of the search and separately select the studies for inclusion. The review authors resolved any disagreement by discussion.

Data extraction and management

The review authors separately extracted, assessed and coded all data for each study using a form that was designed specifically for this review. Differences of opinion between review authors were resolved by discussion. Additional data were sought from the authors of all the trials included in the systematic review.

Where justified clinically, two or more subgroups of a published study, using standard formulae to calculate the weighted mean and to estimate the weighted standard deviation were merged (Altman 1991; Brion 1990; Zahr 1984).

Assessment of risk of bias in included studies

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

In addition, the following issues were evaluated and entered into the the Risk of Bias table:

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

Measures of treatment effect

Statistical analysis was carried out using the standard method of the Cochrane Neonatal Review Group. Specifically, treatment effect was expressed as weighted mean difference (WMD) for continuous variables, and as risk difference (RD) and relative risk (RR) for dichotomous variables, using a fixed effect model for meta-analysis and 95% confidence intervals (CI). For cross-over trials, the inverse variance method, [assuming a correlation coefficient of either 0.4 (as presented in the RevMan Analysis figure), or 0.7 was used (sensitivity analysis)].

Assessment of heterogeneity

Meta-analysis was performed for studies where supplementation was used for similar patients, design and dosage. Tests of between-study heterogeneity (chi-square analysis and I^2 statistic) were used to determine if pooling of data was appropriate; if there was inconsistency in the direction of the effect, the data of the meta-analysis was not presented.

Data synthesis

Statistical analysis was carried out using the standard method of the Cochrane Neonatal Review Group. Specifically, treatment effect was expressed as weighted mean difference (WMD) for continuous variables, and as risk difference (RD) and relative risk (RR) for dichotomous variables, using a fixed effect model for meta-analysis and 95% confidence intervals (CI). For cross-over trials, the inverse variance method, [assuming a correlation coefficient of either 0.4 (as presented in the RevMan Analysis figure), or 0.7 was used (sensitivity analysis)]. Meta-analysis was performed for studies where supplementation was used for similar patients, design and dosage.

Subgroup analysis and investigation of heterogeneity

1. Enteral versus parenteral (cysteine, cystine or N-acetylcysteine) route
2. Gestational age: preterm (< 37 weeks of gestation) versus full-term infants.

3. Adequacy of intake of calories and other amino acids, or lack of adequate ((Kleinman 2004; Roberts 2001) intake of at least one other element
4. TPN versus partial parenteral nutrition (PN)
5. RCTs versus quasi-randomized trials

RESULTS

Description of studies

The MEDLINE search retrieved seven studies and the abstract search four additional studies. The EMBASE and the CCRT search did not retrieve any additional studies. Among these eleven studies, six fulfilled the predetermined criteria established for this review, and five were excluded.

Included studies:

Specific information about these six studies is presented in the table entitled "Characteristics of Included Studies." All six trials allocated individual patients. Five trials used a parallel design, and one (Storm 2003) a cross-over design. Five trials randomized cysteine and one (Ahola 2003) used N-acetylcysteine supplementation. All trials used parenteral supplementation (of cysteine or N-acetylcysteine) versus placebo or control. Five studies enrolled exclusively preterm infants, and (Zlotkin 1981) enrolled preterm infants and full-term infants. Inadequate nitrogen intake was documented in a subset of one study (Malloy 1984). Two studies (Malloy 1984; Zlotkin 1981) used as baseline amino acid solution Aminosyn (Abbott Laboratories), which contains neither tyrosine, nor cysteine in any form. One study (Ahola 2003) used as baseline amino acid solution Vaminolac (Fresenius-Kabi, Uppsala, Sweden), which contains cysteine, cystine and tyrosine. The other three studies (Kashyap 1992; Shew 1999; Storm 2003) presumably (Heird 1988, Uauy 1993; Miller 1996; Shew 2000a) used Trophamine, which contains N-acetyltyrosine and no cysteine. Five studies exclusively enrolled patients on TPN, and one (Ahola 2003) enrolled patients with PN and some enteral feeding. Four trials were RCTs (Ahola 2003; Malloy 1984; Shew 1999; Storm 2003), and two were quasi-randomized trials (Kashyap 1992; Zlotkin 1981).

When all trials were analyzed, it appeared that they belonged to two groups which differed in many characteristics: enrolled patients (large range of birth weight and gestational age versus exclusively extremely low birth weight [ELBW] infants), cysteine content of the baseline parenteral nutrition (PN) (cysteine-free PN versus cysteine-containing PN), intervention (cysteine versus N-acetylcysteine), and PN (TPN versus partial PN). The first group included five small (36 patients or less) trials, involving patients with a wide range of birth weights and gestational ages, which analyzed the effects of short-term cysteine (0.5 - 1 millimoles/kg/day) supplementation of cysteine-free PN (Kashyap 1992; Malloy 1984; Shew 1999; Storm 2003; Zlotkin 1981) on growth (Zlotkin 1981), nitrogen retention (Kashyap 1992; Malloy 1984; Shew 1999; Zlotkin 1981) and plasma levels of cysteine (Kashyap 1992; Malloy 1984; Storm 2003; Zlotkin 1981). The second group included a single large multicenter RCT involving 391 ELBW infants, which analyzed the effects of N-acetylcysteine supplementation of cysteine-containing PN (Ahola 2003) on growth, death, death or bronchopulmonary dysplasia by 36 weeks of postmenstrual age, bronchopulmonary dysplasia, retinopathy of prematurity, necrotizing enterocolitis (operated), periventricular leukomalacia, intraventricular hemorrhage and plasma cysteine levels.

The only outcome variable for the cross-over study that is reported in this systematic review is the plasma level of cysteine.

Cysteine supplementation of cysteine-free PN:

Kashyap 1992: The total number of patients entered into the study was 20. They were allocated to one of three arms: treatment arm (1) n = 7, treatment arm (2) n = 6, and control group n = 7. Entry criteria included: VLBW infants receiving total parenteral nutrition. The mean birth weight was 1010 g and mean gestational age 27.8 weeks. Patients in treatment arm (1) received intravenous cysteine supplementation at a dose of 1 millimole/kg/day in the parenteral solution, those in treatment arm (2) received the same dose of cysteine infused separately, and controls received no supplementation. For the purpose of this systematic review, the two treatment arms were combined as described in the method section. The duration of supplementation was not provided. Outcome variables included nitrogen retention and plasma free cysteine concentration. Caloric and nitrogen intake were not provided.

Malloy 1984: The total number of patients entered into the study was 20, including ten in the treatment group and ten in the control group. Entry criteria included: preterm infants at least two days old, unable to take enteral feeds and unlikely to begin enteral feeding within five to seven days; or without enteral intake for 48 hours and unable to tolerate enteral feeds for at least five to seven days. Birth weight ranged between 900 g and 2500 g, and postnatal age between three and 53 days; gestational age is not provided. Patients were randomized to receive either cysteine-hydrochloride (Cysteine-HCl, Abbott Laboratories, Abbott Park, Chicago, IL) (72 mg/kg/day or 0.46 millimoles/kg/day) added to the parental solution for 6 days, or no cysteine supplementation. Randomization was stratified by the amount of nitrogen intake: those receiving low nitrogen intake (1.5 g/kg/day of amino acids) and those receiving higher nitrogen intake (2.5 g/kg/day of amino acids). In this systematic review, merged data (combining groups with different levels of nitrogen intake) and original data (separate groups by nitrogen intake) were both evaluated. Amino acids were provided by Aminosyn (Abbott Laboratories), which contains neither cysteine, nor tyrosine in any form. Non-protein calories ranged between 63 and 73 kcal/kg/day. Outcome variables included weight gain (no SD provided), nitrogen retention, and plasma levels of total and free cysteine.

Shew 1999: The total number of patients entered into the study was 19, including nine in the treatment group and ten in the control group. Entry criteria included: neonates receiving parenteral nutrition. Patients were randomized to receive either cysteine hydrochloride supplementation (0.78 [SD 0.10] millimoles/kg/day) in parenteral nutrition for 5.9 (SD 1.5) days versus no supplementation for 6.8 (SD 1.6) days. The main outcome variable was the nitrogen balance during the final 48 hours of the study. Caloric intake was 92 (SD 12) kcal/kg/day in the treatment group and 99 (SD 19) kcal/kg/day in the control group, and amino acid intake was 2.9 (SD 0.3) and 2.7 (SD 0.3) g/kg/day, respectively.

Storm 2003: The total number of patients entered into the study was 18. The entry criteria included: low birth weight, gestational age 30 - 37 weeks, postnatal age less than four weeks, requiring PN for at least two weeks. Exclusion criteria included: inborn genetic error or significant organ failure. Target PN dosage was 2.5 g/kg of amino acids with total caloric intake of 135 kcal/kg/day. After a three-day

PN without cysteine, infants were randomly allocated to a series of six three-day dosing schedules of cysteine hydrochloride, ranging between 0 and 40 mg cysteine hydrochloride per g of amino acids. For this systematic review we compared the levels of cyst(e)ine on 40 mg of cysteine hydrochloride per g of amino acid (treatment) versus 0 mg/g (control). Outcome variables included plasma levels of taurine, cystine and methionine.

