

**Evidence-based enteral feeding for
preterm or low birth weight infants-
Systematic review of the use of protein
hydrolysate formula**

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1. Thesis abstract

Infants born preterm, especially those born very preterm, are at elevated risk of mortality and morbidity secondary to organ immaturity and exposure to intensive and invasive care practices and procedures. Although care and outcomes for preterm infants have improved substantially over the past forty years, major challenges remain including the need for better strategies to prevent or treat complications such as necrotising enterocolitis and severe infection. These complications are the most common causes of death and disability after the early neonatal period for preterm infants and are associated with life-long health consequences and costs.

This thesis first presents an overview of the epidemiology, causes, and risk factors for preterm birth, and a summary of the interventions for improving outcomes for preterm infants. I then describe the current understanding of the pathogenesis of necrotising enterocolitis, its impact on growth and development, and the evidence-base for interventions to prevent this condition. This discussion focusses on nutritional strategies, and particularly on how the timing and type of enteral feeding affects gut physiology and health, feed tolerance, and the risk of necrotising enterocolitis in preterm infants.

The main body of the thesis consists of a Cochrane review of a specific enteral feeding option for preterm infants – the use of formula containing hydrolysed protein rather than standard formula. This costly strategy has become widely adopted in high-income countries based on perceptions that protein hydrolysate formulas are tolerated better by the immature gastro-intestinal tract, and are less likely to lead to complications including necrotising enterocolitis. Using Cochrane methods, we conducted the first systematic review of the evidence-base for this intervention. We found ten eligible randomised controlled trials (total participants 600). Meta-analyses did not show any significant differences in feed intolerance or necrotising enterocolitis, calling into question current policies and practice in neonatal units in high-income countries.

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6.Declaration

I confirm that this work is original and that if any passage(s) or diagram(s) have been copied from academic papers, books, the internet or any other sources these are clearly identified by the use of quotation marks and the reference(s) is fully cited. I certify that, other than where indicated, this is my own work and does not breach the regulations of HYMS, the University of Hull or the University of York regarding plagiarism or academic conduct in examinations. I have read the HYMS Code of Practice on Academic Misconduct, and state that this piece of work is my own and does not contain any unacknowledged work from any other sources

Part 1: Background

7. Preterm birth

7.1 Introduction

Preterm birth is defined as the delivery of a baby before 37 weeks' gestation and "very preterm" birth is classed as the delivery before 32 weeks' gestation (1). Preterm birth is a major health problem with the global incidence of preterm live births at approximately 10% and the incidence of very preterm birth is about 1-2% (2). Approximately 15 million babies are born preterm each year and in 2012 alone, around one million babies died as a result of preterm birth (3). Preterm births make up three quarters of perinatal mortality and over 50% of long-term morbidity (4). This poses an enormous challenge for those involved in perinatal health care with most perinatal deaths occurring in preterm infants. In addition to the effect on infants, there are increased costs for health services involved as preterm infants may require several months in hospital for their care on top of the emotional and financial strain on the infants' families.

In most high-income countries, and some middle-income countries, advancements in neonatal care have led to more preterm babies surviving into adulthood. Clinicians and researchers face the challenge of meeting their long-term health problems which were previously uncharted as many very preterm infants did not live long enough to present with problems such as cerebral palsy and neurodevelopmental disabilities. Further investigation and follow-up of preterm survivors are needed to determine long-term effects and problems which may arise later in life due to their preterm birth.

7.2 Epidemiology

The United Nations Millennium Development Goals 4 and 5 targeted a two-thirds reduction of under-five deaths as well as a 75% reduction in the maternal mortality ratio between 1990 and 2015. Despite recent improvements in neonatal and maternal mortality, epidemiological studies show that there is an estimated 3 million neonatal deaths annually accounting for more than 40% of all deaths in children younger than 5 years of age. (5)

The WHO defines preterm birth as the delivery of an infant between 20 and 37 weeks' gestation. The preterm delivery rate is reported at around 11% in the United States and between 5-7% in Europe (4). There have been attempts to subdivide infants born preterm in conjunction to their gestational age (4). The WHO sub-categorises them as follows:

- Extremely preterm (<28 weeks' gestation)
- Very preterm (28 to 31 weeks' gestation)
- Moderate to late preterm (32 to 37 weeks' gestation)

This more distinct classification aids epidemiological studies into the understanding of prevalence and aetiologies of preterm birth as well as searching out improved intervention strategies. However, inconsistent classification of foetal loss, still birth, and early neonatal death in some countries means that infants who are born after very short gestations (less than 24 weeks) are more likely to be categorised as live births, potentially skewing the data (1).

7.3 Low birthweight

Low birthweight is classified by the WHO as the weight at birth of less than 2,500 grams and are based on the epidemiological observations that infants weighing less than 2,500 grams are approximately 20 times more likely to die than heavier babies (6). Most low birthweight infants are as a result of either preterm birth or restricted foetal growth. While most low birthweight babies survive, they are subject to increased risk of multiple impairments and complications including neurodevelopmental, gastrointestinal and respiratory as well as chronic diseases later in life.

Mothers with low socio-economic conditions frequently have low birthweight infants. Of the 20 million infants worldwide, 15.5% are born with low birthweight with a staggering 95.6% of them living in developing countries (3).

7.4 The cost of preterm birth

There is also a huge financial consequence of the high numbers of preterm births as they require the infrastructure, equipment and staffing to provide care and support for the babies and their parents. In 2005, the Institute of Medicine (IOM) estimated that the societal cost of preterm birth in the United States annually was US\$26 billion. This value incorporated medical care costs up to age 5 years for those born preterm, maternal delivery costs and the costs of early intervention. It also included disability-specific costs, special education and lost productivity for four specific developmental disabilities (cerebral palsy, neurocognitive impairment, visual impairment and hearing loss) that are associated with preterm birth. (7) However, this estimate does not include the cost of other long-term implications of preterm birth such as asthma, learning disabilities, attention deficit hyperactive disorder and emotional problems amongst a range of outcomes. Additionally, the costs incurred by the families of the preterm infant such as transportation, accommodation and childcare for other siblings during hospitalizations and outpatient visits (8, 9). It may also affect employment leading to a decline in family income. In a study of the families of 224 preterm low birthweight infants, only 52% of women returned to work six months after their infant was discharged from hospital with the majority of them returning to work later and with fewer hours than originally anticipated (8).

7.5 Aetiology of preterm birth

Preterm birth can be categorised broadly as:

- *Spontaneous* preterm birth, which occurs naturally due to preterm labour or preterm rupture of foetal membranes.
- *Indicated* preterm birth, where labour is medically induced because of pregnancy complications.

7.5.1 Spontaneous preterm birth

Most of preterm births are spontaneous, meaning labour is unexplained or is due to spontaneous preterm pre-labour rupture of the amniotic membranes (PPROM). PPRM is defined as spontaneous rupture of the membranes at fewer than 37 weeks' gestation and at least one hour before the onset of contractions. There is some evidence to suggest that infections are risk factors for PPRM (discussed below) (10). The membranes form a natural barrier to ascending infection, but in many cases of membrane rupture, an asymptomatic intrauterine infection is deemed to have a putative role (11). Other risk factors for spontaneous preterm birth include cervical incompetence, multiple pregnancies and various socioeconomic factors.

A history of preterm birth and low socioeconomic factors of the mother are important indicators for spontaneous preterm delivery (1). Whilst numerous factors such as tobacco smoking and drug misuse have been associated with preterm birth, their association and interaction is complex. Spontaneous preterm birth varies with ethnicity and is most commonly caused by preterm labour in white women, but by PPRM in black women (2). Further research is needed to determine the aetiology of preterm birth.

7.5.2 Indicated preterm birth

In some cases, preterm birth is iatrogenic as it is safer to deliver the infant despite the risks associated with preterm birth (12). This decision is made based on the optimal outcome for the mother, baby, or both requiring weighing up the risks of allowing the pregnancy to continue versus the risk of delivery before term.

Conditions that warrant indicated preterm birth can be divided into placental, maternal or foetal:

- Placenta previa, placenta accreta and chronic abruptio placentae are conditions that carry a significant risk of foetal compromise (12). The risk to the mother in these circumstances include severe obstetrical haemorrhage which could result in shock, a need for transfusion, disseminated intravascular coagulation, and death.
- Maternal and obstetric factors such as pre-eclampsia or a history of stillbirth serve as red flags to consider indicated preterm birth. Up to 8% of stillbirths are recurrent and they are more likely to occur in those with a history of prior loss, accompanied foetal growth restriction and those of black race (13).
- Indicated preterm birth may also be necessary when the foetus presents with conditions or complications which could lead to stillbirth. Foetal growth restriction and multiple gestations are some of the circumstances where removing the foetus from a gradually hostile intrauterine environment may be required. Foetal growth restriction can lead to foetal death and long term neurologic sequelae as the foetus is starved of reaching its potential for intrauterine growth. Multiple gestations also pose a problem for intrauterine growth (14).

The overarching aim of indicated preterm birth is to offset the risks to the mother and baby if the mother proceeds to carry to term. Informed decision-making and family counselling should be offered for this to proceed.

7.6 Risk factors for spontaneous preterm birth

7.6.1 Infection/Inflammation

Bacteria can reach the amniotic fluid by the process of ascending infection by penetrating the cervical barrier and entering the uterus (15). The bacteria causes multiple physiological events associated with preterm birth once in the uterine space. These include raised levels of proinflammatory cytokines, chorioamniotic membrane rupture, cervical ripening and uterine contraction. Group B streptococci (GBS) are one group of bacteria responsible for this phenomenon. Approximately 30% of healthy women are recto-vaginally colonised by GBS making it a leading cause of neonatal morbidity and mortality. However, the mechanism of GBS colonisation and ascending infection is still unclear and research in this area is on-going.

Normal vaginal flora is dominated by lactobacilli, a gram-positive non-sporing rod, which keeps the pH of the vagina below 4.5 by producing lactic acid. *Lactobacillus* spp. stability and dominance are integral to reproductive health in the vagina. A decrease in levels of *Lactobacillus* can lead to vaginal dysbiosis, which is associated with preterm birth.

Women diagnosed with bacterial vaginosis have a two- to six-fold raised risk of preterm birth and late miscarriage (16). The presence and dominance of lactobacilli in the vagina is associated with a reduced risk of bacterial vaginosis and urinary tract infections as it limits the growth of other organisms.

In bacterial vaginosis, a disruption of the balance of flora with a reduction in the quality and/or quantity of lactobacilli, there is a 1,000-fold increase in growth of other organisms. *Mobiluncus* species and anaerobes are organisms linked with bacterial vaginosis. They can produce a keto acid capable of suppressing the chemotactic response leading to an increase in the numbers of organisms in the absence of an inflammatory response. Consequently, there is a large concentration of potentially pathogenic organisms with no distinct cellular host response (17). Certain *Lactobacillus* strains can colonise the vagina after vaginal suppository use with the hope of reducing the risk of urogenital infections. This has led to research into whether restoration by probiotic therapy can restore normal flora and improve the chance of having a healthy term pregnancy (18). Probiotics are live micro-organisms which provides a beneficial health effect on the host when administered in an

adequate amount (19). They have been shown to prevent preterm labour by interfering with the inflammatory response that leads to preterm labour and delivery (20, 21).

Cytomegalovirus (CMV) is the most common infectious cause of congenital and perinatal viral infection in the world (22). Around half of infants born to mothers who acquire a primary CMV infection during pregnancy will be born with a congenital CMV infection (23). CMV infection can result in permanent neurological sequelae including neurodevelopmental delays, motor disabilities, deafness, and blindness (24). Each year, in the United States alone, an estimated 40,000 children are affected with an estimated 400 deaths and 8,000 children left with permanent disabilities costing upwards of USD1-2 billion (25, 26).

Malaria and syphilis are important contributors to a wide range of adverse pregnancy outcomes in low or middle-income countries (LMICs). Around 125 million women become pregnant each year in malaria-endemic areas (27) and there is a strong association between malaria infection in pregnancy and low birthweight infants which is thought to be a result of maternal anaemia caused by erythrocyte invasion by *Plasmodium falciparum* (28). *P. falciparum* is also associated with stillbirth and preterm delivery (29, 30). Despite extensive knowledge on the epidemiology and burden of malaria in pregnancy, there are gaps in the effect of infection in the first trimester and the longer-term effects of malaria in childhood beyond infancy, especially outside Africa where most studies are conducted. This will help attain better estimates of the effects of malaria on maternal and infant morbidity and mortality worldwide (28).

7.6.2 Pregnancy history

As a result of the advancements and increasing use of assisted reproductive techniques, the incidence of multiple pregnancies has increased over the past few decades (1). Multiple pregnancy increases the risk of preterm delivery compared to singleton pregnancies (31). Multiple gestations account for 2-3% of pregnancies but contribute up to 20% of preterm births (11). Over half of twins are born preterm and nearly all higher multiple gestations will result in a preterm delivery. Those with a history of previous preterm deliveries have a more than two-fold risk of preterm birth in their next pregnancy with the exact mechanism of recurrence unknown. One school of thought attributes this to the persistent or recurring intrauterine infection, explaining repetitive spontaneous births (15).

