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[Intervention Review]

Protein substitute for children and adults with phenylketonuria

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ABSTRACT

Background

Phenylketonuria is an inherited metabolic disorder characterised by an absence or deficiency of the enzyme phenylalanine hydroxylase. The aim of treatment is to lower blood phenylalanine concentrations to the recommended therapeutic range to prevent developmental delay and support normal growth. Current treatment consists of a low-phenylalanine diet in combination with a protein substitute which is free from or low in phenylalanine. Guidance regarding the use, dosage, and distribution of dosage of the protein substitute over a 24-hour period is unclear, and there is variation in recommendations among treatment centres. This is an update of a Cochrane review first published in 2005, and previously updated in 2008.

Objectives

To assess the benefits and adverse effects of protein substitute, its dosage, and distribution of dose in children and adults with phenylketonuria who are adhering to a low-phenylalanine diet.

Search methods

We searched the Cochrane Cystic Fibrosis and Genetic Disorders Group Trials Register which consists of references identified from comprehensive electronic database searches and hand searches of relevant journals and abstract books of conference proceedings. We also contacted manufacturers of the phenylalanine-free and low-phenylalanine protein substitutes for any data from published and unpublished randomised controlled trials.

Date of the most recent search of the Group's Inborn Errors of Metabolism Trials Register: 03 April 2014.

Selection criteria

All randomised or quasi-randomised controlled trials comparing: any dose of protein substitute with no protein substitute; an alternative dosage; or the same dose, but given as frequent small doses throughout the day compared with the same total daily dose given as larger boluses less frequently.

Data collection and analysis

Both authors independently extracted data and assessed trial quality.

Main results

Three trials (69 participants) are included in this review. One trial investigated the use of protein substitute in 16 participants, while a further two trials investigated the dosage of protein substitute in a total of 53 participants. Due to issues with data presentation in each

trial, described in full in the review, formal statistical analyses of the data were impossible. Investigators will be contacted for further information.

Authors' conclusions

No conclusions could be drawn about the short- or long-term use of protein substitute in phenylketonuria due to the lack of adequate or analysable trial data. Additional data and randomised controlled trials are needed to investigate the use of protein substitute in phenylketonuria. Until further evidence is available, current practice in the use of protein substitute should continue to be monitored with care.

PLAIN LANGUAGE SUMMARY

The impact of protein substitute on the nutrition status, growth, and neuropsychological performance of children and adults with phenylketonuria

People with phenylketonuria (PKU) who follow a low-phenylalanine diet are required to take protein substitute to ensure adequate consumption of protein, energy, and other nutrients. The need for protein substitute has been established through clinical experience and observational data. Randomised, controlled trials are needed to confirm this need as well as its proper dosage and frequency of use. We performed a systematic review of randomised control trials investigating the impact of the use, dosage, and distribution of protein substitute on physical and neuropsychological outcomes in the treatment of PKU. Trials of children and adults diagnosed with PKU in the newborn period who were treated early and continuously were included. We planned to pool the results of the trials to estimate treatment effect. Three trials met the inclusion criteria for the review. One trial evaluated the impact of protein substitute versus no protein substitute on neuropsychological status, plasma amino acid concentrations, and nutrient intake. The remaining two trials investigated the impact of differing dosages of protein substitute on plasma amino acid concentrations and nutrient intake. No trials investigating daily protein substitute distribution were eligible for inclusion in the review. Results are presented in text form only since adequate information for data pooling was not provided. The investigators will be contacted for further information. Currently data are insufficient to reach any conclusions regarding the use, dosage, and distribution of protein substitute in the treatment of PKU. Further randomized or controlled clinical trials are needed to provide evidence for the effectiveness, dosage, and distribution of protein substitute in the treatment of PKU.

BACKGROUND

Phenylketonuria

Phenylketonuria (PKU) (PKU; OMIM 261600) is a rare, autosomal recessive disorder that, if untreated, can lead to severe mental retardation, eczema, seizures, and behavioural disorders (Paine 1957). PKU is most often caused by a mutation in the phenylalanine hydroxylase (EC 1.14.16.1) (PAH) gene, resulting in little to no liver PAH activity. Consequently, phenylalanine metabolism is impaired, plasma concentrations of phenylalanine rise above 120 $\mu\text{mol/L}$ (2 mg/dL), and the brain and other tissues have increased concentrations of phenylalanine (Scriver 2001). Treatment of PKU necessitates the initiation of dietary phenylalanine restriction and tyrosine supplementation within the first few weeks of life to prevent disease progression (Bickel 1953; Pietz 1998).

Despite successful treatment, lack of diet adherence has been linked to elevated plasma phenylalanine levels and adverse sequelae throughout the lifespan of people with PKU, particularly children and females of childbearing age (Pennington 1985; Fehrenbach 1989; Legido 1993). Relaxation of the diet leads to poor performance in school, decreased attention span, and lower intellectual function (IQ) (Krause 1986; Griffiths 1995). Effects on the unborn foetus of a pregnant mother with uncontrolled PKU are reminiscent of foetal alcohol syndrome: small head size, mental retardation, heart disease, growth retardation, and facial dysmorphism (Lipson 1981; Rouse 1990; Levy 1996).