Zlotkin 1981: The total number of patients entered into the study was 36, including 18 in the treatment group and 18 in the control group. Entry criteria included: Infants (age 2 - 46 days) receiving total parenteral nutrition. The average birth weight in the treatment group and the control group was, respectively, 2231 g (SD 1659 g) and 2141 g (SD 1515 g); average gestational age was, respectively, 34.4 weeks (SD 8.5) and 34.2 weeks (SD 9.3); average postnatal age was 13.7 days (SD 23.3) and 15 days (SD 23.8). Patients were allocated to receive either cysteine hydrochloride supplementation (Abbott Laboratories) (77 mg/kg/day of cysteine or 0.64 millimoles/kg/day) for 6 days or no supplementation. Both groups received an average of 87 kcal/kg/day and 3.2 g/kg/day of protein. Amino acids were provided by Aminosyn (Abbott), which contains neither cysteine, nor tyrosine in any form. Outcome variables included weight gain, changes in length and head circumference, nitrogen balance and plasma levels of cysteine and hemicystine.

N-acetylcysteine supplementation of cysteine-containing PN:

Ahola 2003: The total number of patients entered into the study was 391, including 194 in the treatment group and 197 in the control group. Entry criteria included: Infants weighing 500 to 999 grams and on ventilator or nasal continuous positive airway pressure before the age of 36 hours. Patients were treated according to the routine procedures in each intensive care unit and received PN and some enteral nutrition. Average birth weights in the treatment group and the control group, were respectively, 0.776 kg (SD 0.128 kg) and 0.78 kg (SD 0.134 kg); average gestational ages 26.3 weeks (SD 1.7) and 26.5 weeks (SD 1.8). Patients were randomized to either intravenous N-acetyl-L-cysteine (Parvolex, Evans, UK or Mucomyst, Draco Läkemedel AB, Lund, Sweden) at a constant rate of 16 - 32 mg/kg/day (0.10-0.20 millimole/kg/day, calculated on the basis of birth weight, with the smallest infants receiving the lowest dosages for six days versus placebo (0.9% sodium chloride or solvent of Mucomyst). The dosage was based on a pharmacokinetic study, aiming at a steady-state plasma concentrations of N-acetyl-L-cysteine of 100 - 300 micromoles/L (Ahola 1999). Both groups received a commercial intravenous amino acid solution (Vaminolac, Fresenius-Kabi, Uppsala, Sweden), which contains 1 mg/ml of cysteine + cystine, and 0.5 mg/ml tyrosine. Amino acid intake was started on day one or two, with the first dose 8 ml/kg/day, and increased daily to a maximum of 40 - 60 ml/kg/day (0.33 - 0.50 millimoles/kg/day of cysteine equivalent). The primary outcome was death or bronchopulmonary dysplasia by 36 postmenstrual weeks. Secondary outcomes included death, bronchopulmonary dysplasia, retinopathy of prematurity, necrotizing enterocolitis (operated), periventricular leukomalacia, intraventricular hemorrhage and plasma cysteine levels. Dr. Ahola provided us with the standard deviation of cysteine levels at the end of therapy and with unpublished individual data on weight.

Excluded studies:

Specific information is presented in the table entitled "Characteristics of Excluded Studies."

Reasons for study exclusion included (1) additional intervention (methionine, N-acetyltyrosine or different amino acid solutions) besides cysteine, cystine or N-acetylcysteine, (2) non contemporaneous groups (pre/post design), (3) lack of analysis of any of the outcome variables that had been predefined in this systematic review.

Interventions and outcome variables are briefly described here. Helms 1995 analyzed the effect of sulfur amino acid supplementation on plasma levels of taurine and cyst(e)ine. Laine 1991 analyzed the effect of cysteine chloride supplementation on acidity of the PN solution, serum carbon dioxide content and intake of acetate to compensate metabolic acidosis. This study showed that cysteine supplementation significantly reduced serum carbon dioxide content despite an increased intake of acetate. Pointdexter 2000 analyzed the effect of N-acetyltyrosine and cysteine supplementation on amino acid metabolism and protein balance. Shew 2000 analyzed the effect of cysteine supplementation on amino acid metabolism. Van Goudoever 1994 analyzed the effect of N-acetylcysteine and N-acetyltyrosine on plasma levels and urine excretion of amino acids.

Risk of bias in included studies

Cysteine supplementation of cysteine-free PN:

Among these five small trials, three were randomized, and two of them used blind allocation.
Three trials were RCTs (Malloy 1984; Shew 1999; Storm 2003), one trial used alternate allocation (Zlotkin 1981) and for one trial the method of allocation is not provided (Kashyap 1992).
Blinding of allocation: yes in two trials (Shew 1999; Storm 2003), unclear in one trial (Malloy 1984) and no in two trials (Kashyap 1992; Zlotkin 1981).
Blinding of intervention: yes in two trials (Shew 1999; Storm 2003), unclear in one trial (Malloy 1984) and no in two trials (Kashyap 1992; Zlotkin 1981)
Follow-up complete in all studies.
Blinding of outcome: yes in two trials (Shew 1999; Storm 2003), unclear in one trial (Malloy 1984) and no in two trials (Kashyap 1992; Zlotkin 1981).
Three trials were published in abstract form only (Kashyap 1992; Shew 1999; Storm 2003), thereby limiting our ability to critically and fully assess design and methodology.

N-acetylcysteine supplementation of cysteine-containing PN in ELBW infants:

Ahola 2003: This large multicenter double-blind randomized trial is of very good quality. Blinding of randomization: yes. Blinding of intervention: yes. Complete follow-up: yes. Blinding of outcome measurement: yes.

Effects of interventions

Cysteine supplementation of cysteine-free PN (Comparison 1):

This group included five small trials (three RCTs and two quasi-randomized trials) (Kashyap 1992; Malloy 1984; Shew 1999; Storm 2003; Zlotkin 1981), which compared short-term cysteine (0.5 - 1 millimole/kg/day) with placebo supplementation of cysteine-free PN of cysteine-free PN.

1.1. Primary outcomes:

Growth (Outcomes 1.1 - 1.3):
Effects on growth (gains in weight, length or head circumference) were reported in only two studies (Zlotkin 1981; Malloy 1984). Zlotkin 1981 found no statistically significant effects on either weight gain (mean difference [MD] -4.1 g/kg/day, 95% CI -9.7, +1.5), length gain (MD 0 cm/6 days, 95% CI -0.41, +0.41), or head circumference (MD 0.3 cm/ 6 days, 95% CI -0.11, + 0.71). Malloy stated that there was no statistically significant effect of cysteine supplementation on weight gain, but reported only means and ranges. Thus, we could not combine these data in meta-analysis.

Nitrogen retention (Outcome 1.4):
Effects on nitrogen retention was reported in four studies (Kashyap 1992; Malloy 1984; Shew 1999; Zlotkin 1981). Cysteine supplementation significantly increased nitrogen retention, WMD 31.8 mg/kg/day, 95% CI +8.2, +55.4. Sensitivity analysis did not significantly affect the results.
Subgroup analyses showed significant improvement in nitrogen retention when analyzing infants without documented low intake of other nutrients, RCTs only, preterm infants only, but not in patients with documented low nitrogen intake (Malloy 1984), in those infants with documented lack of tyrosine intake (Malloy 1984; Zlotkin 1981) or in full-term infants (Zlotkin 1981). However, one should note that the single study analyzing full-term infants (Zlotkin 1981) used Aminosyn, which does not contain tyrosine.

1.2. Secondary outcomes:

Mortality and morbidity
No data were reported on death, bronchopulmonary dysplasia, retinopathy of prematurity, necrotizing enterocolitis, periventricular leukomalacia, intraventricular hemorrhage, or osteopenia of prematurity. No exact data were provided on metabolic acidosis (Kashyap 1992) and no data were reported on cholestasis, or calcium and phosphorus retention.

Plasma level of free cyst(e)ine (Outcome 1.5):
The effect of cysteine supplementation on free plasma cyst(e)ine concentration at the end of therapy was reported in four trials (Malloy 1984; Storm 2003; Kashyap 1992; Zlotkin 1981). Cysteine supplementation significantly increased free plasma cyst(e)ine levels (MD 2.48 micromoles/100 ml, 95% CI 1.44,3.53). There was significant heterogeneity between the trials.

Subgroup analyses also showed a significant increase in plasma free cyst(e)ine levels in RCTs only, in patients with demonstrated low nitrogen intake, those patients with lack of tyrosine intake, those patients with no documented low intake of or other nutrients, and in preterm infants, but not in full-term infants. In Storm 2003, average cysteine levels were significantly correlated with the rate of cysteine administration (r = 0.97).

Total plasma cyst(e)ine level (Outcome 1.6):
The effect of cysteine supplementation on total plasma cyst(e)ine concentration was reported in one trial (MD 9.4 micromoles/100 ml, 95% CI 5.1,13.7) (Malloy 1984).

N-acetylcysteine supplementation of cysteine-containing PN in ELBW infants (Comparison 2):
This group included a single multicenter RCT (Ahola 2003), which compared N-acetylcysteine with placebo supplementation of cysteine-containing PN. Since only one trial analyzed this comparison no subgroup analyses could be conducted.

2.1. Primary outcomes:

Growth (Outcomes 2.1 - 2.2):
Weight gain during the first week of life was available in 158 infants in the treatment group and 174 in the placebo group. N-acetylcysteine supplementation did not significantly affect weight gain (MD 0.25 g/kg/day, 95% CI -2.70,+3.20). Weight at 36 weeks postmenstrual age was available in 157 infants in the treatment group and 166 in the placebo group. N-acetylcysteine supplementation did not significantly affect the weight at 36 weeks (MD -4.8 g, 95% CI -90.7, +81.1).