7.6.3 Ethnicity

In the US, there is an increased rate of preterm births amongst black women (16-18% compared to 5-9% for Caucasian women). Asian and Hispanic women have lower preterm birth rates (32). It remains unclear, however, whether these associations are mediated by co-existence of other risk factors linked to socio-economic status such as nutritional status and smoking behaviours.

7.6.4 Smoking

Cigarette smoking is one of the most important and modifiable risk factors associated with preterm birth (33). In the United States, up to 25% of pregnant women smoke which confers an almost two-fold risk of preterm birth (11, 34). It has been suggested that smoking is responsible for 15% of all preterm births. The exact mechanism is unclear as there are more than 3000 chemicals in cigarette smoke and the biological effects of most are unknown. However, the two major compounds in cigarette smoke are nicotine and carbon monoxide. Nicotine can cause a decrease in uteroplacental blood flow and is associated with placental damage (35). Carbon monoxide can cross the placenta and inhibit the availability of oxygen to the foetus (36). The Cardiff Birth Survey, which collected data from more than 50,000 births between 1970 and 1979, found a dose-dependent increase in the risk of preterm birth. Women who smoked >20 cigarettes per day had an adjusted odds ratio for preterm birth of 1.30 (95% CI 1.15-1.7) (37).

Smoking cessation programmes have been at the forefront of efforts to reduce the incidence of preterm birth and Scotland's public smoking ban legislation is a prime example of a successful intervention (38).

7.6.5 Stress

Stress can play a part in increasing the risk of preterm birth. Mothers with raised levels of psychological or social stress are at almost double the risk of preterm birth (11). Stressful situations such as financial and material hardships have been associated with preterm birth (39). Systemic inflammation has been hypothesised as an avenue by which stress can increase the risk of preterm birth (40). Another mechanism proposed to cause preterm birth via maternal stress are neuroendocrine pathways where an early or greater degree of activation of the maternal-placental-foetal endocrine systems that promote parturition (41). The exact mechanism of stress on preterm birth, much like many other risk factors, is unknown.

7.7 Prevention of preterm birth

7.7.1 Primary prevention

Primary prevention aims to prevent or reduce the risk of preterm birth before or during pregnancy and is targeted towards all women (42). Lifestyle adjustments such as smoking cessation, nutrition and body mass index can influence the risk of preterm birth (37, 43).

Cigarette smoking is widely linked to preterm birth and intrauterine growth restriction (44). Both active and second-hand smoking are associated with pregnancy complications. In March 2006, the Scottish government introduced a landmark legislation prohibiting smoking in public space and it has been extremely successful in reducing the exposure to environmental tobacco smoke in public spaces. Additionally, there seemed to be a 11% reduction in the number of preterm deliveries as a result a decrease in the number of smokers due to this intervention (38). This is a fine example of how policies and legislature can influence both immediate and long-term health outcomes.

Decisions regarding the number of embryos transferred in artificial reproductive techniques may affect the risk of preterm birth due to the significantly increased risk of preterm birth in multiple gestations (45).

7.7.2 Secondary prevention

Secondary prevention is aimed at women who have a pre-existing risk of preterm birth. A history of previous preterm birth is the strongest risk factor for preterm birth (46). Much of the research conducted on preterm birth is focused on the preventative measures in this specific 'at risk' group.

Foetal fibronectin

Foetal fibronectin is a glycoprotein produced in foetal tissues which is believed to have a role in implantation and placental attachment to the uterus (47). It can be detected in cervical and vaginal secretions prior to 20 weeks' gestation but its presence after 22 weeks usually suggests a disruption of the uteroplacental interface (48). It has been hypothesised that damage to the foetal membranes may release foetal fibronectin into the cervix and vagina (49) which has led to the thinking that measurement of foetal fibronectin in cervicovaginal secretions may be used as a predictive test for preterm labour. Foetal fibronectin has been shown to have a good negative predictive value.

A Cochrane systematic review conducted by Berghella and colleagues concluded that although foetal fibronectin is commonly used in labour and delivery units to aid in the management of women with symptoms of preterm labour, there is insufficient evidence to recommend its use. They have encouraged further research after finding an association between knowledge of foetal fibronectin results and a lower incidence of preterm birth before 37 weeks (50).

Cervical cerclage

Around 2 million cervical cerclages are performed annually to prevent preterm birth. Cervical cerclage is indicated in women with a history of preterm birth and/or a short cervical length and can reduce the risk of preterm birth by around 20%. Both the American and UK Royal College of Obstetricians and Gynaecologists currently recommend its use (51, 52).

In normal pregnancy, the cervix remains tightly closed for the duration of the pregnancy until near the end where it starts to shorten and progressively becomes softer in preparation for normal labour and delivery. However, in some instances the cervix shortens and dilates too early leading to either miscarriage or preterm birth (53).

A cervical cerclage is a surgical procedure which involves placing a purse-string stitch around the cervix, sometimes dissecting the bladder away from the cervix although there is no evidence of benefit to do so (54). The procedure is thought to provide the cervix with structural support and maintain the endocervical mucus plug as a barrier to ascending infections from the vagina (55).

Cervical cerclage seems to be appropriate only in the setting of a structural defect or deficiency that needs to be repaired, but identification of appropriate candidates has proved difficult (56).

The procedure does not come without risks as the surgical manipulation of the cervix may cause uterine contractions, bleeding and labour which could ultimately end in miscarriage or preterm labour (53). There is also debate on precisely what cervical length to intervene at. However, it is widely acknowledged that cervical cerclages are not appropriate for multiple gestations (57).

A Cochrane review on whether cervical stitches prevented preterm birth in singleton pregnancies demonstrated that the placement of cervical cerclage in women at risk of preterm birth reduced the risk of preterm birth. However, the authors heed caution in interpreting these results as prolonging the pregnancy does not necessarily equate to improvement of outcomes for the baby. They stress the possibility of causing harm by keeping the baby in a 'hostile' uterine environment (53).

Progesterone

Since its synthesis in 1935, progesterone has been proposed and used in treatments of various gynaecological pathologies with many extensive studies conducted. It is proposed that progesterone can prevent preterm birth by counteracting the stimulatory effects of prostaglandins (58), lowering the concentration of oxytocin receptors (59) (leading to relaxation of smooth muscle) and inhibiting gap junction formation (60). A meta-analysis reviewing the use of 17 alpha-hydroxyprogesterone caproate showed no correlation between 17 alpha-hydroxyprogesterone caproate and reduced risk of miscarriage but did conclude that there was a reduction in occurrence of preterm birth (61). Other studies

demonstrated a reduction of preterm births in women with previous preterm delivery who were given progesterone (62, 63).

However, progesterone does not have universal effects in all populations at risk. A randomised placebo-controlled trial reported that 17 alpha-hydroxyprogesterone caproate had no effect on the rate of preterm birth in 600 women with twin pregnancies. The inconsistency of effects amongst women indicates that supplemental progesterone compounds do not influence some pathways to recurrent preterm birth (56). A recent randomised controlled trial investigating the outcomes of progesterone prophylaxis found no reduced risk of preterm birth or associated adverse outcomes. It also followed up children up to two years of age and found no long term benefits or significant harm caused (64). This is a good example of a large, high quality randomised controlled trial which has overturned the findings of the amalgamation of several smaller trials.

Antibiotics

Bacterial vaginosis is the imbalance of vaginal flora because of imbalances in *Lactobacillus* bacteria which normally regulates the growth of other bacteria. Bacterial vaginosis is often asymptomatic and presents in up to 20% of women during pregnancy (65). Evidence suggests that bacterial vaginosis in pregnancy is associated with poor perinatal outcomes such as increased risk of preterm birth and the associated neonatal sequelae (66). The idea of using antibiotics, especially metronidazole and clindamycin, has been proposed to treat bacterial vaginosis in pregnancy. The aim is to reduce the overgrowth of abnormal anaerobic bacteria, restore the lactobacillus bacteria population and ultimately, prevent an inflammatory response which could lead to preterm labour.

The authors of a Cochrane review investigating the use of antibiotics for bacterial vaginosis in pregnancy concluded that although antibiotic treatment could eradicate bacterial vaginosis in pregnancy, the effect on reducing preterm births was not significant (67).

7.7.3 Tertiary prevention

Tertiary preventions involve detection of conditions close to the time of preterm birth and provide a window to improve outcomes, for mother and baby. Some tertiary interventions can prolong pregnancy but not long enough to promote intrauterine growth and maturation. They can, however, provide precious time to transfer the mother and foetus to a facility better equipped to care for preterm infants (56). This has been shown to improve outcomes for preterm infants (68).

Antenatal corticosteroids have significantly reduced the neonatal mortality rate since its widespread inception in the 1980s. Glucocorticoids promote maturation over growth. In the lungs, surfactant synthesis is increased along with other beneficial respiratory outcomes.

Magnesium sulphate has been assessed for its tocolytic properties and a recent Cochrane review determined that magnesium sulphate did not have a significant impact on delaying birth or preventing preterm birth (69). Magnesium sulphate is also used as an effective intervention for women with pre-eclampsia (70). Good-quality randomised controlled trials and meta-analyses have indicated that magnesium sulphate can confer neuroprotective effects on very preterm infants, with the risk of cerebral palsy reduced by one-third (71). The use of magnesium sulphate for neuro-protection of very preterm infants have been adopted in Australia. In the UK, the Royal College of Obstetricians and Gynaecologists have endorsed the practice. Despite this, its use has not yet become established widely due to concerns about side effects, the large number needed to treat for benefit and some uncertainty about the applicability of some data (71).

Tocolytic drugs have been used to prolong pregnancy in pregnant women with an acute risk of preterm birth. The reasoning behind the use of these drugs to delay delivery is to allow transfer to a specialist unit and antenatal corticosteroid administration to reduce neonatal morbidity and mortality (56).

7.8 Complications of preterm birth

Biologically, because of their small size and organ immaturity, preterm infants are more susceptible to a range of complications than their term counterparts. Initially, the main challenge is respiratory and thermal stability, progressing during the first few days and weeks after birth to challenges related to gastro-intestinal immaturity and the need to provide optimal nutritional support (while minimising the risk of necrotising enterocolitis and severe infection). Related to these, the ultimate challenge and aim of care for preterm infants is to ensure survival without adverse neurodevelopmental consequences (cerebral palsy, sensory deficits, cognitive impairment or behavioural difficulties) (72).

7.8.1 Respiratory sequelae

Surfactant

Foetal breathing movements begin as early as 10 weeks of gestation. The breathing of amniotic fluid in and out is essential for the stimulation of lung development. The failure of foetal breathing or lack of amniotic fluid that can be breathed in and out can result in underdeveloped lungs (i.e. pulmonary hypodysplasia), which can be incompatible with extrauterine life. At around 30-32 weeks' gestation, the lungs produce surfactant, a substance which enables the alveoli to remain patent. Infants born before 28-30 weeks' gestation lack surfactant and this deficiency could lead to apnoea and ultimately, death.

Respiratory distress syndrome

Most infants born before 28 weeks' gestation will develop respiratory distress syndrome (73). RDS is associated with surfactant deficiency. Its incidence increases with decreasing gestational age and is higher among white infants than African Americans at each week of gestation. Antenatal glucocorticosteroids for women at risk for preterm delivery reduces the incidence and severity of RDS as well as the rate of mortality. Post-partum, RDS can be treated with respiratory support such as oxygen, positive airway pressure, ventilator, and administration of more surfactant which reduces mortality, air leak and chronic lung disease. However, exogenous surfactant does not influence neurodevelopmental or long-term pulmonary outcomes (74, 75).

Apnoea is another complication of preterm birth where infants may cease to breathe for 20 seconds or more (76), potentially leading to bradycardia or hypoxaemia and can result in longer-term complications. This can be attributed by immaturity of the respiratory system

or a mechanical obstruction. Strategies used to treat apnoea include caffeine, which is the predominant drug used, doxapram and positive pressure ventilation.

Bronchopulmonary dysplasia

Bronchopulmonary dysplasia is a chronic lung disease that can follow RDS in preterm infants which results from inflammation, injury and scarring of the airways and alveoli. There is an association between BPD and neurodevelopmental delays during childhood. The main cause of BPD is lung immaturity but can be accentuated by other factors that contribute to lung injury including high oxygen concentrations, infections and other inflammatory triggers.

The use of systemic postnatal corticosteroids (especially dexamethasone) has been the issue of debate among neonatologists (77). Although over 40 randomised controlled trials showed improved respiratory outcomes, there were also side effects including glucose problems, higher blood pressure and growth failure. Long-term effects of systemic steroids including higher rates of cerebral palsy and cognitive impairment have been reported (78-80).