Phenylketonuria affects people throughout the world with varying incidence and severity, and many countries routinely screen newborns for PKU (Waisbren 2007). Given the more than 531 mutations already identified in the PAH gene (PAH database 2007), there is a wide variation in disease severity, with residual PAH activity ranging from less than 2% up to 70% of full activity (Guldberg 1998). Individuals with classical PKU exhibit very low or no PAH activity, and plasma phenylalanine concentrations exceed 1000 $\mu\text{mol/L}$ when untreated (Scriver 2001), whereas those with milder forms of PKU, termed hyperphenylalaninaemia, have partial PAH activity, and plasma phenylalanine concentrations do not exceed 1000 $\mu\text{mol/L}$ when untreated (Scriver 2001). In the present review the term PKU encompasses both classical PKU and hyperphenylalaninaemia.

Dietary Management of PKU

The ultimate goal of diet management is to achieve plasma phenylalanine within recommended concentrations while maintaining adequate nutrition for normal growth and development (MRC 1993; NIH 2000). First used to manage PKU in 1951 (Bickel 1953), the cornerstone of the treatment is restriction of dietary phenylalanine, plus a protein substitute to supply missing essential amino acids and sometimes other nutrients.

The average dietary intake of phenylalanine in the US among all ages and both sexes is 3.4 g/day (3400 mg/day) (DRIs 2002). For people with PKU, the amount of phenylalanine in the diet must be restricted, depending upon the level of PAH activity, to maintain recommended plasma phenylalanine concentrations. Those with classical PKU must reduce dietary phenylalanine to 0.2 to 0.5 g/day (200 to 500 mg/day) whereas those with hyperphenylalaninaemia can tolerate more than 0.5 g/day (500 mg/day) (Scriver 2001).

Over-restriction of phenylalanine can lead to poor growth, mild to moderate osteoporosis, and other effects of malnutrition (Hanley 1970; Sibinga 1971). Thus, small portions of foods containing low or moderate amounts of protein, including fruits, vegetables, grains, potatoes, and specialized low-protein foods, are recommended to provide the amount of phenylalanine tolerated by each individual. With the exception of many fruits and vegetables, one gram of protein in food contains approximately 50 mg of phenylalanine (Weetch 2006). A diet restriction limiting phenylalanine to 250 mg, therefore, would allow for a total of 5 g of natural (intact) protein per day. To comply with this restriction, foods high in protein, such as meat, poultry, fish, milk, nuts, and eggs, are generally eliminated from the diet and a synthetic protein substitute (called a medical food or amino acid mixture) lacking phenylalanine supplies much of the essential amino acid needs.

Protein substitute typically provides 52% to 80% of total dietary protein (Groppe 1988; Schulz 1995; MacDonald 2000) and the majority of essential and conditionally essential amino acids. In many cases these products also contain carbohydrate, fat, vitamins, and minerals. New varieties of protein substitutes are emerging to improve adherence, touted as having better taste, convenience, energy and nutrient contents, variety of choices, and packaging. Restriction of dietary phenylalanine without an adequate supply of protein substitute has been associated with inadequate intakes of multiple nutrients, including vitamin B12, thiamin, riboflavin, vitamin B6, folate, magnesium, and protein ((Groppe 1988; Schulz 1995), which can lead to deficiencies (Hanley 1993; Aung 1997; Robinson 2000) and poor growth (Arnold 2002).

Whether the protein needs are greater for people with PKU continues to be an area of debate. Observational studies of children with PKU aged two months to fifteen years have shown adequate and consistent growth in height over time when consuming protein, a large proportion of which comes from protein substitutes, at levels at or above general population recommendations. Adequate growth is defined as attaining a height-for-age percentile within the normal range, and consistent growth is defined as following a consistent height-for-age growth curve over time (Kindt 1988; Acosta 2003; Dobbelaere 2003; Huemer 2007). If protein needs are increased in this population, it is likely due to the bioavailability, biological value, and net protein utilization of the protein source (i.e., protein substitute) (Kindt 1985; Przyrembel 2000; Hoeksma 2005). People with PKU consuming free amino acids have shown increased urinary nitrogen (N) loss, and healthy adults have shown increased oxidation and decreased overall protein synthesis compared with comparison groups consuming the same amount of N from intact protein (Jones 1983, Metges 2000). However, a recent study of adults with PKU taking free amino acid-based protein substitute and adults without PKU taking protein from intact sources, both at 0.8 g protein equivalent/kg body weight/day infused intravenously (Note: the gram weight of free amino acids was multiplied by a factor of 0.8 to calculate protein equivalency), showed no differences in protein turnover, oxidation, and net protein balance (van Rijn 2007).

There is considerable agreement that consumption of protein substitute should be evenly spread throughout the day to optimise protein utilization for anabolism and to control plasma phenylalanine concentrations (MacDonald 1999). In one study, people with PKU typically consuming one to two doses of protein

substitute shifted to three equal doses of protein substitute for one day; this shift resulted in a decrease in total urinary N excretion of 4% to 28%, controlling for N intake in eight of ten free-living participants. In turn, N balance increased or stayed constant for nine of ten participants (Schoeffer 1994). Adding support to this finding, Herrmann and colleagues also found decreased total urinary N excretion, controlling for N intake, when a patient consumed a third of her daily protein substitute at a time compared with the entire daily protein substitute at once. Maximum ¹³C-exhalation, indicating amino acid oxidation, was also lower in the five hours after taking only a third of the daily protein for breakfast (Herrmann 1994).

Importance

Since treatment guidelines vary both between and within different countries (MRC 1993; NIH 2000), justification for the use, dosage, and distribution of protein substitute is essential for optimal, cost-effective treatment of PKU. In this review, we will investigate data obtained from relevant randomised, controlled clinical trials in an attempt to resolve discrepancies in treatment guidelines. This is an update of a Cochrane review first published in 2005, and previously updated in 2008 (Rutherford 2005; Yi 2008).