2.2. Secondary outcomes:

Death and bronchopulmonary dysplasia at 36 weeks corrected age (Outcomes 2.3 - 2.5):
The effect of N-acetylcysteine supplementation on death and bronchopulmonary dysplasia at 36 weeks corrected age was available in 391 infants (194 in the treatment group and 197 in the placebo group). N-acetylcysteine supplementation did not significantly affect the risk of death by 36 weeks of corrected age (RR 1.23, 95% CI 0.78,1.95; RD 0.03; 95% CI -0.04, +0.11). N-acetylcysteine supplementation did not affect the risk of bronchopulmonary dysplasia among 328 survivors at 36 weeks corrected age (RR 0.99, 95% CI 0.76,1.29; RD 0.00, 95% CI -0.11, +0.10), or the risk of combined outcome death or bronchopulmonary dysplasia (RR 1.04, 95% CI 0.85,1.27; RD 0.02, 95% CI -0.08, +0.12).

Other morbidity (Outcomes 2.6 - 2.11):
The effect of N-acetylcysteine supplementation on retinopathy of prematurity, necrotizing enterocolitis requiring surgical intervention, periventricular leukomalacia and intraventricular hemorrhage was available in 391 infants, including 194 in the treatment group and 197 in the placebo group (Ahola 2003). N-acetylcysteine supplementation did not significantly affect the risk of retinopathy of prematurity among infants examined (RR 0.89, 95% CI 0.71, 1.12; RD -0.06, 95% CI -0.16, +0.05); severe retinopathy of prematurity among infants examined (RR 1.06, 95% CI 0.59,1.90; RD 0.01, 95% CI -0.06, +0.08); necrotizing enterocolitis requiring surgical intervention (RR 1.08, 95% CI 0.56,2.07; RD 0.01; 95% CI -0.05, +0.06); periventricular leukomalacia among all infants examined (RR 0.72, 95% CI 0.38,1.36; RD -0.03, 95% CI -0.09, +0.03); intraventricular hemorrhage (RR 1.10, 95% CI 0.78, 1.55; RD 0.02, 95% CI -0.06, + 0.11); and severe intraventricular hemorrhage (RR 1.18, 95% CI 0.66,2.11; RD 0.02, 95% CI -0.04, +0.08). Data on metabolic acidosis and osteopenia were not available.

Plasma level of free cyst(e)ine (Outcome 2.12):
The effect of N-acetylcysteine supplementation on free plasma cysteine concentration at the end of therapy was available in 85 infants in the treatment group and 80 in the control group. Infants in the N-acetylcysteine supplementation had a lower plasma cysteine concentration at the end of the study than those in the control group (MD -2.5 micromoles/100 ml, 95% CI -4.61, -0.39). However, the increase from baseline (third day) to the end of the study (day seven) was not significantly different between the two groups (Ahola 2003).

Subgroup analyses: not applicable

3. Cysteine or cystine vs. Nacetylcysteine supplementation of PN

No studies found

DISCUSSION

All trials analyzed the effects of short-term cysteine supplementation on some of the predefined outcomes for this systematic review. All trials but one enrolled preterm infants only. The quality of the trials ranged from high-quality multicenter RCT (Ahola 2003) to small quasi-randomized trials published as abstracts only and providing only some of the important parameters required to assess quality.

As discussed previously, the six trials described and analyzed in this systematic review are best presented in two separate groups. Cysteine-supplementation of cysteine-free PN was analyzed in five small trials in which the authors were mainly interested in nitrogen retention and amino acid levels. Limitations of these trials include small size, lack of adequate or unclear evidence of allocation blinding (n = 3), and abstract publication only (n = 3). In contrast, N-acetylcysteine supplementation of cysteine-containing PN was analyzed in a large high-quality multicenter trial in which the authors analyzed mortality and morbidity.

Cysteine supplementation of cysteine-free PN:

There was a discrepancy between the effects of cysteine supplementation on growth and those on nitrogen balance. One quasi-randomized trial (Zlotkin 1981) showed no effects of cysteine supplementation on weight, length, head circumference or nitrogen retention in full-term and preterm infants. Malloy 1984 showed that cysteine supplementation significantly increased nitrogen balance and stated it did not affect weight gain. In contrast, summary statistics showed that cysteine supplementation significantly increased nitrogen retention. These discrepancies in growth outcome may have resulted from differences in entry criteria, study duration, or nutritional intake other than cysteine. Cysteine is thought to be a conditionally essential amino acid, as shown by a study showing reduction in proteolysis and improvement in protein balance with simultaneous cysteine and tyrosine (or N-acetyltyrosine) supplementation (Uauy 1993; Pointdexter 2000). The effect of cysteine or N-acetyl-cysteine supplementation on growth and nitrogen balance may depend on adequacy of tyrosine (or N-acetyltyrosine) intake (Uauy 1993). Zlotkin 1981and Malloy 1984 used Aminosyn, which contained no tyrosine, as opposed to Aminosyn PF, which contains tyrosine (Kleinman 2004). More recent studies used either Vaminolac (Fresenius-Kabi, Uppsala, Sweden), which contains tyrosine (Ahola 2003) or presumably Trophamine, which contains N-acetyltyrosine (Kashyap 1992; Shew 1999; Storm 2003). The latter yields normal levels for virtually all amino acids except for low levels of cysteine (Heird 1987; Heird 1988). Subgroup analysis supports the hypothesis that the discrepancy between lack of improved nitrogen retention weight gain in two studies published in the 80's and significant improved nitrogen in two more recent studies may have resulted from lack of tyrosine in the first two studies.

Lack of data on mortality, toxicity (especially metabolic acidosis), or other short-term and long-term morbidity, and lack of any study using long-term cysteine supplementation prevent us from giving a strong recommendation about the routine use of cysteine supplementation of cysteine-free PN. Since data are insufficient to make a strong recommendation, we recommend a large randomized trial (see Conclusion).

N-acetylcysteine supplementation of cysteine-containing PN in ELBW infants:

N-acetylcysteine supplementation (6-day course at a dose of 0.1 - 0.2 millimoles/kg/day) of cysteine-containing PN in EVLBW infants had no significant effect on primary and secondary outcomes in a large multicenter trial (Ahola 2003), except for a significant decrease in cysteine levels at the end of therapy. Possible reasons for these findings are multiple. First, glutathione deficiency is unlikely to be the single mechanism of oxidation injury. Second, glutathione levels were similar in both groups, suggesting that cysteine and cystine provided in PN prevented glutathione deficiency in both groups, thereby minimizing any effect of N-acetylcysteine supplementation. Third, lack of efficacy of N-acetylcysteine supplementation in VLBW infants may be due in part to urinary loss of acetylated amino acids (Van Goudoever 1994); however, the dosage used in this study led to adequate plasma levels of N-acetylcysteine. Although plasma cysteine levels were lower in N-acetylcysteine-supplemented infants than in controls, the increase in plasma cysteine levels from day three to day seven, i.e., from baseline to the end of the study was similar in the two arms of the trial. N-acetylcysteine may enter into the cells, where it can be deacetylated into cysteine (Ahola 2003). Fourth, lack of efficacy of N-acetylcysteine supplementation may have resulted from too short an intervention. Finally, sample size was sufficient to detect changes in the primary outcome, but insufficient for secondary outcomes (e.g., periventricular leukomalacia). Lack of any significant effect of N-acetylcysteine supplementation on mortality or any of the other outcomes in this large trial argues against the use of this supplementation in this setting.

AUTHORS' CONCLUSIONS

Implications for practice

Available evidence from RCTs shows that routine short-term cysteine chloride supplementation of cysteine-free PN in preterm infants improves nitrogen balance. However, there is insufficient evidence to assess the risks of this supplementation, especially regarding metabolic acidosis, which has been reported during the first two weeks of cysteine chloride administration. Such acidosis may mostly affect the most immature infants during the first week of life.

Available evidence from one large RCT trial does not support routine N-acetylcysteine supplementation of cysteine-containing PN in extremely low birth weight infants.

Implications for research

A large double-blind RCT would be required to assess whether routine prolonged cysteine supplementation affects quantitative (weight, length, head circumference, nitrogen retention) and qualitative (body composition), and short- and long-term neonatal outcomes in VLBW infants receiving cysteine-free PN. Patients should receive a cysteine-free amino acid solution that yields recommended amino acid concentrations (including tyrosine) except for cysteine, and adequate amounts of calories. To assess the effect of cysteine supplementation on bone mineral content, supplementation should be continued beyond two weeks, until cysteine-induced metabolic acidosis is expected to resolve, thereby

allowing investigators to stop alkalinization of the PN solution and thus increase its mineral content. The design of this double-blind trial should allow the pharmacist to optimize mineral intake, based on solubility curves of calcium and phosphate in PN solutions (taking into account amino acids and pH) (Parikh 2005), without exceeding recommended osmolality.