7.8.2 Neurodevelopmental Sequelae

Preterm or low birth weight infants are at increased risk of developing motor, cognitive and behavioural impairment compared to their term counterparts (81). Although rates of survival for extremely low birth weight infants since the 1990s have improved, the rate of disability has remained fairly constant with up to 50% of these infants exhibiting developmental disabilities such as motor, cognitive or behavioural impairment (82, 83).

Cerebral palsy is the most common development disorder of preterm infants and has many associated long-term disabilities (84). It is a complex syndrome with various contributing aetiologies but the most likely reason is due to brain damage in preterm infants, parenchymal and intraventricular haemorrhage and white matter injuries (85). Children with cerebral palsy often have slower developmental stages compared to their counterparts and early signs usually appear before they reach three years of age (84). Cerebral palsy is classified into different types based on the type and nature of the motor disability as well as neurological signs and symptoms. Prevalence of cerebral palsy has been shown to be

inversely related to gestational age (72). It has been suggested that low birth weight, neonatal encephalopathies and high risk pregnancies are the most important risk factors for cerebral palsy. Maternal cigarette smoking is also linked to cerebral palsy due to the association between smoking and decreased birth weight. (86). Intellectual disability is often present along with another disability state especially cerebral palsy (87).

Intraventricular haemorrhage (IVH) is a major complication of prematurity and presents a critical problem in neonatal intensive care units around the world (88). IVH is instigated in the periventricular germinal matrix which is a highly vulnerable to haemorrhage in the first 48 hours of life. When haemorrhage in the germinal matrix is substantial, the cerebral ventricle can fill up with blood. Infants can vary from being asymptomatic to having a catastrophic deterioration. This reduces the risk of survival and greatly enhances the risk of neurological sequelae developing (89).

Hearing loss is 20 times more likely in preterm infants than their full-term normal weight peers (84). This can lead to difficulties discriminating simple speech sounds and poorer auditory recognition. This may also have the cumulative effect of slower and more difficult acquisition of language skills and learning at school (90). Hearing screening is recommended to all neonates before discharge from hospital (91).

Additional impairments such as delays in perceptual and cognitive, motor development, language development and neurobehavioral development have been observed in children born preterm. These adverse outcomes are an increasing problem for the infants and their families and many require intensive and continuous care.

In a large cohort study conducted by Wood and colleagues, data on all preterm infants born before 26 weeks' gestation in the United Kingdom and Ireland in a ten-month period was collected. An assessment was done at a median of 30 months corrected age which showed approximately 50% of survivors had a disability in the domain of mental and psychomotor development, neuromotor function, or sensory and communication function. A quarter of those involved could also be classified as severely disabled (92).

Symington et al hypothesised that modification of the unfavourable environment in the neonatal intensive care unit (NICU) could help compound the morbidity and minimize the iatrogenic effects and reducing stress for the infant (93).

Spittle et al looked into the effectiveness of early developmental intervention programmes given post hospital discharge to prevent motor or cognitive impairment in preterm infants versus standard medical follow-up of preterms at infancy, preschool age, school age and adulthood (94). They concluded that early intervention programmes for preterm infants have a positive influence on cognitive and motor outcomes during infancy, with cognitive benefits persisting into preschool age.

7.8.3 Gastrointestinal sequelae

The gastrointestinal tract has an important immune and endocrine functions as well as digesting and absorbing food. Preterm infants face challenges in digesting nutrients due to the immaturity of gastrointestinal tract (72). Preterm birth can lead to feed intolerance where preterm infants have difficulty digesting food required for continued growth and development. These infants will require parenteral nutrition and may also need tube feeding because they have not yet developed the ability to coordinate sucking, swallowing and breathing (95).

Along with feed intolerance, gastrointestinal reflux is another common condition in both preterm and term infants which could adversely affect growth. Necrotising enterocolitis is another gastrointestinal complication of preterm birth with high morbidity and mortality which will be discussed in further detail below (96).

8. Growth and nutrition

Advances have been made in the nutrition of infants, particularly those born preterm, in recent years but there remain many areas of concern which lack high quality evidence. Extremely preterm infants have challenging nutrient demands which if not met will result in inadequate growth. This is associated with poor neurodevelopmental outcomes which could lead to long-term problems.

Extremely preterm infants are born at a stage when they would usually be growing rapidly in utero. To match growth in utero, an infant born at 24 week's gestation would need to double its birthweight by 30 weeks' and at 40 weeks, almost increase it five-fold (97). Extremely preterm infants have poor gastrointestinal motility resulting in prolonged gastric emptying time. They are also at risk of gastro-oesophageal reflux, feed intolerance, delayed meconium drainage and intestinal dilation. To further complicate issues, extremely preterm infants have low stores of key nutrients with depleted subcutaneous fat and glycogen stores as these are provided in the third trimester of pregnancy.

The healthcare team looking after the preterm infant face challenges due to the physiological immaturity of the preterm baby. Fluid, glucose and electrolyte imbalances are common occurrences in the first few days coupled with immature organ systems can lead to respiratory distress and thermoregulation problems. The fragile nature and complexity of caring for the preterm infant is highlighted with large amounts of early intake of fluids associated with an increased risk of bronchopulmonary dysplasia and necrotising enterocolitis despite a high demand for energy and fluid.

Furthermore, the structural and functional immaturity of the gastrointestinal tract means that the preterm infant has a poor initial tolerance to enteral feeds. They also may not have coordinated the motions of sucking, swallowing and breathing which does not occur until around 32 to 34 weeks' post-menstrual age.

Current practice is to initiate intravascular fluids immediately after birth and provide parenteral nutrition until full enteral feeds are tolerated. Enteral feeds can be given as small volumes via either an oro-gastric or naso-gastric tube with the volumes incrementally increased. However, this process can be episodic with a reduction of volumes if feeds are

not tolerated and typically take 7 to 14 days and is not uncommon to take even longer. If breastmilk is available to the preterm infant, fortifiers are often added to increase the energy, protein and micronutrient content as breast milk alone cannot fulfil the preterm infants' energy and nutritional demands. Enteral tube feeding will be continued until the infant has matured enough to coordinate sucking, swallowing and breathing which is typically at around 32 to 34 weeks' post-menstrual age.

Nutrition

Preterm and low birth weight infants require extra attention regarding nutrition from birth as it can have a substantial impact on clinically important outcomes such as necrotising enterocolitis, invasive infections and short-term growth. These complications may increase the risk of mortality and other morbidities as well as affecting long-term growth and neurodevelopmental outcomes. (98)

Protein

The amount of protein needed to sustain normal growth varies depending on the infant's growth rate and postconceptional age. It is estimated that at least 3 - 3.5g/kg of amino acid intakes are needed to have sustained nitrogen retention and growth rates similar to the foetus in utero (99). Brain growth and cognitive function are directly related to protein intake during the neonatal period in a preterm infant (100).

Lipids

Lipids have a high energy content per unit volume and a delay in lipid administration in the first week after birth can lead to essential fatty acid deficiency (101).

Glucose

Glucose plays a substantial role in the growth and development of preterm infants, especially in terms of neurodevelopment as it is the main source of fuel for the brain. Hypoglycaemia is a problem for preterm infants due to their limited glycogen and fat stores along with inadequacies of their glucose-insulin axis due to their prematurity (102). Insufficient brain glucose supply can result in brain injury.

Hyperglycaemia has an incidence of up to 80% in very preterm infants (103). Intravenous nutrition delivering glucose at a high rate despite the immature, thus inconsistent, response

to suppress endogenous glucose production can lead to hyperglycaemia (104). Furthermore, these infants have a limited insulin secretory capacity due to small volumes of insulin-sensitive tissues (105).

Clinicians face a delicate scenario when treating hyperglycaemia. Reducing the glucose intake too far can lead to hypoglycaemia and faltering growth. It is also unclear whether common treatment options such as decreasing the intravenous glucose load or administering insulin has any effect on outcomes. Another option is to increase protein or intravenous lipid intake while reducing glucose intake.

Insulin treatment can reduce blood glucose concentrations and improve early weight gain (106). However, it has been demonstrated that insulin treatment increases the risk of hypoglycaemia which can ultimately lead to long-term neurodevelopmental impairment (107).

Maternal breast milk

Maternal breast milk is the gold standard form of enteral nutrition for preterm or low birth weight infants (108). Aside from the nutritional advantages that breast milk confers to the infant, there is strong evidence that maternal breast milk feeding decreases the incidence of feed intolerance and necrotising enterocolitis (98, 109). Breastfeeding has been linked to a reduction in child mortality and morbidity (110). It has also been reported that additional benefits include faster gastric emptying (111) as well as achieving full enteral feeding sooner (112). Further putative benefits of prolonged and exclusive breastfeeding include protection against long-term chronic conditions such as obesity, diabetes, Crohn's disease and lymphoma (113). There are benefits for the mother including weight loss and pregnancy prevention (114). The WHO recommends exclusive breastfeeding for the first 6 months of the infants' lives (115).

In the absence of maternal breast milk, there are several alternatives for preterm or low birth weight infants. There are two common alternatives, artificial formula and donor breast milk.

Donor breast milk

Donor breast milk has a lower energy and protein content than term formula milk (116) and nutritional content depends on the stage of lactation at which it is collected.

Furthermore, evidence shows that donor milk may not always meet the demanding energy requirements for preterm and low birth weight infants due to their relatively depleted reserves as well as being subject to extra metabolic stress compared to their term counterparts. There are however, major putative benefits in the shape of immune-protective and growth factors to the immature gut mucosa which may in turn prevent adverse outcomes such as necrotising enterocolitis and invasive infection (112, 117).

Artificial formula

Artificial formulas are usually adapted from cow's milk. They differ in energy, protein and mineral content and can be broadly split into two groups:

1. Standard 'term' formula: designed for term infants based on the composition of mature breast milk: the typical energy content is between 67 to 70 kcal/100ml
2. Nutrient-enriched 'preterm' formula: designed to provide nutrient intakes to match intrauterine accretion rates: these are energy-enriched (typically up to about 80kcal/100ml) and variably protein- and mineral-enriched.

9. Necrotising enterocolitis

9.1 Epidemiology

Necrotising enterocolitis is one of the most common gastrointestinal emergencies in neonates (118). 90% of infants who develop necrotising enterocolitis are born preterm but those born near term or at full term can also develop the disease (119). It can be a deadly disease that is associated with severe sepsis, intestinal perforation, and significant morbidity and mortality (120).

The term “necrotising enterocolitis” was first used by Mizrahi in 1965 to describe a clinical syndrome involving vomiting, abdominal distention, shock, intestinal haemorrhage and intestinal perforation (121). Since then, dramatic advances in perinatal and neonatal care have led to more preterm neonates living longer resulting in a higher risk of necrotising enterocolitis.

Early epidemiological studies singled out African-Americans and male infants as having an increased risk of necrotising enterocolitis but recent studies have failed to corroborate these observations (122). Necrotising enterocolitis has a heavy economic burden with survivors requiring surgery staying in the NICU more than 90 days making up nearly 20% of NICU costs annually (123). The mortality rate ranges from 20-40% but in infants with the most severe form of the disease, is close to 100% (122, 124). With such severe mortality and associated morbidities, necrotising enterocolitis poses a very real threat to the neonatal population.

9.2 Pathogenesis

Necrotising enterocolitis is characterised as an inflammatory disease of the newborn bowel (125). The pathogenesis is said to be multifactorial although research is still being undertaken to explain the exact mechanism (126). Factors relating to intestinal ischaemia and inflammation, enteral feeding and aberrant bacterial colonisation have been implicated in the development of necrotising enterocolitis in preterm infants (125).

Premature infants are at high risk because of developmental immaturity of key functions, in particular gastrointestinal motility, digestive ability, circulatory regulation, intestinal barrier function, and immune defence (118).

Immature intestinal motility and digestion may predispose preterm infants to necrotising enterocolitis. Studies in both people and animals have suggested that although the development of gastrointestinal motility commences in the second trimester, it does not mature until the third trimester (127, 128) where there is increased gastric emptying (129).

9.3 Risk factors

Growth-restriction in utero is a commonly reported important risk factor for necrotising enterocolitis especially if associated with evidence of compromised placental circulation (absent or reversed end-diastolic flow in the umbilical arteries). These infants often also have poor tolerance of enteral feeding (130).

Patole et al concluded in a systematic review and meta-analysis of observational studies that standardised feeding regimens is possibly the single most important global tool to prevent/minimise necrotising enterocolitis in preterm infants. However, they also concluded that more randomised controlled trials are needed to further substantiate these claims (96).

9.4 Diagnosis

The diagnosis of necrotising enterocolitis at the earliest and least severe stage is a challenge to clinicians as clinical presentation varies between infants (131). It is of

paramount importance that clinicians assign a severity of disease to the diagnosis to guide the treatment of necrotising enterocolitis. Bell staging has been the traditional method in assigning severity of disease to necrotising enterocolitis cases (125).