OBJECTIVES

To assess the benefits and adverse effects of the following in children and adults with PKU who are adhering to a low-phenylalanine diet:

1. protein substitute compared with no protein substitute;
2. a high dose of protein substitute compared with a lower dose;
3. protein substitution given as frequent, small doses throughout the day compared with the same total daily dose given as larger boluses less frequently.

METHODS

Criteria for considering studies for this review

Types of studies

Randomised controlled trials (RCTs), both published and unpublished. Trials where quasi-randomisation methods, such as alternation, are used will be included if there is sufficient evidence that the treatment and control groups are comparable in terms of clinical and nutritional status.

Types of participants

Individuals of any age with PKU and other forms of phenylalanine hydroxylase deficiency diagnosed by the Guthrie test or another recognised, validated screening test, in whom dietary treatment was initiated early in infancy and continued.

Types of interventions

Supplementation of a low-phenylalanine diet with:

1. any dose of protein substitute that has been compared with no protein substitute;
2. a dose of protein substitute compared with an alternative dose;
3. the same dose of protein substitution, but given as frequent, small doses throughout the day, compared with the same total daily dose given as larger boluses less frequently.

A post hoc change was made to the interventions previously outlined in this review. Initially the intervention was to compare a high dose of protein substitute (greater than or equal to 3 g of amino acids per kg per day based on the highest dose recommended by Medical Research Council (MRC) guidelines) with a lower dose (less than 3 g of amino acids per kg per day). This was changed as it was recognised that this practice has changed and continues to change since the publication of the MRC guidelines, and lower protein substitute doses than those quoted in the MRC report are being used (MRC 1993).

Types of outcome measures

Primary outcomes

1. Weight gain, body mass index, z scores, centiles, other indices of nutritional status or growth
2. Measures of neuropsychological performance

Secondary outcomes

1. Blood phenylalanine concentrations
2. Blood amino acid concentrations other than phenylalanine
3. Energy and nutrient intake
4. Brain amino acid concentrations
5. Measures of eating behaviour
6. Measures of quality of life
7. Death

As yet we have been unable to enter data into meta-analyses. However, when this is possible, outcome data from different trials will be compared at using similar time points based on both data availability and relevancy to the outcome measure. For long-term follow up studies, reported outcome data will be grouped at multiple time points during the year and annually thereafter.

Search methods for identification of studies

Electronic searches

Relevant trials were sought from the Group's Inborn Errors of Metabolism Trials Register using the term: PKU.

This Inborn Errors of Metabolism Register is compiled from electronic searches of the Cochrane Central Register of Controlled Trials (CENTRAL) (updated each new issue of *The Cochrane Library*), weekly searches of MEDLINE, and the prospective hand searching of one journal: *The Journal of Inherited Metabolic Disease*. Unpublished work was identified by searching through the abstract books of the Society for the Study of Inborn Errors of Metabolism conference and the SHS Inborn Error Review Series. For full details of all searching activities for the register, please see the relevant section of the [Cochrane Cystic Fibrosis and Genetic Disorders Group Module](#).

Date of most recent search of the Group's Inborn Errors of Metabolism Trials Register: 03 April 2014.

Searching other resources

Additional RCTs were sought from reference lists as well as abstract books from groups and societies interested in inborn errors of metabolism. European and US Manufacturers of the phenylalanine-free and very low-phenylalanine protein substitutes were asked whether they had data from published and unpublished RCTs

on file. Companies contacted for previous versions of this review were: SHS International, Liverpool, UK; Vitaflo Ltd, Liverpool, UK; Mead Johnson, Middlesex, UK; Milupa, Wiltshire, UK; UCB Pharma, Hertfordshire, UK; Glaxo Smithkline, Middlesex, UK; and Ross Products Division, Ohio, USA. Companies contacted for the current version were: Nutricia North America, Maryland, USA; Vitaflo USA, New York, USA; Vitaflo International Ltd, Liverpool, UK; and Abbott Nutrition, Ohio, USA. Contact authors of all included trials were approached for any further information, if appropriate.

Data collection and analysis

Selection of studies

Both authors independently selected the trials to be included in the review.

Data extraction and management

Using standard data acquisition forms, we each independently extracted data. We reached consensus through discussion regarding any disagreements on the suitability of a trial for inclusion in the review or on its quality.

Assessment of risk of bias in included studies

We each assessed the methodological quality of the included trial, using a method described by Jüni (Jüni 2001). In particular, we examined details of the randomisation method, method of allocation concealment, the degree of blinding, whether intention-to-treat analyses were possible from the available data, and whether the number of participants lost to follow up or subsequently excluded from the trial was recorded.

Measures of treatment effect

When data are available we plan to undertake the following analyses.

For binary outcomes, we aim to calculate a pooled estimate of the treatment effect for each outcome across trials (the odds of an outcome among treatment allocated participants to the corresponding odds among controls). For continuous outcomes, we plan to record either mean change from baseline for each group or mean post-treatment/intervention values and standard deviation for each group. Then, where appropriate, we will calculate a pooled estimate of treatment effect by calculating the mean difference.

Unit of analysis issues

Three trials were included in the review. Data from each trial were not presented in a way that could be used for formal statistical analysis, therefore results are presented in the text of this review only. Issues with the data provided included combining outcome data across trial arms or different studies, omitting data, and providing descriptive statistics that are incompatible with the method of analysis used for this review (Prince 1997; MacDonald 2006b; Schindeler 2007).