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* Indicates the major publication for the study

CHARACTERISTICS OF STUDIES

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

Ahola 2003

Methods	Multicenter double-blind randomized trial. Blinding of randomization: yes. Blinding of intervention: yes. Complete follow-up: yes. Blinding of outcome measurement: yes.
Participants	Total number of patients entered into the study: 391. Entry criteria: Infants weighing 500 to 999 grams and on ventilator or nasal continuous positive airway pressure before the age of 36 hours. Average birth weight in the treatment group and the control group, respectively, 776 g (SD 128 g) and 780 g (SD 134 g) ; average gestational age 26.3 weeks (SD 1.7) and 26.5 weeks (SD 1.8); average postnatal age 24 hours (SD 9) and 23 hours (SD 9).
Interventions	Intravenous N-acetyl-L-cysteine (Parvolex, Evans, UK) or Mucomyst (Draco Läkemedel AB, Lund, Sweden) 16-32 mg/kg/day (0.10-0.20 millimole/kg/day) for 6 days (n = 194); versus placebo (0.9% sodium chloride or solvent of Mucomyst) (n = 197).
Outcomes	Primary outcome of the main manuscript: death or bronchopulmonary dysplasia by 36 postmenstrual weeks. Secondary outcomes: death, bronchopulmonary dysplasia, retinopathy of prematurity, necrotizing enterocolitis (operated), periventricular leukomalacia, intraventricular hemorrhage and plasma cysteine levels on days 3 and 7. In addition to the original report, the authors also analyzed sub-

Ahola 2003 *(Continued)*

	groups, one in which they found no effect of N-acetyl-L-cysteine supplementation on pulmonary function (Sandberg 2004), and one in which they found evidence for lipid peroxidation (Ahola 2004). Dr. Ahola has generously provided us with data on weight and on plasma cysteine levels. Long-term follow-up data are not available yet. No data were available on bone accretion or level of acidosis.
Notes	Both groups received an amino acid solution (Vaminolac, Fresenius-Kabi, Uppsala, Sweden) containing 1 mg/ml of cysteine + cystine, and 0.5 mg/ml tyrosine, and providing a dose of cysteine starting at 8 mg/kg/day on day 1 or 2 and increasing daily up to 40-60 mg/kg/day (0.33-0.50 millimoles/kg/day of cysteine equivalent). Average nitrogen and caloric intake not provided. Setting: Finland, Sweden, Iceland, Denmark, Norway.

Risk of bias

Bias	Authors' judgement	Support for judgement
Adequate sequence generation?	Low risk	Multicenter double-blind randomized trial. Randomization stratified for each center in blocks of 10 patients Blinding of randomization: yes
Allocation concealment?	Low risk	Multicenter double-blind randomized trial Blinding of randomization: yes
Blinding? All outcomes	Low risk	Blinding of intervention: yes Blinding of outcome measurement: yes
Incomplete outcome data addressed? All outcomes	Low risk	Complete follow-up: yes
Free of selective reporting?	Low risk	
Free of other bias?	Low risk	

Kashyap 1992

Methods	Single-site 3-arm trial. Method of allocation: not provided. Blinding of allocation: no. Blinding of intervention: no. Complete follow-up: yes. Blinding of outcome measurement: no.
Participants	Total number of patients entered into the study: 20. Entry criteria: Very low birth weight infants receiving total parenteral nutrition. Mean birth weight: 1010 g, mean gestational age 27.8 weeks; postnatal age not provided.
Interventions	Intravenous cysteine supplementation 1 millimole/kg/day (either in the parenteral solution [n=7] or separately [n=6]) versus no supplementation (n=7). Duration not provided.
Outcomes	Nitrogen retention; plasma free cysteine concentration.
Notes	Abstract only. Caloric and nitrogen intake not provided. Patients in treatment arm (1) required twice as much base to maintain normal acid-base status as those in treatment arm (2). Controls required no base. For the purpose of this systematic review, the two treatment arms were combined as described in the method section. Nitrogen retention and plasma free cysteine concentration were not affected by the type of cysteine supplementation. Amino acid solution: contains N-acetyltyrosine.

Kashyap 1992 *(Continued)*

	Setting: United States.

Risk of bias

Bias	Authors' judgement	Support for judgement
Adequate sequence generation?	Unclear risk	Single-site 3-arm trial Method of allocation: not provided
Allocation concealment?	High risk	Blinding of allocation: no
Blinding? All outcomes	High risk	Blinding of intervention: no. Blinding of outcome measurement: no
Incomplete outcome data addressed? All outcomes	Low risk	Complete follow-up: yes

Malloy 1984

Methods	Single-site randomized trial. Blinding of allocation: can't tell. Blinding of intervention: can't tell. Complete follow-up : yes. Blinding of outcome measurement: can't tell.
Participants	Total number of patients entered into the study: 20. Entry criteria: preterm infants at least 2 days old, unable to take enteral feeds and unlikely to begin enteral feeding within 5-7 days; or without enteral intake for 48 hours and would be unable to tolerate enteral feeds for at least 5-7 days. Birthweight ranged between 900 g and 2500 g, and postnatal age between 3 and 53 days; gestational age is not provided.
Interventions	Cysteine-chloride (Abbott Laboratories) (72 mg/kg/day or 0.46 millimoles/kg/day) added to the parental solution for 6 days (n=10) versus no cysteine supplementation (n=10).
Outcomes	Weight gain (no SD provided), nitrogen retention, plasma levels of total and free cysteine.
Notes	Randomization stratified by nitrogen intake: those receiving low nitrogen intake (1.5 g/kg/day of amino acids) and those receiving high nitrogen intake (2.5 g/kg/day of amino acids). Amino acids: provided by Aminosyn (Abbott Laboratories), which contains neither cysteine, nor tyrosine in any form. Non protein calories ranged between 63 and 73 kcal/kg/day. Setting: United States.

Risk of bias

Bias	Authors' judgement	Support for judgement
Adequate sequence generation?	Unclear risk	Single-site randomized trial. Blinding of allocation: can't tell
Allocation concealment?	Unclear risk	Single-site randomized trial. Blinding of allocation: can't tell
Blinding? All outcomes	Unclear risk	Blinding of intervention: can't tell Blinding of outcome measurement: can't tell
Incomplete outcome data addressed? All outcomes	Low risk	Complete follow-up : yes

Shew 1999

Methods	Single site randomized trial. Blinding of randomization: yes. Blinding of intervention: yes. Complete follow-up: yes. Blinding of outcome measurement: yes.
Participants	Total number of patients entered into the study: 19. Entry criteria: neonates receiving parenteral nutrition. Average birth weight in the treatment group and the control group, respectively, 1470 g (SD 750 g) and 1540 g (SD 510 g); average gestational age 30.8 weeks (SD 3.0) and 29.9 weeks (SD 4.4); average postnatal age not provided.
Interventions	Cysteine hydrochloride supplementation (0.78 [SD 0.90] millimoles/kg/day) in parenteral nutrition for 5.9 (SD 1.5) days (n = 9) versus no supplementation for 6.8 (SD 1.6) days (n =10).
Outcomes	Nitrogen balance during the final 48 hours of the study.
Notes	Abstract only. Caloric intake was 92 (SD 12) kcal/kg/day in the treatment group and 99 (SD 19) kcal/kg/day in the control group, and amino acid intake was 2.9 (SD 0.3) and 2.7 (SD 0.3) g/kg/day, respectively. There was no difference in glutathione concentration between the two groups. Setting: United States.

Risk of bias

Bias	Authors' judgement	Support for judgement
Adequate sequence generation?	Unclear risk	Single site randomized trial. Blinding of randomization: yes
Allocation concealment?	Low risk	Single site randomized trial. Blinding of randomization: yes
Blinding? All outcomes	Low risk	Blinding of intervention: yes Blinding of outcome measurement: yes
Incomplete outcome data addressed? All outcomes	Low risk	Complete follow-up: yes

Storm 2003

Methods	Single site cross-over randomized trial. Blinding of randomization: yes. Blinding of intervention: yes. Complete follow-up: yes. Blinding of outcome measurement: yes.
Participants	Total number of patients entered into the study: 18. Entry criteria: low birth weight premature infants, gestational age ranged 30-37 weeks, postnatal age less than 4 weeks, requiring PN for at least two weeks. Exclusion criteria: inborn genetic error or significant organ failure.
Interventions	After a 3-day PN without cysteine, infants were randomly allocated to one of six successive schedules dosing schedules of cysteine hydrochloride for 3 days, ranging between 0 and 40 mg/g amino acids. For this systematic review we compared the levels of cyst(e)ine at the end of the schedule with the highest amount of cysteine intake (40 mg of cysteine hydrochloride per g of amino acid) (treatment, n = 18) versus the levels at the end of the lowest, i.e., 0 mg/g (control, n = 18).
Outcomes	Plasma levels of taurine, cystine and methionine at the end of each successive schedule.
Notes	Abstract only. Target PN dosage was 2.5 g/kg of amino acids with total caloric intake of 135 kcal/kg/day.

Storm 2003 *(Continued)*

	Setting: United States.

Risk of bias

Bias	Authors' judgement	Support for judgement
Adequate sequence generation?	Unclear risk	Single site cross-over randomized trial Blinding of randomization: yes
Allocation concealment?	Low risk	Blinding of randomization: yes
Blinding? All outcomes	Low risk	Blinding of intervention: yes Blinding of outcome measurement: yes
Incomplete outcome data addressed? All outcomes	Low risk	Complete follow-up: yes

Zlotkin 1981

Methods	Single site trial. Alternate allocation to treatment versus control. Blinding of allocation: no. Blinding of intervention: no. Complete follow-up: yes. Blinding of outcome: no.
Participants	Total number of patients entered into the study: 36. Entry criteria: Infants (age 2-46 days) receiving total parenteral nutrition. Average birth weight in the treatment group and the control group, respectively, 2231 g (SD 1659 g) and 2141 g (SD 1515 g); average gestational age 34.4 weeks (SD 8.5) and 34.2 weeks (SD 9.3); average postnatal age 13.7 days (SD 23.3) and 15 days (SD 23.8).
Interventions	Cysteine hydrochloride (Abbott Laboratories) supplementation (77 mg/kg/day of cysteine or 0.64 millimoles/kg/day) for 6 days (n = 18) versus no supplementation (n = 18).
Outcomes	Weight gain, changes in length and head circumference, nitrogen balance, plasma cysteine levels.
Notes	Both groups received an average of 87 kcal/kg/day and 3.2 g/kg/day of amino acids, provided by Aminosyn (Abbott Laboratories), which contains neither cysteine, nor tyrosine in any form. Setting: Canada.