Bell's stages of necrotising enterocolitis were introduced, by its namesake, to clinically stage infants with uniformity in 1978. It classifies infants as having stage I (suspect), stage II (definite), or stage III (advanced) disease through certain criterion including systemic, intestinal and radiological signs as well as laboratory signs and changes (132). Current guidelines for management of necrotising enterocolitis are based on diagnosis according to the aforementioned criterion (118).

The clinical presentation of necrotising enterocolitis spans a wide spectrum from non-specific signs that progress insidiously over several days to multi-organ system failure and shock with fulminant onset of gastrointestinal signs (133). In stage I, the diagnosis of necrotising enterocolitis is often questionable and can only be diagnosed after other gastrointestinal disorders have been ruled out. Symptoms include temperature instability, lethargy, apnoea and bradycardia (125). The infant may also have feed intolerance characterised by poor feeding, vomiting, mildly distended abdomen, and increased gastric residuals. Radiographic evidence may show distended bowel loops with mild ileus (132).

Infants with signs of pneumatosis intestinalis are classed as Stage II. They often present with marked abdomen distension as well as persistent occult or frank blood in their stools. Aside from pneumatosis intestinalis, radiological signs include persistent or unchanging bowel loops and the development of portal venous gas (132).

In Stage III, the advanced stage, the infant's vital signs will deteriorate with marked GI bleeding and there may be evidence of septic shock. If bowel necrosis has occurred by the time clinicians have diagnosed the infant, then surgical intervention is required. Abdominal films may also show pneumoperitoneum at this stage (132).

Changes have been made to the case definition of necrotising enterocolitis by breaking each stage into two subcategories to better differentiate milder and more severe courses of the disease (134). Ultimately, radiological tests must be used in conjunction with clinical

course, laboratory studies and consultations with radiologists and surgeons to definitively diagnose necrotising enterocolitis in an infant (135).

9.5 Management

Management of necrotising enterocolitis can be divided into medical and surgical interventions. Early identification and intervention of developing necrotising enterocolitis is paramount to reducing the devastating effects and adverse outcomes that can ensue.

When an infant is suspected to have necrotising enterocolitis, all enteral feedings and medications should be stopped with immediate effect. Parenteral nutrition should be initiated with sufficient protein to encourage the repair of injured tissue and maintain positive nitrogen balance (136, 137). In infants with apnoea, endotracheal intubation is the preferred method of management. Fluid maintenance and vigilant monitoring of the infant's vital signs are critical. Furthermore, strict infection control measures should be observed.

9.6 Prevention

Human breast milk

The Cochrane review of donor breast milk versus formula for feeding preterm and low birthweight infants indicates that formula feeding approximately doubles the risk of necrotising enterocolitis (138). Although there have not been any randomised controlled trials of feeding preterm infants with formula versus their own mother's expressed breast milk, observational data suggest an even larger protective effect against necrotising enterocolitis (139).

Human breast milk therefore remains the best strategy to lower the risk of necrotising enterocolitis and feed intolerance. However, when maternal breast milk or donor milk is not available, uncertainty exists about which type of formula is best tolerated and least likely to contribute to the risk of necrotising enterocolitis in preterm infants. Protein hydrolysate formulas, with hydrolysed protein, are used in some settings because of the potential beneficial effects with regards to feed intolerance and necrotising enterocolitis (140, 141).

Trophic feeding

Many clinicians advocate the initiation of early trophic feeds instead of extended bowel rest citing concerns with gut atrophy and a worsened inflammatory response (118).

Trophic feeding, also referred to as minimal enteral nutrition, hypocaloric feeding and gut priming, is an alternative to complete enteral fasting for very preterm or very low birth weight infants (142). It involves introducing small volumes of milk, typically 12 to 24 ml/kg/day, intragastrically via a nasogastric or orogastric tube for the first few days after birth without increasing the feed volume in the first week (143).

Trophic feeding is designed to accelerate gastrointestinal, physiological, endocrine and metabolic maturity in the hope of a quicker transition to full enteral feeding without the aid of parenteral nutrition, which carries infectious and metabolic complications (144).

However, a recent systematic review concluded that there is not sufficient evidence to support trophic feeding for preterm or very low birth weight infants. The authors concluded that further randomised controlled trials would be required to clearly determine the extent of benefits to whether trophic feeding is better than enteral fasting (144).

Probiotics

Within the neonatal community, there is a growing interest in the proactive colonization of the gastrointestinal tract of the preterm infants. Bacterial colonisation can affect the course of many intestinal diseases including necrotising enterocolitis (145). Probiotics are live microbial supplements that colonise the gastrointestinal tract and may provide benefit to the host (146). *Lactobacilli*, *Bifidobacterium* and *Saccharomyces* are the most commonly used probiotics.

It is believed that probiotic supplements can protect infants from necrotising enterocolitis by improving host immune function and an increased barrier to migration bacteria and their associated products across the mucosa (147). A recent Cochrane systematic review identified that probiotics reduce the incidence of necrotising enterocolitis and associated adverse outcomes in preterm infants weighing less than 1500 grams and advocated a change in practise. However, they also highlighted that there is insufficient data regarding the effects of probiotics on at risk infants, those weighing less than 1000 grams at birth (145).

Prebiotics

Another strategy that has been considered is prebiotics, a non-digestible dietary supplement. Prebiotics can promote the proliferation of beneficial commensal bacteria in the gastrointestinal tract (148). Prebiotics have the benefit of not containing live micro-organisms so they carry a lesser risk of infection than probiotic therapies (148). Studies have shown promising commensal bacteria colonisation and a decreased pathogenic colonisation of the gut in preterm infants fed a prebiotic formula compared to those fed a control formula (149). However, prebiotics have been associated with adverse effects such as flatulence, bloating and diarrhoea (118, 148).

Others

Antenatal steroids, IgA supplementation, lactoferrin, erythropoietin and oral antibiotics are amongst the strategies that have been hypothesised to reduce the incidence or severity of necrotising enterocolitis. Currently, there is insufficient evidence to change or shape guidelines to incorporate them into standard care.

10. Role of Cochrane Systematic Reviews

The Cochrane Collaboration, formed in 1993, is an independent, non-profit organisation geared towards the pursuit of creating evidence-based summaries to guide clinical practice (150). Originally formed in response to Archie Cochrane's efforts to create systematic summaries of all available randomised controlled trials related to pregnancy, childbirth and care of the newborn infant, the Cochrane Collaboration has grown to involve over 37,000 contributors from more than 130 countries. It has generated hundreds of reviews pertaining to pregnancy and perinatal health alone. This herculean task involves regularly updating current knowledge and allows for "credible, accessible health information that is free from commercial sponsorship and other conflicts of interest" (151).

Cochrane reviews have influenced the use of therapies and care guidelines. In neonatology, individual randomised controlled trials are often underpowered to exclude modest effects and Cochrane reviews can assimilate data from multiple trials to allow for a more precise estimation of effect size. This is most aptly demonstrated regarding antenatal corticosteroids. Prior to a systematic review published in 1990 showing that antenatal corticosteroids significantly reduced neonatal mortality and morbidity without raising the rate of adverse maternal outcomes (152), previous randomised controlled trials conducted in the 1970s and 1980s reporting the same benefits were largely ignored. The systematic review led to recommendations by national bodies to incorporate antenatal corticosteroids into the treatment of all women at risk of anticipated preterm delivery (153). Another important role of Cochrane reviews is to identify gaps in knowledge to highlight and guide further research in those areas.

What is a Cochrane review?

Cochrane reviews gather all relevant information to help answer clinical questions or develop practice guidelines. Previously, narrative reviews in journals or textbooks were used but may be subject to various biases which could hinder the implementation of effective interventions. Alternatively, they could continue to promote and suggest potentially harmful or ineffective practices (154). To prevent this, Cochrane reviews have the following characteristics:

- A protocol which pinpoints a precise clinical question as well as setting out the search strategy, inclusion and exclusion criteria, and an analysis plan. This must be peer reviewed and published before the review can continue.
- Risk of bias is assessed by the reviewers to identify all eligible studies including unpublished trials, abstracts and those published in other languages.
- Each trial is critically evaluated on their methodological features.
- Meta-analysis is performed to enhance the precision of the overall estimate of effect size where possible.
- Cochrane reviews are published online free of charge and reviewers are encouraged to update their reviews at least every two years or in the event of new trials being found.

Cochrane reviews have greatly influenced practice, especially in the field of neonatology where single, large trials are rare and difficult to undertake. It has led to the adoption of antenatal corticosteroids as standard care for women with impending preterm delivery. It has also allowed for focus on all important outcomes by pooling numerous trials together.

While the Cochrane Collaboration has achieved a lot in the past 20 years, it faces several obstacles to maintain its influence on clinical care and guidelines. The key challenges include making the reviews more user friendly as they may not be used to steer guidance development despite being readily available (155), ensuring reviews are kept up to date with the most topical and clinically relevant information as well as increasing its relevance to middle- and low-income settings (150, 156). This is especially important with regards to utilising scarce resources in the most appropriate manner.

10.1 Using GRADE in the Cochrane Review

Grading of Recommendations Assessment, Development and Evaluation (GRADE) is a process to rate the quality of evidence adopted by many organisations worldwide, including the Cochrane Group, because of its methodologically rigorous and user friendly grading system (157).

GRADE separates the quality of evidence in one of four levels – high, moderate, low, and very low. Evidence derived from randomised controlled trials starts as high quality evidence but can be downgraded for several reasons such as study limitations, inconsistency of results, indirectness of evidence, imprecision and reporting bias. In contrast, evidence based on observational studies begin with a low-quality rating but can be upgraded.

GRADE is useful for giving ratings for each patient important outcome as opposed to the quality of individual trials in the systematic review. It does this by identifying five factors that can downgrade the quality of evidence (158).

The GRADE approach instructs guideline developers to consider the quality of evidence across outcomes as identical to that of the lowest quality of evidence. However, there is some flexibility to exclude outcomes that are deemed not important as the judgement requires the consideration of the context.

GRADE is an extremely useful tool that is increasingly being taken up by organisations and has become the gold standard for evaluating quality of evidence. It can aid clinicians and patients alike to understand the evidence provided to them via a clear and transparent system.

In our Cochrane review, we assessed the quality of evidence for the main comparisons at the primary outcomes level using the GRADE approach, as outlined in the GRADE handbook (159). Two authors independently assessed the quality of the evidence for outcomes identified as critical or important for clinical decision-making (feed tolerance and incidence of necrotising enterocolitis). We considered evidence from randomised controlled trials as high quality but downgraded the evidence one level for serious (or two levels for very serious) limitations based upon the following: design (risk of bias), consistency across studies, directness of the evidence, precision of estimates and presence of publication bias.

Part 2: Cochrane Systematic Review

11. Abstract

11.1 Background

When human milk is not available for feeding preterm infants, protein hydrolysate rather than standard cow's milk formulas (with intact proteins) are often used because they are perceived as being tolerated better and less likely to lead to complications. Protein hydrolysate formulas, however, are more expensive than standard formulas, and concern exists that their use in practice is not supported by high-quality evidence.

11.2 Objectives

To determine whether feeding preterm infants with protein hydrolysate versus standard cow's milk formula affects the risk of feed intolerance, necrotising enterocolitis, and other morbidity and mortality.

11.3 Search methods

We used the standard Cochrane Neonatal search strategy including electronic searches of the Cochrane Central Register of Controlled Trials (CENTRAL; 2017, Issue 4), Ovid MEDLINE, Embase, and the Cumulative Index to Nursing and Allied Health Literature (CINAHL) (until April 2017), as well as conference proceedings and previous reviews.

11.4 Selection criteria

Randomised and quasi-randomised controlled trials that compared feeding preterm infants with protein hydrolysate versus standard (non-hydrolysed) cow's milk formula.

11.5 Data collection and analysis

Two review authors assessed trial eligibility and risk of bias and extracted data independently. We analysed treatment effects as described in the individual trials and reported risk ratios and risk differences for dichotomous data, and mean differences for continuous data, with respective 95% confidence intervals. We used a fixed-effect model in meta-analyses and explored potential causes of heterogeneity in sensitivity analyses. We assessed quality of evidence at the outcome level using the Grading of Recommendations Assessment, Development and Evaluation approach.

11.6 Main results

We identified ten trials for inclusion in the review. All trials were small (total participants 600) and had various methodological limitations including uncertainty about methods to ensure allocation concealment and blinding. Most participants were clinically stable preterm infants of gestational age less than about 34 weeks or birth weight less than about 1800 g. Fewer participants were extremely preterm, extremely low birth weight, or growth-restricted. The trials did not show any effects on feed intolerance assessed variously as average pre-feed gastric residual volume, incidence of concerning gastro-intestinal signs, or time taken to achieve full enteral feeds (low quality evidence). Meta-analysis did not show a significant effect on the risk of necrotising enterocolitis: typical risk ratio 1.10 (95% confidence interval 0.36 to 3.34); risk difference 0.00 (95% confidence interval -0.04 to 0.04) (low quality evidence).