Dealing with missing data

In a future update, it may be possible to include more data from the included trials, and the lead investigators have been or will be contacted to provide individual patient data.

In order to allow an intention-to-treat analysis, we will seek data on the number of participants, by allocated treatment group, irrespective of compliance and whether or not the participant will be later thought to be ineligible or otherwise excluded from treatment or follow up.

Assessment of heterogeneity

Statistical heterogeneity between trial results will be quantified by using the I^2 statistic (Higgins 2003).

Subgroup analysis and investigation of heterogeneity

Trials of the use of protein substitute in participants who have 'relaxed' their diet will be analysed separately, if sufficient trials are identified to allow a subgroup analysis. This group will consist of adolescents/adults with PKU who consistently have blood phenylalanine concentrations above 700 $\mu\text{mol/L}$.

Sensitivity analysis

We plan to perform a sensitivity analysis based on the methodological quality of the trials, including and excluding quasi-randomised trials.

RESULTS

Description of studies

Results of the search

The searches identified 88 references, of which three trials (seven references), including a total of 69 participants, met the inclusion criteria for this review (Prince 1997; MacDonald 2006b; Schindeler 2007). To the best of our knowledge, there are currently no ongoing studies eligible for this review.

Included studies

Supplementation of a low-phenylalanine diet with any dose of protein substitute that has been compared with no protein substitute

One trial, conducted by Schindeler and colleagues, provided data on the impact of protein substitute usage (Schindeler 2007). Although the primary objective of the Schindeler trial was to study the impact of large neutral amino acid supplementation, the interventions in two of the four trial arms met the criteria for inclusion in this review. This trial investigated the neuropsychological and biochemical outcomes of 16 participants ages 11 to 45 years when on and off protein substitute each for 14 days.

Supplementation of a low phenylalanine diet with a dose of protein substitute compared with an alternative dose

Two trials investigated variation in dosage of protein substitute (Prince 1997; MacDonald 2006b). Prince employed a two-phase trial assessing the effects of a new protein substitute which had a lower protein content (10 g protein equivalent to 400 kcal/100 g dry powder) than the control product (a 50% reduction in protein equivalent/100 g dry powder) in 28 children with PKU aged 4 years to 10 years. All children were prescribed protein intakes meeting the US RDA 1989. Only phase one (the first two of five years) was an RCT (Prince 1997). MacDonald conducted a RCT with a cross-over design that compared the effects of a higher dose of protein from protein substitute (2 g protein equivalent/kg body weight/day) with

a lower dose (1.2 g/kg/day) using the same protein substitute. A total of 25 children aged 2 to 10 years remaining on their current protein substitute participated (MacDonald 2006b).

Supplementation of a low-phenylalanine diet with the same dose of protein substitution, but given as frequent small doses throughout the day compared with the same total daily dose given as larger boluses less frequently

No trials were eligible for inclusion.

Excluded studies

Of the remaining 81 references identified by the search, 50 were discounted as not being relevant to the review and three were duplicate references. On further examination another 25 references (24 trials) were excluded as not relevant and are listed in the 'Excluded studies' section of the review. Three of the 25 references were excluded because they were not RCTs or quasi-randomised trials (Clemens 1991; Acosta 1994; SHS 2001); 18 references (17 studies) were excluded as the interventions did not meet the inclusion criteria (MacDonald 2003a; Baumgartner 2004; MacDonald 2005; Rose 2005; Agostoni 2006; Cleary 2006; MacDonald 2006a; Matalon 2006; Koletzko 2007; Levy 2007; Matalon 2007; MacDonald 2008; Ahring 2010; Ahring 2012; Gokmen-Ozel 2011; Stroem 2011; ten Hoedt 2011); one was excluded because the participants did not meet inclusion criteria (Kalkanoglu 2005); two were excluded because the participants and intervention did not meet inclusion criteria (Cleary 2003; Marsden 2005); and one trial was excluded because the participants, intervention, and outcomes did not meet inclusion criteria (MacDonald 2006c).

Characteristics of studies awaiting classification

For the other three references (three trials), summary details for these trials, which are awaiting assessment, are available in the [Characteristics of studies awaiting classification](#) table. One trial is awaiting assessment as three participants were introduced to the trial after the initial randomisation phase (MacDonald 2003b). The second and third trials were published as abstracts only and eligibility is unclear (Giovannini 2006; Lange 2004). The lead investigators of these trials will be contacted to request further information.

Risk of bias in included studies

Assessment of methodological quality was based on a method described by Jüni (Jüni 2001). Additional information on the trials conducted by Prince, MacDonald, and Schindeler have been or will be requested by personal communication with the lead investigators, and it is hoped that further details can be included in a future update (Prince 1997; MacDonald 2006b; Schindeler 2007). Summary details including methodological quality of included studies are available in the [Characteristics of included studies](#) table.

Supplementation of a low-phenylalanine diet with any dose of protein substitute that has been compared with no protein substitute

Generation of allocation sequence

Generation of randomisation sequence was unclear in the included trial (Schindeler 2007).

Concealment of allocation

Allocation was concealed until the participant completed each arm of the trial (Schindeler 2007).

Blinding

Participants, investigators, and the operator who processed the MRS results were blinded to the treatment allocation (Schindeler 2007).

Intention-to-treat analysis

All stated participants completed the trial arms (Schindeler 2007). All participant data were included except the dietary data for two participants in one trial who did not return their diet records (Schindeler 2007).