Risk of bias

Bias	Authors' judgement	Support for judgement
Adequate sequence generation?	High risk	Single site trial Alternate allocation to treatment versus control
Allocation concealment?	High risk	Alternate allocation to treatment versus control Blinding of allocation: no
Blinding? All outcomes	High risk	Blinding of intervention: no Blinding of outcome: no
Incomplete outcome data addressed? All outcomes	Low risk	Complete follow-up: yes

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

Study	Reason for exclusion
Helms 1995	The intervention was either methionine supplementation, or methionine and cysteine supplementation.
Laine 1991	The study did not include two simultaneous groups but had a before/after design.
Pointdexter 2000	The intervention was N-acetyltyrosine and cysteine hydrochloride supplementation
Shew 2000	None of the predetermined outcome variables for this systematic review were analyzed.
Van Goudoever 1994	The intervention was not limited to cysteine supplementation; it included different amounts of cysteine and tyrosine, or N-acetyl-L-tyrosine and N-acetyl-L-cysteine.

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

NCT00254176

Trial name or title	Cysteine Supplementation in Critically Ill Neonates
Methods	Treatment, Randomized, Double Blind (Subject, Caregiver, Investigator), Placebo Control, Single Group Assignment, Bio-availability Study
Participants	Mechanically ventilated neonates of all gestational ages and birth weights, less than 1 month of postnatal age admitted to the NICU, SNAP (Score of Neonatal Acute Physiology) > 10, projected requirement for continued parenteral nutrition of at least 1 week duration
Interventions	Random allocation to receive a cysteine or an isonitrogenous cysteine-free supplement to their TPN (total parenteral nutrition) regimen
Outcomes	Primary Outcome Measures:Total RBC glutathione Secondary Outcome Measures: Tumor necrosis factor (TNF), l Interleukin-6 (IL-6), Oxygen dependency, Ventilation dependency, Erythrocyte oxidized: reduced glutathione ratio, In vivo erythrocyte glutathione synthetic rate, Plasma malondialdehyde concentration
Starting date	September 2006
Contact information	sshew@mednet.ucla.edu
Notes	

Cysteine supplementation in critically ill neonates
Sponsor: University of California, Los Angeles NCT 00254176

DATA AND ANALYSES

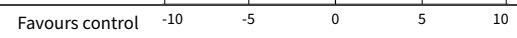
Comparison 1. Cyst(e)ine vs. placebo in added to cysteine-free PN

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Weight gain during the study (g/kg/day)	1	36	Mean Difference (IV, Fixed, 95% CI)	-4.1 [-9.72, 1.52]
2 Increase in length (cm/ 6 days)	1	36	Mean Difference (IV, Fixed, 95% CI)	0.0 [-0.41, 0.41]
3 Increase in head circumference (cm/ 6 days)	1	36	Mean Difference (IV, Fixed, 95% CI)	0.30 [-0.11, 0.71]
4 Nitrogen retention (mg/kg/day)	4		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
4.1 All studies	4	95	Mean Difference (IV, Fixed, 95% CI)	31.81 [8.18, 55.43]
4.2 RCTs only	2	39	Mean Difference (IV, Fixed, 95% CI)	51.97 [18.21, 85.73]
4.3 Documented low nitrogen intake	1	10	Mean Difference (IV, Fixed, 95% CI)	36.20 [-20.90, 93.30]
4.4 Documented lack of tyrosine intake	2	56	Mean Difference (IV, Fixed, 95% CI)	24.54 [-1.56, 50.65]
4.5 No documented low intake of other nutrients	2	39	Mean Difference (IV, Fixed, 95% CI)	64.64 [9.12, 120.16]
4.6 Preterm only	4	73	Mean Difference (IV, Fixed, 95% CI)	40.53 [13.52, 67.55]
4.7 Full-term only	1	16	Mean Difference (IV, Fixed, 95% CI)	-14.0 [-79.24, 51.24]
5 Plasma level of free cyst(e)ine (micromoles/100 ml)	4		WMD (Fixed, 95% CI)	Subtotals only
5.1 All studies	4	112	WMD (Fixed, 95% CI)	2.48 [1.44, 3.53]
5.2 RCTs only	2	56	WMD (Fixed, 95% CI)	2.21 [0.86, 3.57]
5.3 Document low nitrogen intake	1	10	WMD (Fixed, 95% CI)	6.88 [1.58, 12.18]
5.4 Documented lack of tyrosine intake	2	56	WMD (Fixed, 95% CI)	3.19 [1.50, 4.88]
5.5 No documented low intake of other nutrients	2	56	WMD (Fixed, 95% CI)	2.05 [0.72, 3.38]
5.6 Preterm only	4	90	WMD (Fixed, 95% CI)	2.46 [1.28, 3.64]
5.7 Full-term only	1	16	WMD (Fixed, 95% CI)	1.79 [-1.76, 5.34]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
6 Total plasma cyst(e)ine level (micromoles/ 100 ml)	1	20	Mean Difference (IV, Fixed, 95% CI)	9.40 [5.13, 13.67]

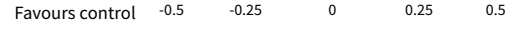
Analysis 1.1. Comparison 1 Cyst(e)ine vs. placebo in added to cysteine-free PN, Outcome 1 Weight gain during the study (g/kg/day).

Study or subgroup	Treatment N	Treatment Mean(SD)	Control N	Control Mean(SD)	Mean Difference Fixed, 95% CI	Weight	Mean Difference Fixed, 95% CI
Zlotkin 1981	18	6.1 (9.8)	18	10.2 (7.2)		100%	-4.1[-9.72,1.52]
Total ***	**18**		**18**			**100%**	**-4.1[-9.72,1.52]**
Heterogeneity: Not applicable							
Test for overall effect: Z=1.43(P=0.15)							



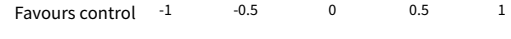
Analysis 1.2. Comparison 1 Cyst(e)ine vs. placebo in added to cysteine-free PN, Outcome 2 Increase in length (cm/ 6 days).

Study or subgroup	Treatment N	Treatment Mean(SD)	Control N	Control Mean(SD)	Mean Difference Fixed, 95% CI	Weight	Mean Difference Fixed, 95% CI
Zlotkin 1981	18	0.6 (0.4)	18	0.6 (0.8)		100%	0[-0.41,0.41]
Total ***	**18**		**18**			**100%**	**0[-0.41,0.41]**
Heterogeneity: Not applicable							
Test for overall effect: Not applicable							



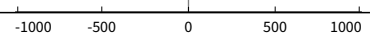
Analysis 1.3. Comparison 1 Cyst(e)ine vs. placebo in added to cysteine-free PN, Outcome 3 Increase in head circumference (cm/ 6 days).

Study or subgroup	Treatment N	Treatment Mean(SD)	Control N	Control Mean(SD)	Mean Difference Fixed, 95% CI	Weight	Mean Difference Fixed, 95% CI
Zlotkin 1981	18	0.9 (0.8)	18	0.6 (0.4)		100%	0.3[-0.11,0.71]
Total ***	**18**		**18**			**100%**	**0.3[-0.11,0.71]**
Heterogeneity: Not applicable							
Test for overall effect: Z=1.42(P=0.15)							



Analysis 1.4. Comparison 1 Cyst(e)ine vs. placebo in added to cysteine-free PN, Outcome 4 Nitrogen retention (mg/kg/day).