11.7 Authors' conclusions

The available trial data do not provide evidence that feeding preterm infants with protein hydrolysate versus standard formula reduces the risk of feed intolerance or necrotising enterocolitis. Further large, pragmatic trials are needed to provide more precise estimates of effectiveness and cost-effectiveness.

11.8 Plain Language Summary

Hydrolysed formula for preterm infants

Review question: Does feeding preterm infants with cow's milk formula containing pre-digested (hydrolysed) proteins rather than whole proteins improve digestion and reduce the risk of severe bowel problems?

Background: Preterm infants often find cow's milk formula more difficult to digest than human milk, and cow's milk formula may increase the risk of severe bowel problems for preterm infants. If preterm infants are fed with cow's milk formula (when human milk is not available), then using a formula in which the protein is already partially-digested (hydrolysed) rather than a standard formula (with intact proteins) might reduce the risk of these problems. Hydrolysed formulas, however, are more expensive than standard formulas, and may have specific side-effects not seen with standard formulas. Given these concerns, we have reviewed all of the available evidence from clinical trials that compared these types of formula for feeding preterm infants.

Study characteristics: We found ten trials; most were small (involving 600 infants in total) and had methodological weaknesses.

Key results: The data from these trials did not provide any strong or consistent evidence that feeding preterm infants with hydrolysed formula rather than standard formula improved digestion or reduced the risk of severe bowel problems.

Conclusions: The currently available evidence suggests that feeding preterm infants with hydrolysed formula (rather than standard formula) during their initial hospital admission does not have any important benefits. This finding, however, is not yet conclusive, and further, larger and better quality trials would be needed to provide evidence to help clinicians and families resolve make informed choices about this issue.

12. Background

Hydrolysed cow's milk formulas, originally developed for infants with cow's milk protein allergy or intolerance, are used as enteral feeding alternatives for preterm infants for whom human milk is not available. These formulas contain hydrolysed rather than intact proteins, and may also differ from standard cow's milk formulas in carbohydrate, lipid, and micronutrient type and content (160). Their use as a sole or supplemental enteral feed source for preterm infants has increased over the past 20 years, particularly in high-income countries, because they are perceived as being tolerated better, and less likely to lead to complications, than standard cow's milk formulas (161). Hydrolysed formulas, however, are more expensive than standard formulas, and concern exists that their use in practice is not supported by high-quality evidence (162).

12.1 Description of the condition

Human breast milk is recommended as the best form of enteral nutrition for preterm infants (163). Breast milk contains many non-nutrient factors including immunoglobulins and lactoferrin that promote intestinal adaptation and maturation, improve enteral feed tolerance, and protect against infection and inflammatory disorders (109, 164).

When sufficient human breast milk is not available, cow's milk-based formulas are used for feeding preterm infants, either as the sole enteral diet or as a supplement to human breast milk (142). Feeding preterm infants with standard cow's milk formulas rather than human breast milk is, however, associated with higher rates of feed intolerance and necrotising enterocolitis (138). Feed intolerance and interruption of enteral feeds is a major contributor to cumulative nutrient deficits and postnatal growth restriction in very preterm infants (165, 166). Slow postnatal growth is associated with neurodevelopmental impairment in later childhood and with poorer cognitive and educational outcomes (167-169). Necrotising enterocolitis affects about 5% of very preterm infants. Infants who develop necrotising enterocolitis experience more infections, have lower levels of nutrient intake, grow more slowly, have longer durations of intensive care and hospital stay, and are more likely to die or be disabled than gestation-comparable infants who do not develop necrotising enterocolitis (170-172).

12.2 Description of the intervention

Standard cow's milk formulas can be grouped broadly as 'term' formulas (designed for term infants; nutrient content based on the composition of mature breast milk) and nutrient-enriched 'preterm' formulas (designed for preterm or low birth weight infants; energy-enriched and variably protein- and mineral-enriched) (173). Concern exists that standard cow's milk formulas (either 'term' or 'preterm') are poorly tolerated, especially by very preterm infants, because the immature infant's gastrointestinal tract is less efficient than that of term infants at digesting intact cow's milk proteins and fats (111, 174).

12.3 Hydrolysed formulas

'Hydrolysed' protein formulas, containing protein digested chemically (acid/alkali) or enzymatically (protease) to oligopeptides, are often used for feeding preterm infants, especially infants with feed intolerance or clinical features (such as episodic apnoea, oxygen desaturation, or bradycardia) that are attributed to gastro-oesophageal reflux, or following gastrointestinal surgery or necrotising enterocolitis (161).

Several brands of hydrolysed formulas (both 'term' and 'preterm') are available commercially and these are grouped broadly depending on degree of hydrolysis:

- Extensively-hydrolysed: residual free amino acids and peptides with molecular weights < 1.5 to 3.0 kDa;
- Partially-hydrolysed: residual peptides with molecular weights of 3.0 to 10.0 kDa.

This distinction is mainly relevant to the putative hypo-allergenic properties of hydrolysed formulas and there are limited data regarding its functional relevance to preterm infants. Formulas also vary by the predominant protein source (casein versus whey-casein) as well as by carbohydrate (lactose, maltodextrin) and fat (cow, vegetable) type and content (175).

12.4 How the intervention might work

Although developed as hypo-allergenic alternatives to standard cow's milk formulas for infants at risk of cow's milk protein intolerance or allergy, the evidence for this effect in term infants is very weak (176, 177). In preterm infants, hydrolysed formulas are mostly used for their perceived benefits in reducing the risk of feed intolerance and necrotising enterocolitis. When human milk is unavailable, hydrolysed formulas may be used empirically (starter formula) or therapeutically to improve feeding tolerance or reduce gastro-oesophageal reflux. The possible mechanisms for these effects include accelerated gastric emptying and intestinal transit, more efficient enteric peptide digestion, and stimulation of small intestinal enzymatic and motilin activity (141, 161). If better feed tolerance reduces the time taken to establish full enteral feeding in very preterm infants, this may reduce the adverse infectious or metabolic consequences of prolonged exposure to parenteral nutrition.

Several potential adverse effects of hydrolysed formulas are recognised. Osmolality increases when protein is hydrolysed into smaller peptides, and these higher osmolarity fluids delivered to the small intestine may increase the risk of necrotising enterocolitis. Furthermore, if bio-active proteins such as immunoglobulin or lactoferrin are hydrolysed, this may reduce their putative benefits in reducing the risk of infection or necrotising enterocolitis. It is possible that some peptides created by artificial hydrolysis have diminished or harmful functional activities (178). Concern about micro-nutrient bio-availability in hydrolysed formulas also exists, particularly whether bone minerals are less well absorbed in the absence of intact casein proteins (161).

12.5 Why it is important to do this review

Given the potential for protein hydrolysate formulas (rather than standard cow's milk formulas) to improve enteral feed tolerance and prevent adverse outcomes in preterm infants, we undertook a systematic review of the randomised trial data to help to inform practice and research.

13. Objectives

To assess the effect of feeding preterm infants with hydrolysed formula (versus standard cow's milk formulas) on the risk of feed intolerance, necrotising enterocolitis, and other morbidity and mortality in preterm infants.

14. Methods

14.1 Inclusion criteria

14.1.1 Types of studies

Randomised or quasi-randomised controlled trials, including cluster-randomised controlled trials.

14.1.2 Types of participants

Preterm (< 37 weeks' gestation) newborn infants who receive cow's milk formula as their sole or supplemental enteral diet.

14.1.3 Types of interventions

Hydrolysed cow's milk formula versus standard (non-hydrolysed) cow's milk formula or another type of hydrolysed cow's milk formula. Formula was to be allocated as at least 20% of intended enteral diet for at least two weeks to allow measurable effects on growth rates and episodes of feed intolerance. Trials should have compared formulas with similar energy and protein levels (that is,

hydrolysed 'preterm' formula versus non-hydrolysed 'preterm' formula, or hydrolysed 'term' formula versus non-hydrolysed 'term' formula). We planned separate comparisons of trials that assessed:

- *empirical* use of hydrolysed formulas;
- *indicated* (therapeutic) use of hydrolysed formulas to treat infants with feed intolerance, gastro-oesophageal reflux (and associated apnoea, desaturation, or bradycardia), or following gastro-intestinal surgery or necrotising enterocolitis (as defined by the primary investigators).

14.1.4 Types of outcome measures

Primary outcomes

1. Number of infants with at least one episode of feed intolerance that results in cessation or reduction in enteral feeding (enteral feeds reduced or ceased for > 4 hours), or average number of episodes of feed intolerance during trial period, or both.
2. Infants with at least one episode of necrotising enterocolitis (modified Bell stage

2/3) (179) (unless indicated use following necrotising enterocolitis).

Secondary outcomes

3. Time to full enteral feeding independent of parenteral fluids (days).
4. Growth: time to regain birth weight, and subsequent rates of weight (g/kg/day), length (mm/week), and head growth (mm/ week) during hospital admission.
5. Duration of hospital admission (days).
6. Measures of bone mineralization: (i) serum alkaline phosphatase level at 36 to 40 weeks postmenstrual age, or (ii) bone mineral content assessed post-term by dual energy x-ray absorptiometry (DEXA), or (iii) clinical or radiological evidence of rickets on long- term follow-up.
7. Late-onset invasive infection diagnosed more than 72 hours after birth as determined by culture from a normally sterile site: cerebrospinal fluid, blood, bone or joint, peritoneum, pleural space, or central venous line tip; or findings on autopsy examination consistent with invasive microbial infection.
8. Mortality: all-cause until 28 days and during hospital admission.
9. Neurodevelopmental outcomes assessed by a validated test after 12 months' post-term: neurological evaluations, developmental scores and classifications of disability, including auditory and visual disability.
10. Allergy or atopy diagnosed after 12 months' post-term: asthma, eczema, allergic rhinitis or conjunctivitis, food allergy, allergic sensitisation (skin prick, or specific or total immunoglobulin E level) (176)

14.2 Search methods for identification of studies

14.2.1 Electronic searches

We searched the Cochrane Central Register of Controlled Trials (CENTRAL, 2017, issue 4), Ovid MEDLINE (1946 to April 2017), OVID Embase (1974 to April 2017), OVID Maternity & Infant Care Database (1971 to April 2017), and CINAHL (1982 to April 2017) using a combination of the following text words and MeSH terms described in Appendix 20.6 Electronic Search Strategy. We limited the search outputs with the relevant search filters for clinical trials as recommended in the *Cochrane Handbook for Systematic Reviews of Interventions* (180). We did not apply any language restrictions.

We searched ClinicalTrials.gov and the World Health Organization's International Trials Registry and Platform for completed or ongoing trials.

14.2.2 Searching other resources

We examined reference lists in previous reviews and included studies. We searched the proceedings of the annual meetings of the Pediatric Academic Societies (1993 to 2016), the European Society for Paediatric Research (1995 to 2016), the Royal College of Paediatrics and Child Health (2000 to 2017), and the Perinatal Society of Australia and New Zealand (2000 to 2016). Trials reported only as abstracts were eligible if sufficient information was available from the report, or from contact with the authors, to fulfil the inclusion criteria.

14.3 Data collection and analysis

We used the standard methods of the Cochrane Neonatal Review Group.

14.3.1 Selection of studies

We screened the title and abstract of all studies identified by the above search strategy and two review authors independently assessed the full articles for all potentially relevant trials. We excluded those studies that did not meet all of the inclusion criteria and we stated the reason for exclusion. We discussed any disagreements until consensus was achieved.

14.3.2 Data extraction and management

Two authors (DN and WM) extracted data independently using a data collection form to aid extraction of information on design, methodology, participants, interventions, outcomes and treatment effects from each included study. We discussed any disagreements until we reached a consensus. If data from the trial reports were insufficient, we contacted the trialists for further information.

14.3.3 Assessment of risk of bias in included studies

We used the criteria and standard methods of Cochrane Neonatal to assess the methodological quality of any included trials. Two authors (DN and JKA) assessed risk of bias across key domains (Appendix 20.2) and resolved disagreements in consultation with a third author (WM). We requested additional information from the trial authors to clarify methodology and results when necessary. We did not exclude trials on the basis of risk of bias, but we did plan to conduct sensitivity analyses if applicable to explore the consequences of synthesizing evidence of variable quality (180).

Risk of bias assessment criteria:

Random sequence generation: We categorised the method used to generate the allocation sequence as:

- Low risk of bias: Any random process. e.g. random number table; computer random number generator; coin tossing; shuffling of cards or envelopes; throwing of dice; drawing of lots; minimization (may be implemented without a random element; this is considered equivalent to being random).
- High risk of bias: any non-random process. e.g. sequence generated by odd or even date of birth; date (or day) of admission; sequence generated by hospital or clinic record number; allocation by judgment of the clinician; allocation by preference of the participant; allocation based on results of a
- laboratory test or series of tests; allocation based on availability of the intervention.
- Unclear risk of bias: insufficient information about the sequence generation process to permit judgment.