Supplementation of a low phenylalanine diet with a dose of protein substitute compared with an alternative dose

Generation of allocation sequence

Generation of randomisation sequence was considered to be adequate in the Prince trial as the sequence was generated using random number tables (Prince 1997); however, it was unclear in the MacDonald trial (MacDonald 2006b).

Concealment of allocation

Allocation of the intervention was concealed until the participants had been randomised in the two trials (Prince 1997; MacDonald 2006b).

Blinding

Neither participant nor investigator was blinded in the two trials (Prince 1997; MacDonald 2006b).

Intention-to-treat analysis

In one trial, one participant did not complete the intervention arm of the trial, and all data for this participant were excluded from the final analysis (Prince 1997). In the MacDonald trial, all stated participants completed the trial arms (MacDonald 2006b).

Supplementation of a low-phenylalanine diet with the same dose of protein substitution, but given as frequent small doses throughout the day compared with the same total daily dose given as larger boluses less frequently

No trials were eligible for inclusion.

Effects of interventions

As stated in the 'Methods' section, data for the trials are not currently available for analysis, therefore results are presented narratively (Prince 1997; MacDonald 2006b; Schindeler 2007). Authors will be contacted for data needed to conduct analyses.

Supplementation of a low-phenylalanine diet with any dose of protein substitute that has been compared with no protein substitute

Primary outcomes

1. Weight gain, body mass index, z scores, centiles, other indices of nutritional status or growth

This outcome was not measured in the included trial ([Schindeler 2007](#)).

2. Measures of neuropsychological performance

Attention, reaction time, response inhibition, generativity, self-monitoring, cognitive flexibility, planning, immediate span, and working memory were assessed. Measures of attention were improved when taking protein substitute compared with not taking protein substitute. Data for protein substitute phases are not presented separately from LNAA arms; the author will be contacted for these data ([Schindeler 2007](#)).

Secondary outcomes

1. Blood phenylalanine concentrations

The median (range) plasma phenylalanine concentrations were significantly higher when participants did not take protein substitute (1180 (641 to 1744) $\mu\text{mol/L}$) compared with when they did take protein substitute (734 (19 to 1231) $\mu\text{mol/L}$) ($P = 0.001$) ([Schindeler 2007](#)).

2. Blood amino acid concentrations other than phenylalanine

Plasma amino acids other than phenylalanine were measured; however, only phenylalanine:tyrosine ratio data were presented. The median (range) plasma phenylalanine: tyrosine ratio was significantly higher when not taking protein substitute (30 (11.9 to 52.1)) compared with when taking protein substitute (14 (0.2 to 27.5)) ($P < 0.001$). The authors stated that plasma amino acid concentrations of large neutral amino acids were increased when consuming MF compared with not consuming MF ([Schindeler 2007](#)).

3. Energy and nutrient intake

Median (range) intakes of total protein were higher (1.43 (0.88 to 1.85) g/kg/day versus 0.51 (0.17 to 0.62) g/kg/day) and protein from protein substitute were higher (0.94 (0.13 to 1.45) g/kg/day versus 0 (0 to 0) g/kg/day) in the arm on protein substitute compared with off protein substitute. Median (range) intakes of phenylalanine (18.5 (6.4 to 43.9) mg/kg/day versus 21.8 (6.2 to 27.9) mg/kg/day) and protein from food (0.40 (0.14 to 0.94) g/kg/day versus 0.51 (0.17 to 0.62) g/kg/day) were lower in the arm on protein substitute. The authors did not report the statistical significance of these differences. As noted earlier, the analysed dietary data include 14 of the 16 participants ([Schindeler 2007](#)).

4. Brain amino acid concentrations

Concentrations of phenylalanine in the brain between all arms of the trial ranged between 176 and 365 $\mu\text{mol/L}$. The authors reported no significant difference between trial arms, and arm-specific data are not presented ([Schindeler 2007](#)).

5. Measures of eating behaviour

This outcome was not measured in the included trial ([Schindeler 2007](#)).

6. Measures of quality of life

This outcome was not measured in the included trial ([Schindeler 2007](#)).

7. Death

This outcome was not measured in the included trial ([Schindeler 2007](#)).

Supplementation of a low phenylalanine diet with a dose of protein substitute compared with an alternative dose

Primary outcomes

1. Weight gain, body mass index, z scores, centiles, other indices of nutritional status or growth

Height and weight centiles and protein status were measured during the Prince trial; however, it is unclear at which time points these were measured, and data were not presented. The authors commented that there were no clinical concerns of reduced growth or protein status during the combined phases of the trial ([Prince 1997](#)). Other outcomes of nutritional status were not measured ([MacDonald 2006b](#); [Prince 1997](#)).

2. Measures of neuropsychological performance

This outcome was not measured in either of the included trials ([Prince 1997](#); [MacDonald 2006b](#)).

Secondary outcomes

1. Blood phenylalanine concentrations

Blood phenylalanine concentrations were measured in both trials. The MacDonald trial showed a significant decrease in median plasma phenylalanine concentrations when on the higher dose of protein substitute ([MacDonald 2006b](#)). Data for the RCT phase of the Prince trial were not presented separately ([Prince 1997](#)).

2. Blood amino acid concentrations other than phenylalanine

Select serum amino acids other than phenylalanine (cystine, histidine, isoleucine, leucine, lysine, methionine, threonine, tyrosine, valine) were measured in one of the trials; however, data were not presented ([Prince 1997](#)). The author commented that no significant differences in mean serum amino acid concentrations were found between the control and experimental groups at entry or at the end of phase one ([Prince 1997](#)).