Study or subgroup	Treatment N	Treatment Mean(SD)	Control N	Control Mean(SD)	Mean Difference Fixed, 95% CI	Weight	Mean Difference Fixed, 95% CI
1.4.1 All studies							
Kashyap 1992	13	228 (54)	7	168 (81)		12.51%	60[-6.8,126.8]
Malloy 1984	10	220.9 (50.6)	10	171.9 (28.1)		43.38%	49[13.13,84.87]
Shew 1999	9	282 (105)	10	207 (117)		5.6%	75[-24.82,174.82]
Zlotkin 1981	18	280 (67.9)	18	283 (46.7)		38.51%	-3[-41.07,35.07]
Subtotal ***	**50**		**45**			**100%**	**31.81[8.18,55.43]**
Heterogeneity: Tau^2=0; Chi^2=5.5, df=3(P=0.14); I^2=45.42%							
Test for overall effect: Z=2.64(P=0.01)							
1.4.2 RCTs only							
Malloy 1984	10	220.9 (50.6)	10	171.9 (28.1)		88.56%	49[13.13,84.87]
Shew 1999	9	282 (105)	10	207 (117)		11.44%	75[-24.82,174.82]
Subtotal ***	**19**		**20**			**100%**	**51.97[18.21,85.73]**
Heterogeneity: Tau^2=0; Chi^2=0.23, df=1(P=0.63); I^2=0%							
Test for overall effect: Z=3.02(P=0)							
1.4.3 Documented low nitrogen intake							
Malloy 1984	5	150.2 (62)	5	114 (20.1)		100%	36.2[-20.9,93.3]
Subtotal ***	**5**		**5**			**100%**	**36.2[-20.9,93.3]**
Heterogeneity: Not applicable							
Test for overall effect: Z=1.24(P=0.21)							
1.4.4 Documented lack of tyrosine intake							
Malloy 1984	10	220.9 (50.6)	10	171.9 (28.1)		52.97%	49[13.13,84.87]
Zlotkin 1981	18	280 (67.9)	18	283 (46.7)		47.03%	-3[-41.07,35.07]
Subtotal ***	**28**		**28**			**100%**	**24.54[-1.56,50.65]**
Heterogeneity: Tau^2=0; Chi^2=3.8, df=1(P=0.05); I^2=73.66%							
Test for overall effect: Z=1.84(P=0.07)							
1.4.5 No documented low intake of other nutrients							
Kashyap 1992	13	228 (54)	7	168 (81)		69.07%	60[-6.8,126.8]
Shew 1999	9	282 (105)	10	207 (117)		30.93%	75[-24.82,174.82]
Subtotal ***	**22**		**17**			**100%**	**64.64[9.12,120.16]**
Heterogeneity: Tau^2=0; Chi^2=0.06, df=1(P=0.81); I^2=0%							
Test for overall effect: Z=2.28(P=0.02)							
1.4.6 Preterm only							
Kashyap 1992	13	228 (54)	7	168 (81)		16.35%	60[-6.8,126.8]
Malloy 1984	10	220.9 (50.6)	10	171.9 (28.1)		56.7%	49[13.13,84.87]
Shew 1999	9	282 (105)	10	207 (117)		7.32%	75[-24.82,174.82]
Zlotkin 1981	7	283 (58.2)	7	296 (58.2)		19.63%	-13[-73.97,47.97]
Subtotal ***	**39**		**34**			**100%**	**40.53[13.52,67.55]**
Heterogeneity: Tau^2=0; Chi^2=3.96, df=3(P=0.27); I^2=24.23%							
Test for overall effect: Z=2.94(P=0)							
1.4.7 Full-term only							
Zlotkin 1981	8	290 (79.2)	8	304 (50.9)		100%	-14[-79.24,51.24]
Subtotal ***	**8**		**8**			**100%**	**-14[-79.24,51.24]**
Heterogeneity: Not applicable							

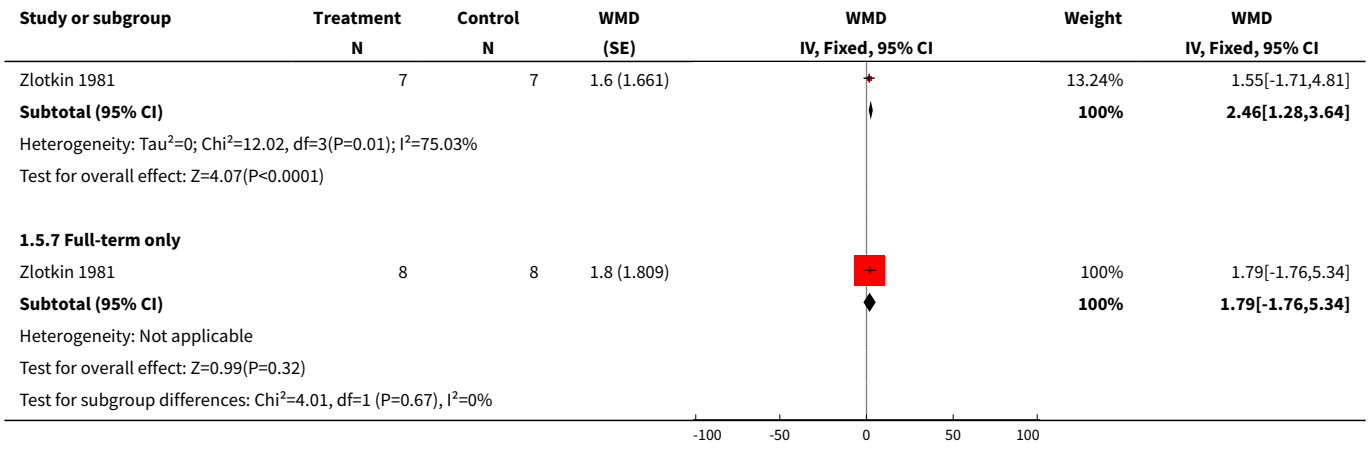


Favours control — Favours treatment

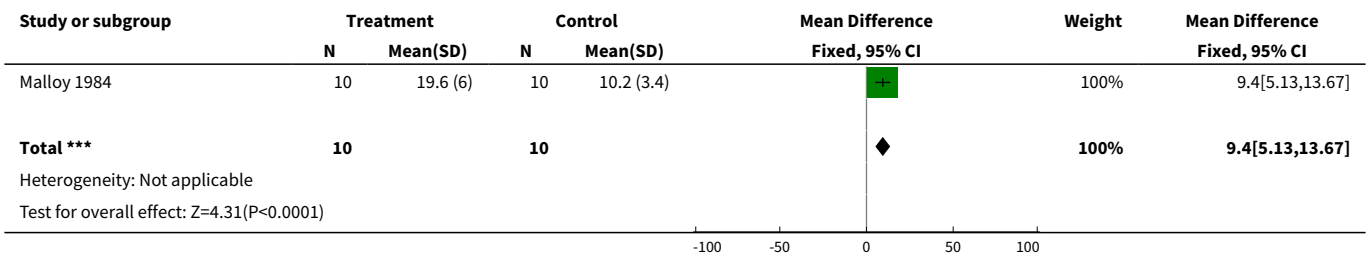
Study or subgroup	Treatment N	Treatment Mean(SD)	Control N	Control Mean(SD)	Mean Difference Fixed, 95% CI	Weight	Mean Difference Fixed, 95% CI
Test for overall effect: Z=0.42(P=0.67)							
Test for subgroup differences: Chi²=5.08, df=1 (P=0.53), I²=0%							

Analysis 1.5. Comparison 1 Cyst(e)ine vs. placebo in added to cysteine-free PN, Outcome 5 Plasma level of free cyst(e)ine (micromoles/100 ml).

Study or subgroup	Treatment N	Control N	WMD (SE)	WMD IV, Fixed, 95% CI	Weight	WMD IV, Fixed, 95% CI
1.5.1 All studies						
Kashyap 1992	13	7	5.5 (1.894)		7.92%	5.5[1.79,9.21]
Malloy 1984	10	10	8.6 (2.237)		5.68%	8.6[4.22,12.98]
Storm 2003	18	18	1.5 (0.726)		53.9%	1.54[0.12,2.96]
Zlotkin 1981	18	18	2.2 (0.935)		32.5%	2.24[0.41,4.07]
Subtotal (95% CI)					**100%**	**2.48[1.44,3.53]**
Heterogeneity: Tau²=0; Chi²=11.77, df=3(P=0.01); I²=74.51%						
Test for overall effect: Z=4.66(P<0.0001)						
1.5.2 RCTs only						
Malloy 1984	10	10	8.6 (2.237)		9.53%	8.6[4.22,12.98]
Storm 2003	18	18	1.5 (0.726)		90.47%	1.54[0.12,2.96]
Subtotal (95% CI)					**100%**	**2.21[0.86,3.57]**
Heterogeneity: Tau²=0; Chi²=9.01, df=1(P=0); I²=88.9%						
Test for overall effect: Z=3.2(P=0)						
1.5.3 Document low nitrogen intake						
Malloy 1984	5	5	6.9 (2.706)		100%	6.88[1.58,12.18]
Subtotal (95% CI)					**100%**	**6.88[1.58,12.18]**
Heterogeneity: Not applicable						
Test for overall effect: Z=2.54(P=0.01)						
1.5.4 Documented lack of tyrosine intake						
Malloy 1984	10	10	8.6 (2.237)		14.87%	8.6[4.22,12.98]
Zlotkin 1981	18	18	2.2 (0.935)		85.13%	2.24[0.41,4.07]
Subtotal (95% CI)					**100%**	**3.19[1.5,4.88]**
Heterogeneity: Tau²=0; Chi²=6.88, df=1(P=0.01); I²=85.47%						
Test for overall effect: Z=3.69(P=0)						
1.5.5 No documented low intake of other nutrients						
Kashyap 1992	13	7	5.5 (1.894)		12.81%	5.5[1.79,9.21]
Storm 2003	18	18	1.5 (0.726)		87.19%	1.54[0.12,2.96]
Subtotal (95% CI)					**100%**	**2.05[0.72,3.38]**
Heterogeneity: Tau²=0; Chi²=3.81, df=1(P=0.05); I²=73.76%						
Test for overall effect: Z=3.02(P=0)						
1.5.6 Preterm only						
Kashyap 1992	13	7	5.5 (1.894)		10.18%	5.5[1.79,9.21]
Malloy 1984	10	10	8.6 (2.237)		7.3%	8.6[4.22,12.98]
Storm 2003	18	18	1.5 (0.726)		69.29%	1.54[0.12,2.96]



Analysis 1.6. Comparison 1 Cyst(e)ine vs. placebo in added to cysteine-free PN, Outcome 6 Total plasma cyst(e)ine level (micromoles/ 100 ml).