Allocation concealment: We categorised the method used to conceal the allocation sequence as:

- Low risk of bias: randomisation method described that would not allow investigator/participant to know or influence the intervention group before eligible participants entered the study (i.e. central allocation, including telephone, web-based, and pharmacy-controlled randomisation; sequentially numbered drug containers of identical appearance; sequentially numbered, opaque, sealed envelopes).
- High risk of bias: open random allocation schedule (i.e. list of random numbers); assignment envelopes used without appropriate safeguards (e.g. if envelopes were unsealed or non-opaque or were not sequentially numbered); alternation or rotation; date of birth; case record number; any other explicitly unconcealed procedure.
- Unclear risk of bias: randomisation stated but no information provided on method used.

Blinding of participants and personnel: We assessed blinding of participants, clinicians and caregivers, and outcome assessors separately for different outcomes and categorised the methods as

- Low risk of bias: no blinding or incomplete blinding, but review authors judged that the outcome was not likely to be influenced by lack of blinding; blinding of participants and key study personnel ensured, and unlikely that blinding could have been broken.
- High risk of bias: no blinding or incomplete blinding, and the outcome was likely to be influenced by lack of blinding; blinding of key study participants and personnel attempted, but likely that blinding could have been broken, and the outcome was likely to be influenced by lack of blinding.
- Unclear risk of bias: insufficient information to permit judgment.

Incomplete outcome data: We described the completeness of data including attrition and exclusions from the analysis for each outcome and any reasons for attrition or exclusion where reported. We assessed whether missing data were balanced across groups or were

related to outcomes. We categorised completeness as:

- Low risk of bias: no missing outcome data; reasons for missing outcome data unlikely to be related to true outcome (for survival data, censoring unlikely to introduce bias); missing outcome data balanced in numbers across intervention groups, with similar reasons for missing data across groups; for dichotomous outcome data, the proportion of missing outcomes compared with observed event risk not enough to have a clinically relevant impact on the intervention effect estimate; for continuous outcome data, plausible effect size (difference in means or standardized difference in means) among missing outcomes not sufficient to have a clinically relevant impact on observed effect size; missing data imputed by appropriate methods.
- High risk of bias: reason for missing outcome data likely to be related to true outcome, with imbalance in numbers or reasons for missing data across intervention groups; for dichotomous outcome data, the proportion of missing outcomes compared with observed event risk enough to induce clinically relevant bias in intervention effect estimate; for continuous outcome data, plausible effect size (difference in means or standardized difference in means) among missing outcomes enough to induce clinically relevant bias in observed effect size; analysis done with substantial departure of the intervention received from that assigned at randomisation; potentially inappropriate application of simple imputation.
- Unclear risk of bias: insufficient information to permit judgment.

Selective reporting: We assessed reporting bias due to selective outcome reporting as

- Low risk of bias: the study protocol is available, and all of the study's prespecified (primary and secondary) outcomes that were of interest in the review had been reported in the prespecified way; the study protocol was not available, but it was clear that published reports included all expected outcomes, including those that were prespecified.
- High risk of bias: not all of the study's prespecified primary outcomes had been reported; one or more primary outcomes had been reported by measurements,

analysis methods, or subsets of data (i.e. subscales) that had not been prespecified; one or more reported primary outcomes were not prespecified (unless clear justification for their reporting is provided, such as an unexpected adverse effect); one or more outcomes of interest in the review had been reported incompletely, so that they could not be entered into a meta-analysis; the study report failed to include results for a key outcome that would be expected to have been reported for such a study.

- Unclear risk of bias: insufficient information to permit judgment.

Other bias: We analysed bias due to problems not covered elsewhere in the table.

- Low risk of bias: the study appears to be free of other sources of bias.
- High risk of bias: the study had a potential source of bias related to the specific study design used; stopped early because a data-dependent study design was used; stopped early as the result of a data-dependent process (including a formal stopping rule); had extreme baseline imbalance; was claimed to be fraudulent; had some other problem.
- Unclear risk of bias: insufficient information to assess whether an important risk of bias existed; insufficient rationale or evidence to suggest that an identified problem would introduce bias.

14.3.4 Measures of treatment effect

We analysed the treatment effects in the individual trials using Review Manager 5 and reported risk ratio (RR) and risk difference (RD) for dichotomous data and mean difference (MD) for continuous data, with respective 95% confidence intervals (CI). We determined the number needed to treat for an additional beneficial outcome (NNTB) or an additional harmful outcome (NNTH) for analyses with a statistically significant difference in the RD.

14.3.5 Unit of analysis issues

The unit of analysis was the participating infant in individually randomised trials and the neonatal unit (or sub-unit) for cluster- randomised trials. For cluster-randomised trials, we planned to undertake analyses at the level of the individual while accounting for the clustering in the data using the methods recommended in the *Cochrane Handbook for Systematic Reviews of Interventions* (180).

14.3.6 Dealing with missing data

Where data were missing, and could not be derived as described, we approached the analysis of missing data as follows:

- We contacted the original study investigators to request the missing data.
- Where possible, we imputed missing standard deviations (SDs) using the coefficient of variation (CV) or calculated from other available statistics including standard errors, confidence intervals, t values and P values.
- If the data were assumed to be missing at random, we analysed the data without imputing any missing values.
- If this could not be assumed then we planned to impute the missing outcomes with replacement values, assuming all to have a poor outcome. We planned sensitivity analyses to assess any changes in the direction or magnitude of effect resulting from data imputation.

14.3.7 Assessment of heterogeneity

Two authors assessed clinical heterogeneity, with a meta-analysis conducted only when both authors agreed that study participants, interventions and outcomes were sufficiently similar. We examined the treatment effects of individual trials and heterogeneity between trial results by inspecting the forest plots. We calculated the I^2 statistic for each analysis to quantify inconsistency across studies and described the percentage of variability in effect estimates that may be due to heterogeneity rather than to sampling error. If we detected moderate or high heterogeneity ($I^2 > 50\%$), we would explore the possible causes (for example, differences in study design, participants, interventions or completeness of outcome assessments).

14.3.8 Assessment of reporting biases

If more than 10 trials were included in a meta-analysis, we planned to examine a funnel plot for asymmetry.

14.4 Data synthesis

We used the fixed-effect model in Review Manager 5 for meta-analyses (as per Cochrane Neonatal recommendations). Where moderate or high heterogeneity existed, we planned to examine the potential causes in subgroup and sensitivity analyses.

14.4.1 Subgroup analysis and investigation of heterogeneity

We planned subgroup analyses by:

- Gestational age at birth: very preterm (< 32 weeks) infants versus infants born at 32 weeks or later.
- Indication (for therapeutic use): post-surgery versus post-necrotising enterocolitis versus feeding intolerance or gastro-oesophageal reflux.
- The extent of protein hydrolysis (as defined by manufacturers): extensively versus partially hydrolysed formula.

14.4.2 Sensitivity analysis

We planned sensitivity analyses to determine if the findings were affected by including only studies of adequate methodology (low risk of bias), defined as adequate randomisation and allocation concealment, blinding of intervention and measurement, and less than 10% loss to follow-up.

15. Results

15.1 Description of studies

See Characteristics of included studies and Characteristics of excluded studies.

15.1.1 Results of the search

We included ten trials, excluded seven trials, and identified four on-going trials. Two trials await English language translation to allow assessment of eligibility for inclusion.

15.1.2 Included studies

Ten trials fulfilled the review eligibility criteria (141, 181-189). Most of these trials were undertaken during the 1990s and early 2000s by investigators in neonatal units in Europe (mainly Germany and Italy) and North America. For further details, see Characteristics of included studies.

15.1.3 Participants

In total, 600 infants participated in the included trials. Most participants were clinically stable preterm infants of gestational age less than about 34 weeks or birth weight less than about 1800 g. Fewer participants were extremely preterm, extremely low birth weight, or growth-restricted. Most of the trials specifically excluded infants with congenital anomalies, or gastrointestinal or neurological problems.

Interventions

All the trials assessed the *empirical* use of protein hydrolysate formulas; none assessed *indicated* use.

Trials varied according to brand of formula studied. All trials except one assessed a “preterm” (nutrient-enriched) hydrolysed formula; Schweizer 1993 assessed a “term” hydrolysed formula (182). Most trials used a whey-casein based hydrolysate. Two trials

(Huston 1992; Riezzo 2001) used a predominantly casein-based hydrolysate (181, 186). Control diets were preterm non-hydrolysed formulas in all except Riezzo 2001 where the control diet was a standard term formula (186). Trial participants received the intervention or control formulas on commencing enteral feeds either as a sole diet or a supplement when mother's own milk was not available or insufficient. One trial (Mihatsch 2002) specifically excluded participants post hoc if mother's own milk formed more than 10% of enteral intake (141). In general, trial feeds were allocated for several weeks, or until participating infants reached a specified weight (typically about 1.8 kg).

15.1.4 Outcomes

The outcomes reported most commonly were feed intolerance (reported in various ways but often without accompanying numerical data), growth parameters during the study period or until hospital discharge, and adverse events (including mortality and necrotising enterocolitis). None of the trials reported long-term growth and neurodevelopmental outcomes.

15.1.5 Excluded studies

We excluded seven studies (Rigo 1994b; Rigo 1994a; Mihatsch 1999; Mihatsch 2001; Agosti 2003; Corvaglia 2013; Logarajaha 2015) (140, 190-195). The reasons for exclusion are described in Characteristics of excluded studies.

15.1.6 Risk of bias in included studies

Quality assessments are detailed in Characteristics of included studies and summarised in Figure 1.

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
Florendo 2009	+	+	+	+	+	?	?
Huston 1992	?	?	-	-	+	?	?
Maggio 2005	?	+	+	+	+	?	?
Mihatsch 2002	+	?	?	?	-	?	?
Pauls 1996	?	?	-	-	+	?	?
Picaud 2001	?	?	?	?	+	?	?
Raupp 1995	?	?	-	-	+	?	?
Riezzo 2001	?	?	-	-	+	?	?
Schweizer 1993	?	?	+	+	+	?	?
Szajewska 2004	?	+	?	?	+	?	?

Figure 1 Risk of bias summary: review authors' judgements about each risk of bias item for each included study.

Allocation

Three trials reported adequate allocation concealment methods (sealed, numbered envelopes; central randomisation in blocks) (Szajewska 2004; Maggio 2005; Florendo 2009). None of the remaining trials reported sufficient details to assess if or how allocation concealment was achieved.

Blinding

Three trials reported adequate blinding of investigators, caregivers, or parents (Schweizer 1993; Maggio 2005; Florendo 2009). It is probable that the other trials were not blinded as the reports did not describe any methods that might achieve this.

Incomplete outcome data

Most trials are likely to be at low risk of bias because of incomplete assessment of the trial cohort. In one trial (Mihatsch 2002) the investigators recruited 129 infants initially then excluded 42 participants post hoc because they had received more than 10% of their enteral intake as human milk (141).

Selective reporting

We were not able to assess reliably whether selective reporting occurred as we did not have protocols or other indicators of pre-specified outcomes for any of the trials.

Other potential sources of bias

We did not identify any other potential sources of bias in the reports.

15.2 Effects of interventions

See: 20.1 **Summary of findings for the main comparison** Hydrolysed compared to non-hydrolysed formula for feeding preterm infants

15.2.1 Comparison 1: Empirical use of protein hydrolysate versus standard formula

1. Feed intolerance (Outcome 1.1)

Two trials reported numerical data on the incidence of feed intolerance (Maggio 2005; Florendo 2009). Meta-analysis did not show a statistically significant effect: typical RR 2.71 (95% CI 0.29 to 25.00); RD 0.04 (95% CI -0.05 to 0.12) (I^2 not applicable). The other trials did not report any numerical data but described their findings narratively. These did not show differences in measures of gastric residual volumes (Pauls 1996; Mihatsch 2002), frequency of regurgitation (Riezzo 2001), or vomiting or diarrhoea (Szajewska 2004). Raupp 1995 reported that “both formulas were well tolerated”. The remaining trials did not report any measures of feed intolerance (Huston 1992; Schweizer 1993; Picaud 2001)

2. Incidence of necrotising enterocolitis (Outcome 1.2)

Meta-analysis of data from four trials (325 infants) did not show a statistically significant effect: typical RR 1.10 (95% CI 0.36 to 3.34); RD 0.00 (95% CI -0.04 to 0.04) ($I^2 = 0\%$) (Figure 2).