3. Energy and nutrient intake

Energy and nutrient intake were measured in both trials. Data for the RCT phase of the Prince trial were not presented separately ([Prince 1997](#)). In the MacDonald trial, protein intake was only presented for the lower-dose interventions, and energy was presented only as the difference between the two different interventions. The author has been contacted for the participant-level data ([MacDonald 2006b](#)).

4. Brain amino acid concentrations

This outcome was not measured in either of the included trials ([Prince 1997](#); [MacDonald 2006b](#)).

5. Measures of eating behaviour

This outcome was not measured in either of the included trials ([Prince 1997](#); [MacDonald 2006b](#)).

6. Measures of quality of life

This outcome was not measured in either of the included trials (Prince 1997; MacDonald 2006b).

7. Death

This outcome was not measured in either of the included trials (Prince 1997; MacDonald 2006b).

Supplementation of a low-phenylalanine diet with the same dose of protein substitution, but given as frequent small doses throughout the day compared with the same total daily dose given as larger boluses less frequently

No trials were eligible for inclusion.

DISCUSSION

Each trial included in this review contained small numbers of participants. In a future update of the review, it may be possible to include more data from these trials if provided by the authors.

Although the Schindler trial conducted an intent-to-treat analysis on the primary outcome measures, two participants were excluded from the analysis of nutrient intakes due to missing data. The exclusion of these data may increase the risk for bias in these results, however the impact on the overall trial findings is likely to be low.

Although little evidence has been found to support the use, the dosage, and the distribution of doses of protein substitute, this does not mean that these products are ineffective. Rather, the data available are insufficient to reach any conclusions regarding its use. Clinical experience and observational data have revealed that without protein substitute many people with PKU would not take sufficient protein to facilitate normal growth or body maintenance and control of blood phenylalanine. It is important to ascertain the

quantity and quality of protein substitute required, as well as the frequency of administration to ensure optimal health, quality of life, and efficient use of healthcare resources. Improving the evidence to guide the use of protein substitute in PKU is helpful not only for the treatment of PKU, but as a model for the treatment of other aminoacidopathies.

AUTHORS' CONCLUSIONS

Implications for practice

No conclusions can be made about the short- or long-term use of protein substitute in PKU due to the lack of adequate trial data. Current practice in the use of protein substitute should continue to be observed and monitored with care until further evidence is available from current and future included trials. Likewise, people with PKU should continue to take the amount of protein substitute as advised by their healthcare professionals.

Implications for research

Large RCTs are needed to investigate the use of protein substitute in PKU. Due to the relatively small numbers of people with this condition, it is recommended that a multicentre approach be adopted. It is important that both long- and short-term outcomes are considered in future trials. To assist in ensuring the appropriateness of future trials, it would be useful to involve people with PKU and their caregivers in the design of the trials.

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REFERENCES

References to studies included in this review

MacDonald 2006b {published data only}

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* Indicates the major publication for the study

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

MacDonald 2006b

Methods	Randomised controlled cross-over trial.
Participants	Number of participants: 25 enrolled, ages 2 to 10 years. Inclusion criteria: well controlled plasma phenylalanine concentrations over the past 6 months; ages 2 to 10 years; parents able to collect blood specimens. Exclusion criteria: none indicated.
Interventions	Two doses of protein substitute for 14 days each with a 14-day washout period. 1. 2 g/kg body weight/day 2. 1.2 g/kg body weight/day
Outcomes	1. Plasma phenylalanine concentrations 2. Intakes of total protein, protein from protein substitute, protein from food, and phenylalanine exchanges 3. Intakes of total energy and energy from protein substitute
Notes	Location: UK

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Randomization process not described.
Allocation concealment (selection bias)	Low risk	Adequate.
Blinding (performance bias and detection bias) All outcomes	High risk	Not used.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Inclusion of all randomized participants in the final analysis.

Prince 1997

Methods	Two-phase trial (phase 1 trial length: 2 years; phase 2 study length: 3 years). Only phase 1 was an RCT. Phase 1: Randomised controlled parallel trial. Inclusion of all randomized participants in the final analysis: Phase I: unclear, data not shown;
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Prince 1997 (Continued)

Not included in this review: Phase 2: historic control trial. Only 25 of 28 randomized participants were included.

Participants	<p>Phase 1: Number of participants: 28 enrolled, ages 4 to 10 years. Inclusion criteria: diagnosed with moderate to severe PKU; ages 4 to 10 years; currently treated by a protein-restricted diet supplemented with protein substitute. Exclusion criteria: none indicated.</p> <p>Not included in this review: Phase 2: historic control trial.</p>
Interventions	<p>Phase 1: 1. Novel protein substitute: 100% US RDA for protein (1989) for 2 years. The novel protein substitute contained 10 g protein equivalent for 400 kcal (100 g dry powder) and included a reduction in methionine and cystine, and eliminated aspartic acid and glutamic acid. 2. Control protein substitute (PhenylFree, Mead-Johnson Co., Evansville, IN, USA): 100% US RDA for protein (1989) for two years.</p> <p>Not included in this review: Phase 2: historic control trial.</p>
Outcomes	<p>Phase 1: 1. Growth 2. Serum protein (prealbumin and transferrin) concentrations 3. Serum phenylalanine concentrations 4. Serum essential amino acid concentrations 5. Protein substitute intake assessed by 4-day diet records 6. Protein substitute intake assessed by product acquisition records</p> <p>Not included in this review: 7. Compliance rating Phase 2: historic control trial.</p>
Notes	Location: USA

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Randomization process not described.
Allocation concealment (selection bias)	Unclear risk	Unclear if allocation concealment was used.
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Unclear if blinding was used.
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Unclear as data not shown whether all randomized participants were included in the final analysis.