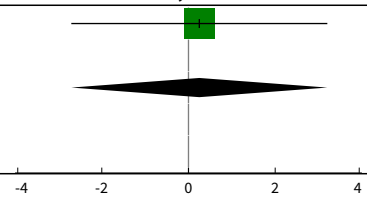
Comparison 2. N-acetylcysteine vs. placebo added to cysteine-containing PN

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Weight gain during the study (g/kg/day)	1	332	Mean Difference (IV, Fixed, 95% CI)	0.25 [-2.70, 3.20]
2 Weight (g) at 36 weeks of postmenstrual age	1	323	Mean Difference (IV, Fixed, 95% CI)	-4.80 [-90.66, 81.06]
3 Death by 36 weeks of postmenstrual age	1	391	Risk Ratio (M-H, Fixed, 95% CI)	1.23 [0.78, 1.95]
4 Bronchopulmonary dysplasia among survivors at 36 weeks of postmenstrual age	1	328	Risk Ratio (M-H, Fixed, 95% CI)	0.99 [0.76, 1.29]
5 Death or Bronchopulmonary Dysplasia (36 weeks postmenstrual age)	1	391	Risk Ratio (M-H, Fixed, 95% CI)	1.04 [0.85, 1.27]
6 Retinopathy of prematurity (any stage) among those examined	1	332	Risk Ratio (M-H, Fixed, 95% CI)	0.89 [0.71, 1.12]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
7 Severe (stage 3 or more) retinopathy of prematurity among those examined	1	332	Risk Ratio (M-H, Fixed, 95% CI)	1.06 [0.59, 1.90]
8 Necrotizing enterocolitis (operated)	1	391	Risk Ratio (M-H, Fixed, 95% CI)	1.08 [0.56, 2.07]
9 Periventricular leukomalacia in all examined	1	388	Risk Ratio (M-H, Fixed, 95% CI)	0.72 [0.38, 1.36]
10 Intraventricular hemorrhage (any grade)	1	388	Risk Ratio (M-H, Fixed, 95% CI)	1.10 [0.78, 1.55]
11 Severe intraventricular hemorrhage (grade 3-4)	1	386	Risk Ratio (M-H, Fixed, 95% CI)	1.18 [0.66, 2.11]
12 Plasma level of free cyst(e)ine (micromoles/ 100 ml)	1	165	Mean Difference (IV, Fixed, 95% CI)	-2.50 [-4.61, -0.39]

Analysis 2.1. Comparison 2 N-acetylcysteine vs. placebo added to cysteine-containing PN, Outcome 1 Weight gain during the study (g/kg/day).

Study or subgroup	Treatment N	Treatment Mean(SD)	Control N	Control Mean(SD)	Mean Difference Fixed, 95% CI	Weight	Mean Difference Fixed, 95% CI
Ahola 2003	158	-8.1 (12.7)	174	-8.4 (14.8)		100%	0.25[-2.7,3.2]
Total ***	**158**		**174**			**100%**	**0.25[-2.7,3.2]**
Heterogeneity: Not applicable							
Test for overall effect: Z=0.17(P=0.87)							

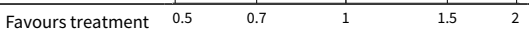


Analysis 2.2. Comparison 2 N-acetylcysteine vs. placebo added to cysteine-containing PN, Outcome 2 Weight (g) at 36 weeks of postmenstrual age.

Study or subgroup	Treatment N	Treatment Mean(SD)	Control N	Control Mean(SD)	Mean Difference Fixed, 95% CI	Weight	Mean Difference Fixed, 95% CI
Ahola 2003	157	1886.9 (393.1)	166	1891.7 (393.9)		100%	-4.8[-90.66,81.06]
Total ***	**157**		**166**			**100%**	**-4.8[-90.66,81.06]**
Heterogeneity: Not applicable							
Test for overall effect: Z=0.11(P=0.91)							

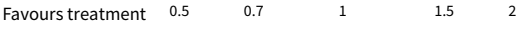
Analysis 2.3. Comparison 2 N-acetylcysteine vs. placebo added to cysteine-containing PN, Outcome 3 Death by 36 weeks of postmenstrual age.

Study or subgroup	Treatment n/N	Control n/N	Risk Ratio M-H, Fixed, 95% CI	Weight	Risk Ratio M-H, Fixed, 95% CI
Ahola 2003	34/194	28/197		100%	1.23[0.78,1.95]
Total (95% CI)	**194**	**197**		**100%**	**1.23[0.78,1.95]**
Total events: 34 (Treatment), 28 (Control)					
Heterogeneity: Not applicable					
Test for overall effect: Z=0.89(P=0.37)					



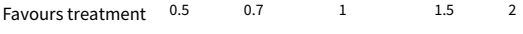
Analysis 2.4. Comparison 2 N-acetylcysteine vs. placebo added to cysteine-containing PN, Outcome 4 Bronchopulmonary dysplasia among survivors at 36 weeks of postmenstrual age.

Study or subgroup	Treatment n/N	Control n/N	Risk Ratio M-H, Fixed, 95% CI	Weight	Risk Ratio M-H, Fixed, 95% CI
Ahola 2003	64/160	68/168		100%	0.99[0.76,1.29]
Total (95% CI)	**160**	**168**		**100%**	**0.99[0.76,1.29]**
Total events: 64 (Treatment), 68 (Control)					
Heterogeneity: Not applicable					
Test for overall effect: Z=0.09(P=0.93)					



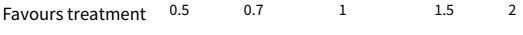
Analysis 2.5. Comparison 2 N-acetylcysteine vs. placebo added to cysteine-containing PN, Outcome 5 Death or Bronchopulmonary Dysplasia (36 weeks postmenstrual age).

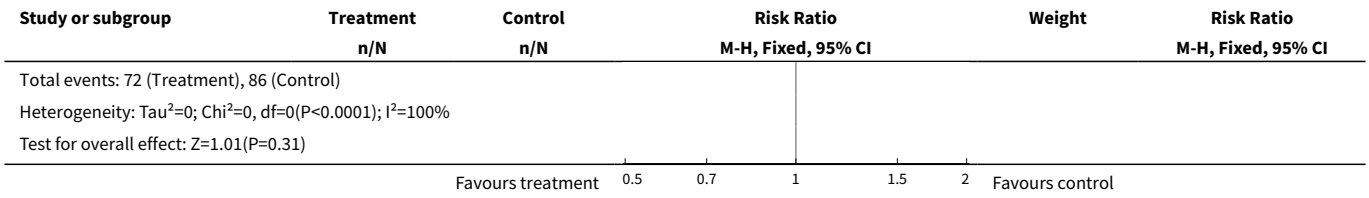
Study or subgroup	Treatment n/N	Control n/N	Risk Ratio M-H, Fixed, 95% CI	Weight	Risk Ratio M-H, Fixed, 95% CI
Ahola 2003	98/194	96/197		100%	1.04[0.85,1.27]
Total (95% CI)	**194**	**197**		**100%**	**1.04[0.85,1.27]**
Total events: 98 (Treatment), 96 (Control)					
Heterogeneity: Not applicable					
Test for overall effect: Z=0.35(P=0.72)					



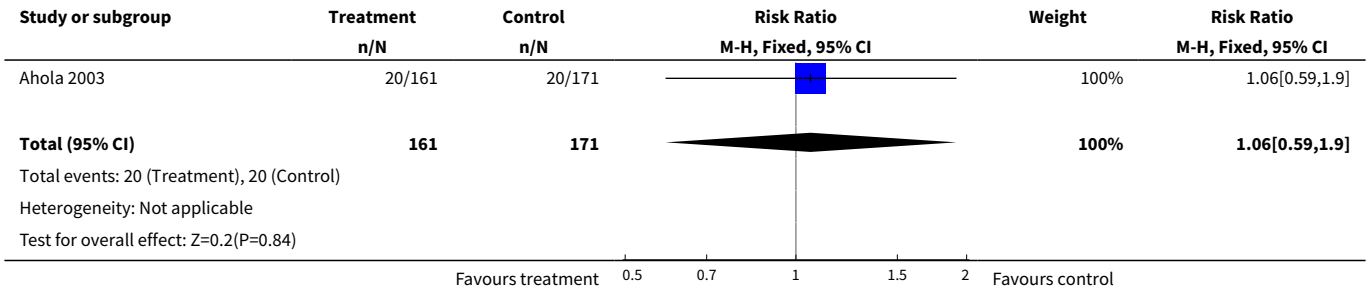
Analysis 2.6. Comparison 2 N-acetylcysteine vs. placebo added to cysteine-containing PN, Outcome 6 Retinopathy of prematurity (any stage) among those examined.