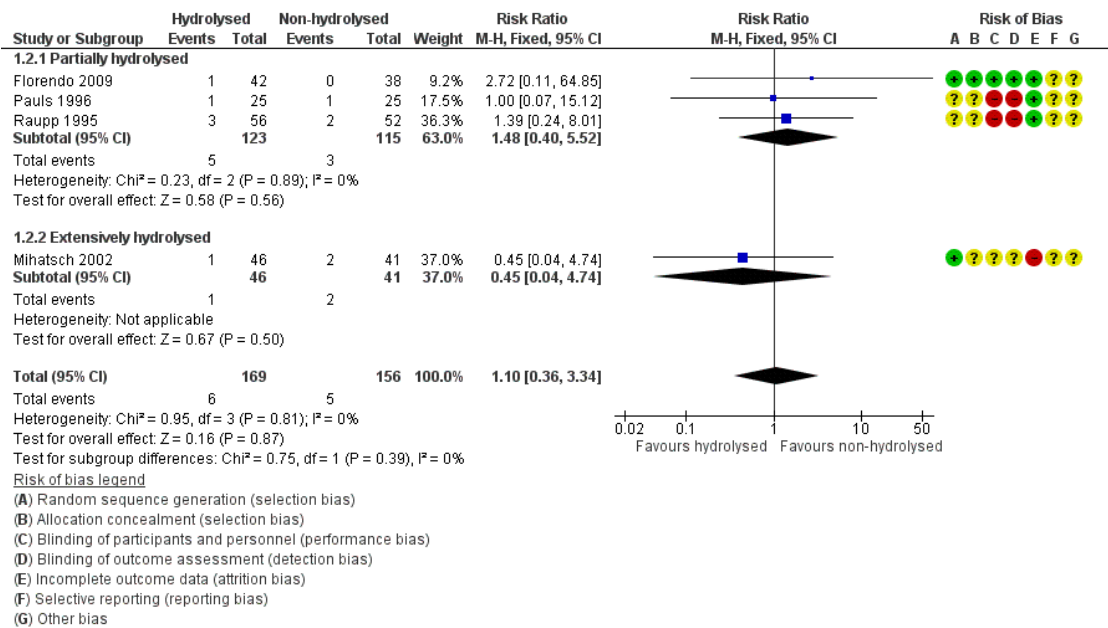


Figure 2: Forest plot of comparison: 1 Hydrolysed vs non-hydrolysed formula, outcome: 1.2 Necrotising enterocolitis.

The other trials did not report this outcome, although in most it is likely that none of the participants developed necrotising enterocolitis.

3. Time to full enteral feeding (Outcome 1.3).

Most trials did not report time to full enteral feeds (Huston 1992; Raupp 1995; Riezzo 2001; Szajewska 2004; Maggio 2005; Florendo 2009). Mihatsch 2002 reported that the median time to full enteral feeding was shorter in the intervention group (10 days versus 12 days in the control group).

Three trials reported no statistically significant difference:

- Pauls 1996; no data reported
- Schweizer 1993: 24 versus 25 days (SD not reported)
- Picaud 2001: 16 (SD 8) versus 17 (SD 8) days: MD -1.00 (95% CI -8.36, 6.36) days

4. Growth: time to regain birth weight, and subsequent rates of growth during hospital admission (Outcomes 1.4- 1.6).

Three trials did not report any growth data (Pauls 1996; Riezzo 2001; Szajewska 2004). The other trials reported some data on growth parameters during the study period or until hospital discharge, but most did not provide sufficient data for inclusion in the meta-analysis (Huston 1992; Schweizer 1993; Raupp 1995; Mihatsch 2002).

Time to regain birth weight

One trial reported days to regain birth weight (Schweizer 1993). This trial did not show a statistically significant difference: 10 versus 9 days (SD not reported).

Weight gain

Three trials reported rates of weight gain over the study period or until hospital discharge (Picaud 2001; Maggio 2005; Florendo 2009). Meta-analysis showed that weight gain was slower in the infants fed with hydrolysed formula: MD -3.02 (95% CI -4.66 to -1.38) g/kg/day (Figure 3).

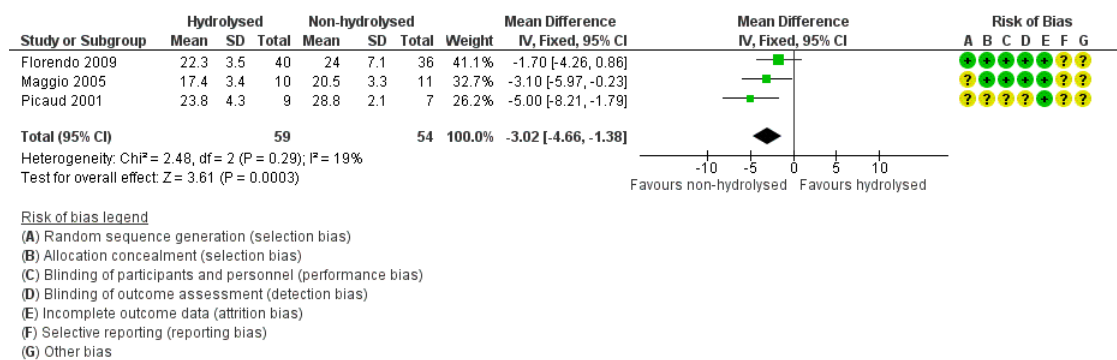


Figure 3 Forest plot of comparison: 1 Hydrolysed vs non-hydrolysed formula, outcome: 1.4 Weight gain (g/kg/day).

Length change

Meta-analysis of data from two trials (97 infants) did not show a difference: MD -0.04 (95% CI -1.24 to 1.15) mm/week (Maggio 2005; Florendo 2009).

Head circumference growth

Meta-analysis of data from two trials (97 infants) did not show a difference: MD 0.27 (95% CI -0.39 to 0.94) mm/week (Maggio 2005; Florendo 2009).

5. Duration of hospital admission.

None of the trials reported the duration of hospital admission.

6. Measures of bone mineralisation (Outcome 1.7).

Two trials reported measures of bone mineralization (Raupp1995; Florendo 2009). Neither trial, nor a meta-analysis of data from both trials, showed a difference in serum alkaline phosphatase level at 36 to 40 weeks' postmenstrual age: MD 16.6 IU/L (95% CI - 34.1 to 67.3) (Figure 4). None of the trials reported bone mineral content assessed post-term or clinical or radiological evidence of rickets on long-term follow-up.

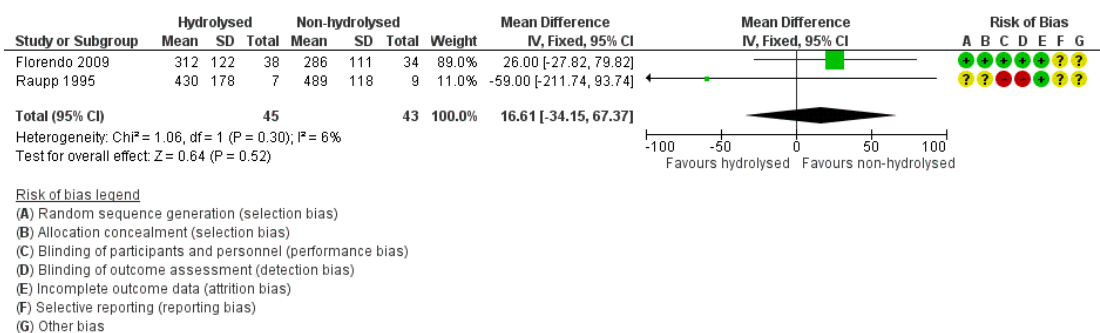


Figure 4 Forest plot of comparison: 1 Hydrolysed vs non-hydrolysed formula, outcome: 1.7 Serum alkaline phosphatase (IU/L).

7. Late-onset invasive infection.

None of the trials reported the incidence of late-onset invasive infection.

8. Mortality.

None of the trials reported the incidence of mortality.

9. Neurodevelopmental outcomes.

None of the trials reported neurodevelopmental outcomes.

10. Allergy or atopy diagnosed after 12 months' post-term (Outcome 1.8).

One trial assessed this outcome (Szajewska 2004). The trial did not show a difference in the incidence of “any allergic disease” (atopic dermatitis, gastrointestinal symptoms, wheezing) at 12 months: RR 0.62 (95% CI 0.27 to 1.42); RD -0.13 (95% CI -0.36 to 0.10) (Figure 5).

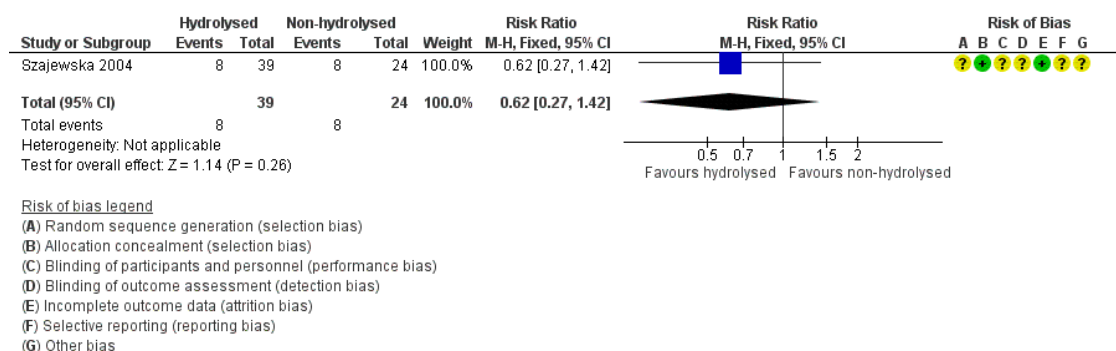


Figure 5 Forest plot of comparison: 1 Hydrolysed vs non-hydrolysed formula, outcome: 1.8 Any allergic disease.

15.2.2 Subgroup analyses

- Gestational age at birth: very preterm (< 32 weeks) infants versus infants born at 32 weeks or later: Subgroup data not available.
- Indication (for therapeutic use): post-surgery versus post-necrotising enterocolitis versus feeding intolerance or gastro-oesophageal reflux: Not applicable- all trials assessed empirical use.
- The extent of protein hydrolysis (as defined by manufacturers): Data for subgroup analysis sufficient for outcome 1.2 (necrotising enterocolitis) only: No evidence of a subgroup effect (Test for subgroup differences: $\text{Chi}^2 = 0.75$, $\text{df} = 1$ ($\text{P} = 0.39$), $I^2 = 0\%$) (Figure 2).

15.2.3 Comparison 2: Indicated use of protein hydrolysate versus standard formula

We did not find any trials that assessed this comparison.

16. Discussion

16.1 Summary of main results

These data from ten small randomised controlled trials do not provide strong or consistent evidence that feeding preterm infants (typically stable infants of gestational age less than 34 weeks at birth) with protein hydrolysate rather than standard cow's milk formula affects the risk of feed tolerance, necrotising enterocolitis, or other adverse outcomes. Limited data do not indicate any important effects on growth, although a meta-analysis of data from three trials suggests that weight gain may be slower in infants fed with protein hydrolysate compared with isocaloric preterm formula. There are currently not any data available to assess the effects on growth and neurodevelopmental outcomes beyond the initial hospital admission.

16.2 Overall completeness and applicability of evidence

These findings should be interpreted and applied cautiously. The primary outcome, feed intolerance, was reported in various different ways, and together with the paucity of numerical data, this precluded meta-analysis. Trials generally reported that feeding with protein hydrolysate did not affect measures such as the pre-feed gastric residual volume, or the need to cease enteral feeding. Similarly, few trials reported the impact of the intervention on the time to achieve full enteral feeding, and the three trials that reported this outcome did not show statistically significant or clinically important effects.

Although a meta-analysis of four trials (325 participants) does not show a substantial effect on the risk of necrotising enterocolitis, there are insufficient data to exclude a more modest but still important effect size. The lower bound of the 95% CI is consistent with a reduced risk of more than 60% (or with one fewer infant developing necrotising enterocolitis for every 25 infants who receive protein hydrolysate formula). Because necrotising enterocolitis is a relatively rare outcome, affecting about 5% of very preterm infants, much larger trials would be needed to provide a more precise estimate of the effect of feeding with protein hydrolysate versus standard formula (172).

Data on growth parameters are limited, as are data on other adverse outcomes.

Furthermore, uncertainty remains about longer-term impact on growth or development. As concerns exist that hydrolysed proteins may be utilised less efficiently than intact proteins by preterm infants, and that concomitant mineral uptake may be lower, trials that assess the effects on both short- and long-term growth and body composition (including bone health) may help to inform policy and practice (196).

Another major applicability limitation of this review is that all of the included trials were undertaken at healthcare facilities in high-income countries, and none in low-income countries. This evidence therefore may be of limited applicability to practices in the resource-limited settings where, globally, most preterm and low birth weight infants are cared for (197).

All of the included trials assessed the effect of empirical (primary) use of protein hydrolysate for feeding preterm infants. We did not find any trials that assessed the indicated use of protein hydrolysate versus standard formula for preterm infants with feed intolerance, gastro-oesophageal reflux (and associated apnoea, desaturation, or bradycardia), or following gastro-intestinal surgery or necrotising enterocolitis. Although indicated use of protein hydrolysate is common, based on perceptions that formulas with intact proteins may be tolerated poorly by infants with intestinal trauma or compromise, no evidence is available from trials to inform this practice (198).