Schindeler 2007

Methods	Randomised controlled cross-over trial.
Participants	Number of participants: 16 enrolled, ages 11 to 45 years.

Protein substitute for children and adults with phenylketonuria (Review)

Schindeler 2007 (Continued)

Inclusion criteria: diagnosed with classical PKU; early initiation of treatment; currently treated by a protein-restricted diet supplemented with protein substitute.
Exclusion criteria: none indicated.

Interventions 4-arm trial; each arm lasted 14 days separated with washout periods of at least 4 weeks. Participants were instructed to remain on a low-phenylalanine diet through the entirety of the trial.

1. Placebo without protein substitute
2. Placebo plus protein substitute

Not included in this review:

3. LNAAs plus protein substitute
4. LNAAs without protein substitute

Outcomes

1. Neuropsychological performance (attention, reaction time, response inhibition, generativity, self-monitoring, cognitive flexibility, planning, immediate span, and working memory)
2. Plasma phenylalanine concentrations
3. Plasma phenylalanine: tyrosine ratio
4. Intakes of total protein, protein from protein substitute, protein from food, and phenylalanine
5. Brain phenylalanine concentrations

Not included in this review:

6. Self-reported ratings of mood
7. LNAA intake

Notes Location: Australia

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Generation of allocation sequence: randomization process not described.
Allocation concealment (selection bias)	Low risk	Adequate.
Blinding (performance bias and detection bias) All outcomes	Low risk	Participants, investigators, and outcome assessor were blinded to treatment allocation.
Incomplete outcome data (attrition bias) All outcomes	Low risk	All randomized participants are included in the final analysis of neuropsychological performance, plasma phenylalanine and tyrosine concentrations, and brain phenylalanine concentrations. Two participants were excluded from diet analyses due to failure to submit diet records in at least one of the trial arms.

LNAA: large neutral amino acid
PKU: phenylketonuria
RCT: randomised controlled trial
RDA: recommended daily allowance

Characteristics of excluded studies [ordered by study ID]

Study	Reason for exclusion
Acosta 1994	Not a CCT or RCT. Ascertained by communication with current head of metabolic products who contacted the author (her predecessor in post). Infants fed Analog XP were studied first, and Phenex study began after this.
Agostoni 2006	Intervention not eligible for inclusion.
Ahring 2010	Intervention not eligible for inclusion.
Ahring 2012	Intervention not eligible for inclusion.
Baumgartner 2004	Intervention not eligible for inclusion.
Cleary 2003	Participants and intervention not eligible for inclusion.
Cleary 2006	Intervention not eligible for inclusion.
Clemens 1991	Not a CCT or RCT.
Gokmen-Ozel 2011	Intervention not eligible for inclusion.
Kalkanoglu 2005	Group of participants not eligible for inclusion.
Koletzko 2007	Intervention not eligible for inclusion.
Levy 2007	Intervention not eligible for inclusion.
MacDonald 2003a	Intervention not eligible for inclusion. The trial did not set out to compare two different doses of protein substitute intake, but examined a group of 20 participants with PKU and studied the effects of giving participants at least 40% of their daily protein substitute requirements as tablets for at least 12 weeks. There was better compliance with protein substitute as tablets, therefore, in this trial, different intakes of protein substitute were only measured as outcomes.
MacDonald 2005	Intervention not eligible for inclusion.
MacDonald 2006a	Intervention not eligible for inclusion.
MacDonald 2006c	Participants, intervention, and outcomes not eligible for inclusion.
MacDonald 2008	Intervention not eligible for inclusion.
Marsden 2005	Participants and intervention not eligible for inclusion.
Matalon 2006	Intervention not eligible for inclusion.
Matalon 2007	Intervention not eligible for inclusion.
Rose 2005	Intervention not eligible for inclusion.
SHS 2001	Not a CCT or RCT (ascertained by communication with co-ordinator of original study at SHS).
Stroem 2011	Intervention not eligible for inclusion.
ten Hoedt 2011	Intervention not eligible for inclusion.

CCT: controlled clinical trial

PKU: phenylketonuria
 RCT: randomized controlled trial

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

Giovannini 2006

Methods	Randomised parallel trial. Generation of allocation sequence: randomization process not described. Allocation concealment: unclear. Blinding: unclear. Inclusion of all randomized participants in the final analysis: unclear.
Participants	Number of participants: 13 enrolled, ages 5 to 26 years. Inclusion criteria: none indicated; title and text indicate diagnosis with phenylketonuria as a criterion. Exclusion criteria: none indicated.
Interventions	1. 100% of total nitrogen needs from slow-release protein substitute (6 months) 2. 80% of total nitrogen needs from slow-release protein substitute (6 months)
Outcomes	1. Plasma phenylalanine concentrations 2. Plasma amino acid concentrations 3. Plasma protein concentrations (albumin, total protein, and transferrin)
Notes	Location: Italy. The lead investigator will be contacted for further information needed to complete the assessment of trial eligibility.