Study or subgroup	Treatment n/N	Control n/N	Risk Ratio M-H, Fixed, 95% CI	Weight	Risk Ratio M-H, Fixed, 95% CI
Ahola 2003	72/161	86/171		100%	0.89[0.71,1.12]
Total (95% CI)	**161**	**171**		**100%**	**0.89[0.71,1.12]**

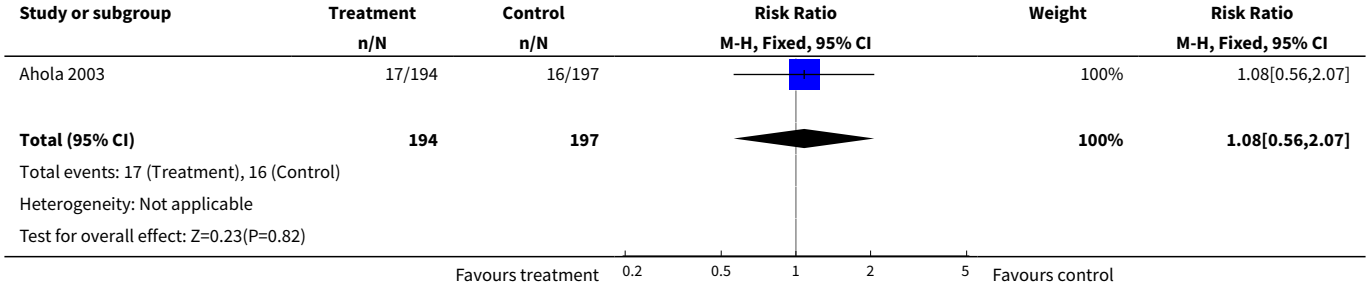




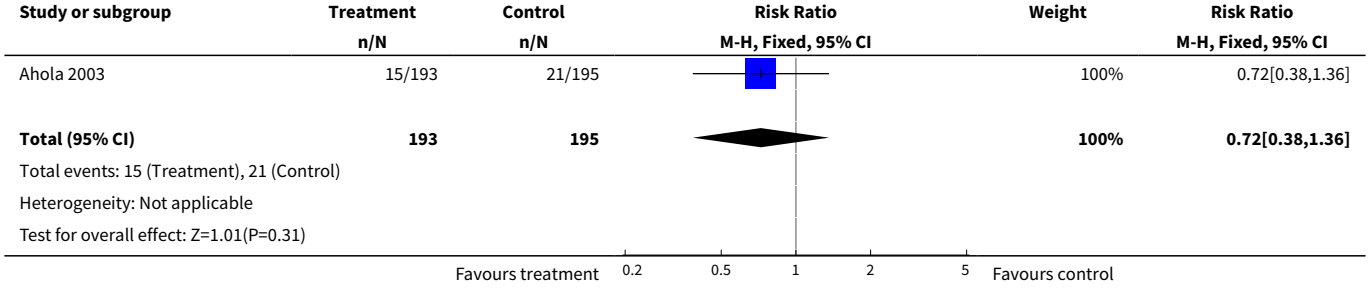
Analysis 2.7. Comparison 2 N-acetylcysteine vs. placebo added to cysteine-containing PN, Outcome 7 Severe (stage 3 or more) retinopathy of prematurity among those examined.



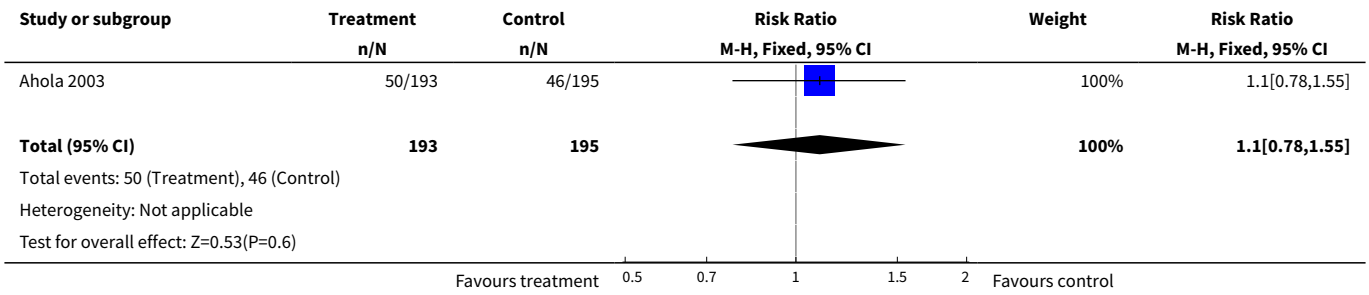
Analysis 2.8. Comparison 2 N-acetylcysteine vs. placebo added to cysteine-containing PN, Outcome 8 Necrotizing enterocolitis (operated).



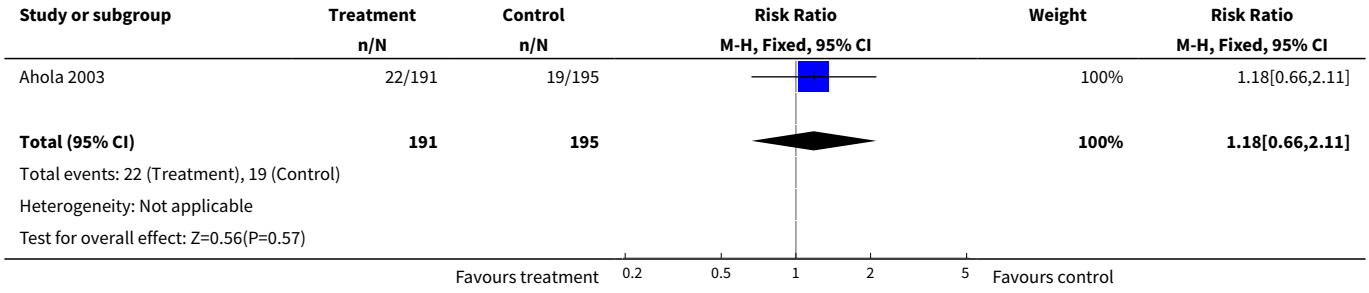
Analysis 2.9. Comparison 2 N-acetylcysteine vs. placebo added to cysteine-containing PN, Outcome 9 Periventricular leukomalacia in all examined.



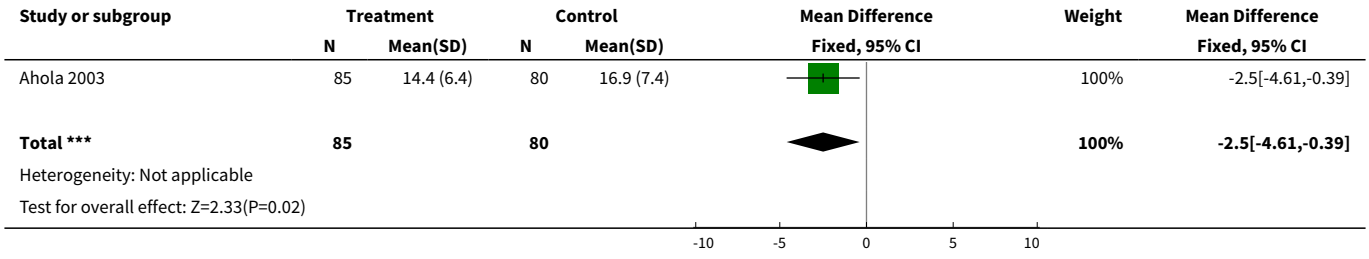
Analysis 2.10. Comparison 2 N-acetylcysteine vs. placebo added to cysteine-containing PN, Outcome 10 Intraventricular hemorrhage (any grade).



Analysis 2.11. Comparison 2 N-acetylcysteine vs. placebo added to cysteine-containing PN, Outcome 11 Severe intraventricular hemorrhage (grade 3-4).



Analysis 2.12. Comparison 2 N-acetylcysteine vs. placebo added to cysteine-containing PN, Outcome 12 Plasma level of free cyst(e)ine (micromoles/ 100 ml).



FEEDBACK

Comment from Jamie Kirkham, 7 August 2007

Summary

In the review you specify two primary outcomes: 1) Growth 2) Nitrogen Retention. However, in the review protocol, Growth is listed as the single most important primary outcome, while nitrogen retention was a secondary outcome.

Reply

In the protocol there was a typographical error: there was a mismatch between aim, objectives and outcomes: nitrogen retention was listed as primary in aim and objectives and as secondary in outcome measures. This mismatch was corrected in the review.

Contributors

Dr. Jamie Kirkham, MRC funded project ORBIT (Outcome Reporting Bias in Trials) (G0500952)

WHAT'S NEW

Date	Event	Description
19 August 2009	New search has been performed	This updates the review "Cysteine, cystine or N-acetylcysteine supplementation in parenterally fed neonates" published in the Cochrane Database of Systematic Reviews, Issue 4, 2006 (Soghier 2006a). Updated search conducted in August 2009 did not identify any new completed studies. One ongoing study was identified in Clinicaltrials.gov. The background in the Abstract and in the text was amended based on new information on cysteine in very low birth weight infants (Riedijk 2007; Riedijk 2008). Amendment made to text at the end of 'Types of outcome measures' section.

HISTORY

Protocol first published: Issue 3, 2004
Review first published: Issue 4, 2006

Date	Event	Description
12 March 2008	Amended	Converted to new review format.
10 July 2006	New citation required and conclusions have changed	Substantive amendment

CONTRIBUTIONS OF AUTHORS

Lamia Soghier wrote the first draft of the protocol and searched the databases.
Luc Brion searched the abstracts and wrote the first draft of the review.

Luc Brion (LB) updated the review in 2006.
The August 2009 update was conducted centrally by the Cochrane Neonatal Review Group staff (Yolanda Montagne, Roger Soll, Diane Haughton) and reviewed and approved by LB.

DECLARATIONS OF INTEREST

None

SOURCES OF SUPPORT

Internal sources

- Albert Einstein College of Medicine, Children's Hospital at Montefiore, Division of Neonatology, USA.

External sources

- No sources of support supplied

INDEX TERMS

Medical Subject Headings (MeSH)

*Dietary Supplements; *Parenteral Nutrition; Acetylcysteine [administration & dosage]; Cysteine [*administration & dosage]; Cystine [administration & dosage]; Infant, Newborn [*growth & development]; Infant, Premature; Randomized Controlled Trials as Topic

MeSH check words

Humans