Lange 2004

Methods	Open, parallel trial. Method of allocation not described. Inclusion of all participants in the final analysis: unclear.
Participants	Number of participants: 20, ages 6 to 8 years Inclusion criteria: none indicated; title and text indicate diagnosis with PKU as a criterion. Exclusion criteria: none indicated.
Interventions	1. Treatment with both Milupa PKU 2 and PKU 2 mix for 6 months 2. Treatment with both Milupa PKU 2 only for 6 months
Outcomes	1. Anthropometric parameters (not otherwise described) 2. Diet intake of chosen nutrients (not otherwise described)
Notes	Location: Poland. The lead investigator will be contacted for further information needed to complete the assessment of trial eligibility.

MacDonald 2003b

Methods	Randomised controlled cross-over trial.
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Protein substitute for children and adults with phenylketonuria (Review)

MacDonald 2003b (Continued)

Generation of allocation sequence: randomization process not described.
Allocation concealment: adequate.
Blinding: not used.
Inclusion of all randomized participants in the final analysis: No.

Participants	Number of participants: 16 enrolled, 15 included in analysis, ages 1 to 10 years. Inclusion criteria: well controlled plasma phe concentrations over the past 6 months; parents able to collect blood specimens. Exclusion criteria: none indicated.
Interventions	1. Protocol A: protein substitute administered in 3 equal doses over a 10-hour period (7 days) 2. Protocol B: protein substitute administered in 3 equal doses over a 14-hour period (7 days) 3. Protocol C: protein substitute administered in 4 equal doses over a 14-hour period (7 days) 4. Protocol D: protein substitute administered in 6 equal doses over a 24-hour period (3 days)
Outcomes	1. 24-hour plasma phenylalanine variation 2. 24-hour dietary phenylalanine distribution 3. Energy intake (% EAR) Outcomes not reported in manuscript: 4. Other nutrient intakes (carbohydrates, fat)
Notes	Location: UK. Initially only 13 participants were randomised into protocols A, B, and C. A further 3 participants were randomized into protocols A, B, and D. Since the 3 participants were introduced after the initial randomisation phase, their results may not be included in the review. At the present time only information from protocol C can be used as the extra participants did not enter this group. Individual patient data must be sought from the author. This would enable the 3 extra participants to be identified within groups A and B and D and excluded from the analysis.

EAR: estimated average requirements

Phe: phenylalanine

PKU: phenylketonuria

RCT: randomised controlled trial

WHAT'S NEW

Date	Event	Description
28 January 2015	New search has been performed	A search of the Cystic Fibrosis and Genetic Disorders Group's Inborn Errors of Metabolism Register identified 51 references. A total of 43 records were excluded after preliminary screening of titles or abstracts (or both). Of the remaining eight potentially eligible trials, six were evaluated and then added to the 'Excluded studies' section, a further trial is already listed in the 'Studies awaiting classification' section (Giovannini 2006) and the remaining trial has been added to 'Studies awaiting classification' (Lange 2004).
28 January 2015	New citation required but conclusions have not changed	Minor changes have been made throughout the text for this update.

HISTORY

Protocol first published: Issue 2, 2004

Protein substitute for children and adults with phenylketonuria (Review)

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Review first published: Issue 4, 2005

Date	Event	Description
11 March 2011	New search has been performed	A search of the Group's Inborn Errors of Metabolism Trials Register identified eleven potentially relevant references, although only one was eligible for inclusion in the review. This was an additional reference to an already excluded study (MacDonald 2006c).
13 May 2009	Amended	No changes - republished to fix technical problem.
18 July 2008	New citation required but conclusions have not changed	New review team in place.
18 July 2008	New search has been performed	<p>Two new trials have been included in the review (MacDonald 2006b; Schindeler 2007). Note: MacDonald 2006b was previously listed in 'Ongoing studies'.</p> <p>Fourteen new trials have been added to the 'Excluded studies' section of this review (Agostini 2006; Baumgartner 2004; Cleary 2003; Cleary 2006; Kalkanoglu 2005; Koletzko 2007; Levy 2007; MacDonald 2003a; MacDonald 2005; MacDonald 2006c; Marsden 2005; Matalon 2006; Matalon 2007; Rose 2005). Note: Cleary 2003 was previously listed in 'Studies awaiting assessment'.</p> <p>One new study has been added to 'Studies awaiting assessment' (Giovannini 2006).</p>
10 July 2008	Amended	Converted to new review format.
1 September 2006	New search has been performed	Five new trials have been identified by the latest search. Four of these are now listed in 'Excluded studies' (Baumgartner 2004; Kalkanoglu 2005; MacDonald 2005; Rose 2005) and one in 'Studies awaiting assessment' (Marsden 2005).

CONTRIBUTIONS OF AUTHORS

Sarah H L Yi drafted the review.
 Rani H Singh commented on the content of the review.
 Both authors undertook trial selection and data extraction.
 Rani H Singh acts as the guarantor of the review.

DECLARATIONS OF INTEREST

Sarah H L Yi : none known.
 Rani H Singh has served on an international advisory board (Nutricia).

SOURCES OF SUPPORT

Internal sources

- No sources of support supplied

External sources

- National Institute for Health Research, UK.

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INDEX TERMS**Medical Subject Headings (MeSH)**

*Food, Formulated; Dietary Proteins [*administration & dosage]; Phenylalanine [*blood]; Phenylalanine Hydroxylase [deficiency]; Phenylketonurias [diet therapy] [*therapy]; Randomized Controlled Trials as Topic

MeSH check words

Adult; Child; Humans