16.3 Quality of the evidence

The GRADE assessments indicated that the quality of evidence for the primary outcomes was “low” because of methodological limitations in the included trials (including uncertainty about allocation concealment and blinding), and imprecision of effect size estimates (Summary of findings for the main comparison).

Most of the included trials were funded or supported by the manufacturers of the formulas being assessed but the funders were not involved in trial design or analysis. There remains some concern, however, that formula manufacturers may promote study findings of trials of specialist formulas selectively as part of a marketing strategy that subverts UNICEF Baby Friendly Initiative regulations (199).

16.4 Potential biases in the review process

It is possible that our findings are subject to publication and other reporting biases. We attempted to minimise this threat by screening the reference lists of included trials and related reviews and searching the proceedings of major international perinatal conferences to identify trial reports that are not (or are not yet) published in full form in academic journals. The meta-analyses that we performed did not contain sufficient trials to explore symmetry of funnel plots as a means of identifying possible publication or reporting bias.

17. Conclusions

Preterm birth remains an immense health problem with consequences for the preterm infant, their families and the healthcare system. Infants born preterm are faced with a plethora of challenges ranging from temperature regulation to inadequate nutrient reserves for growth and development. In addition to these immediate adverse outcomes, they are at further risk of long term effects such as neurodevelopmental and respiratory disorders. They also have an increased risk of developing necrotising enterocolitis which could ultimately lead to mortality and morbidities. We conducted this systematic review to determine whether novel techniques such as protein hydrolysate formula can aid in preventing the development of necrotising enterocolitis and its associated adverse effects.

17.1 Implications for practice

This review did not find strong or consistent evidence of benefits of feeding protein hydrolysate versus standard formula. There are no trial data to suggest an effect on the risk of feed intolerance or necrotising enterocolitis in preterm infants, although the total number of infants studied was small (N= 600), and the data that could be abstracted from published studies for inclusion in meta-analyses were limited.

17.2 Implications for research

Further, high-quality randomised controlled trials are needed to assess the benefits and safety of protein hydrolysate versus standard cow's milk formulas for feeding very preterm infants when maternal breast milk is insufficient or not available. Trials could assess (i) primary (empirical) use, and (ii) secondary (indicated) use in infants with feed intolerance or gastro-oesophageal reflux, or following gastro-intestinal surgery or necrotising enterocolitis. Trials should aim to ensure the participation of extremely preterm, extremely low birth weight, or growth-restricted infants so that subgroup analyses can be planned for these infants at higher risk of necrotising enterocolitis. Given that protein hydrolysate is more expensive than standard formula, trials could justifiably include a cost-benefit analysis.

18. Declaration of Interest

None.

19. References

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20. Appendices

20.1 Summary of findings for the main comparison

Table 1 Summary of findings for the main comparison

Hydrolysed compared to non-hydrolysed formula for feeding preterm infants						
Patient or population: feeding preterm infants						
Setting:						
Intervention: hydrolysed						
Comparison: non-hydrolysed formula						
Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	No. of participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with non-hydrolysed formula	Risk with hydrolysed				
Feed intolerance	Study population		RR 2.71 (0.29 to 25.00)	101 (2 RCTs)	LOW	Limited data from two small trials with imprecise estimate of effect size
		55 per 1,000 (6 to 510)				
Necrotising enterocolitis	Study population		RR 1.10 (0.36 to 3.34)	325 (4 RCTs)	LOW	Methodological limitations in included trials, and imprecise effect size estimate
	32 per 1,000	35 per 1,000 (12 to 107)				

20.2 Characteristics of included studies

Table 2 Characteristics of included studies

Florendo 2009

Methods	RCT	
Participants	Preterm infants (≤ 32 weeks', ≤ 1750 g at birth) receiving $\leq 25\%$ breast milk as total enteral intake	
Interventions	Empirical use of partially hydrolysed whey-casein preterm formula (N= 42) versus intact preterm formula (N= 38)	
Outcomes	Feed intolerance (interruption of enteral feeds) Necrotising enterocolitis	
Notes	Division of Neonatology, University of Tennessee Center for Health Sciences, Memphis, TN, USA. 2004-5.	
<i>Risk of bias</i>		
Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer-generated
Allocation concealment (selection bias)	Low risk	Sequentially labelled, sealed opaque envelopes
Blinding of participants and personnel (performance bias) All outcomes	Low risk	"Double-blinded" – ready-to-feed colour coded cartons
Blinding of outcome assessment (decision bias) All outcomes	Low risk	"Double blind"
Incomplete outcome data (attrition bias) All outcomes	Low risk	Complete outcome data for 74 of 80 participants [One infant in the control group developed sepsis and one infant from the hydrolysed formula group developed NEC and was withdrawn]
Selective reporting (reporting bias)	Unclear risk	Protocol not available
Other bias	Unclear risk	Funded by Nestle (manufacturer of the trial formula)

Huston 1992

Methods	RCT	
Participants	Preterm very low birth weight infants (≤ 1500 g)	
Interventions	Empirical use of casein hydrolysate formula (with either 40% or 60% medium chain triglyceride) versus non-hydrolysed preterm formula (total N= 60)	
Outcomes	Food tolerance and growth rates	
Notes	Department of Pediatrics, Emanuel Children's Health Care Centre, Portland, OR, USA Early 1990s Reported as abstract only	
<i>Risk of bias</i>		
Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Insufficient information – only abstract available
Allocation concealment (selection bias)	Unclear risk	Insufficient information – only abstract available
Blinding of participants and personnel (performance bias) All outcomes	High risk	Unlikely to be blinded
Blinding of outcome assessment (decision bias) All outcomes	High risk	Unlikely to be blinded
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcomes reported for all participants
Selective reporting (reporting bias)	Unclear risk	Protocol not available
Other bias	Unclear risk	Funder: Mead Johnson Nutritional Group

Maggio 2005

Methods	RCT	
Participants	Preterm infants (≤ 34 weeks', ≤ 1750 g at birth)	
Interventions	Empirical use of hydrolysed whey-based formula* (N= 10) versus conventional preterm formula* (N= 11)	
Outcomes	Growth rates from inclusion until hospital discharge. Feed intolerance (no infants had enteral feeds interrupted)	
Notes	Division of Neonatology, Department of Paediatrics, Catholic University of the Sacred Heart, Rome, Italy 1998- 2000 *Energy content of both formulas = 75 kCal/100 mL	
<i>Risk of bias</i>		
Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Randomised schedule generated - unspecified how
Allocation concealment (selection bias)	Low risk	Sealed opaque envelopes
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Study and control formulas identical in colour and smell
Blinding of outcome assessment (decision bias) All outcomes	Low risk	Study and control formulas identical in colour and smell
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcomes reported for all participants
Selective reporting (reporting bias)	Unclear risk	Protocol not available
Other bias	Unclear risk	Funded by Humana (manufacturer of the trial formula)

Mihatsch 2002

Methods	RCT	
Participants	Very low birth weight (<1500g) infants	
Interventions	Empirical use of extensively hydrolysed (whey-casein) preterm formula* (N= 41) versus standard preterm formula* (N= 46)	
Outcomes	Necrotising enterocolitis Proportion of enteral feeds with gastric residual volumes > 5 mL/kg birth weight	
Notes	Division of Neonatology and Pediatric Critical Care, Department of Pediatrics, Ulm University, 89070 Ulm, Germany 1999- 2001 *Energy content of both formulas = 80 kCal/100 mL	
<i>Risk of bias</i>		
Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer-generated
Allocation concealment (selection bias)	Unclear risk	Sealed envelopes - unclear if opaque
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	"Double-blind"- same appearance, but investigators acknowledge taste different
Blinding of outcome assessment (decision bias) All outcomes	Unclear risk	"Double-blind"
Incomplete outcome data (attrition bias) All outcomes	High risk	129 infants were recruited initially, then 42 were excluded post hoc because they received >10% of their enteral intake as human milk.
Selective reporting (reporting bias)	Unclear risk	Protocol not available
Other bias	Unclear risk	Funder: Milupa GmbH, Germany (manufacturer of the trial formula)

Pauls 1996

Methods	RCT	
Participants	Very low birth weight (<1500g) infants	
Interventions	Empirical use of partially hydrolysed whey-casein formula* (N= 25) versus non-hydrolysed protein formula* (N= 25)	
Outcomes	Average gastric residual volume (as a percentage of intake) Time to full enteral feeds Necrotising enterocolitis	
Notes	Kinderklinik, Freie Universitat Berlin, Germany (early 1990s) Reported as an abstract only *Energy content of both formulas = 80 kCal/100 mL *Protein content: Hydrolysed formula 2.9 g/100 mL versus non-hydrolysed formula 2.7 g/100 mL	
<i>Risk of bias</i>		
Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Insufficient information - only abstract available
Allocation concealment (selection bias)	Unclear risk	Insufficient information - only abstract available
Blinding of participants and personnel (performance bias) All outcomes	High risk	Unlikely to be blinded
Blinding of outcome assessment (decision bias) All outcomes	High risk	Unlikely to be blinded
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcomes reported for all participants
Selective reporting (reporting bias)	Unclear risk	Protocol not available
Other bias	Unclear risk	Funder: not stated

Picaud 2001

Methods	RCT	
Participants	Preterm newborns with a birth weight below 1500 g and who are fewer than 15 days old when commencing enteral feeds	
Interventions	Empirical use of partially hydrolyzed formula* (N= 9) vs standard preterm formula* (N= 7) until 40 weeks' post-menstrual age	
Outcomes	Rate of weight gain during initial hospital admission [Nitrogen balance studies]	
Notes	Edouard Herriot Hospital, Claude Bernard University, Lyon, France. Late 1990s *Energy content of both formulas = 80 kCal/100 mL, but nitrogen content 10% higher in standard preterm formula	
<i>Risk of bias</i>		
Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Not stated
Allocation concealment (selection bias)	Unclear risk	Sealed envelopes – unclear if opaque
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	Investigators unaware of formula, unclear if carers or parents aware.
Blinding of outcome assessment (decision bias) All outcomes	High risk	Investigators unaware of formula, unclear if carers or parents aware.
Incomplete outcome data (attrition bias) All outcomes	Low risk	All infants assessed for primary outcomes
Selective reporting (reporting bias)	Unclear risk	Protocol not available
Other bias	Unclear risk	Funder: Nestle (manufacturer of the trial formula)

Raupp 1995

Methods	RCT	
Participants	Neonates (1000-1799 g)	
Interventions	Empirical use of partially hydrolysed whey-casein formula* (N=56) versus non-hydrolysed preterm formula* (N=52)	
Outcomes	Biochemistry, bone mineralisation, blood/serum, necrotising enterocolitis	
Notes	University Children's Hospital of Düsseldorf *Energy content of both formulas = 80 kCal/100 mL	
<i>Risk of bias</i>		
Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Information not available
Allocation concealment (selection bias)	Unclear risk	Information not available
Blinding of participants and personnel (performance bias) All outcomes	High risk	Unblinded
Blinding of outcome assessment (decision bias) All outcomes	High risk	Unblinded
Incomplete outcome data (attrition bias) All outcomes	Low risk	All infants assessed for primary outcomes
Selective reporting (reporting bias)	Unclear risk	Protocol not available
Other bias	Unclear risk	Funder: Nestle (manufacturer of the trial formula)

Riezzo 2001

Methods	RCT	
Participants	Preterm infants (N= 36)	
Interventions	Hydrolysed casein preterm formula* (N=18) versus standard (whey-casein) formula* (N=18)	
Outcomes	Proportion of infants who had > 1 episode of regurgitation or vomiting per day	
Notes	Department of Pediatrics, Neonatology Section, University of Bari, Bari, Italy 2000. NB. Energy content of hydrolysed formula (80 kCal/100 mL) higher than control standard term formula (68 kCal/100 mL).	
<i>Risk of bias</i>		
Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Unclear evidence provided - only states that infants were randomly assigned
Allocation concealment (selection bias)	Unclear risk	Unclear evidence provided - only states that infants were randomly assigned
Blinding of participants and personnel (performance bias) All outcomes	High risk	Unblinded
Blinding of outcome assessment (decision bias) All outcomes	High risk	Unblinded
Incomplete outcome data (attrition bias) All outcomes	Low risk	All infants assessed for primary outcomes
Selective reporting (reporting bias)	Unclear risk	Protocol not available
Other bias	Unclear risk	Funder: Not stated

Schweizer 1993

Methods	RCT	
Participants	Preterm infants (formula fed)	
Interventions	Hydrolysed whey-casein term formula (Alfare)* (N= 26) versus non-hydrolysed preterm formula (Prematil)* (N= 26)	
Outcomes	Weight gain, time to regain birth weight Time to full enteral feeding Average number of high gastric residual volumes per day	
Notes	Kinderklinik der Stadt, Kliniken, Dortmund (1991-3) * NB. Energy content of hydrolysed formula (70 kCal/100 mL) lower than control standard preterm formula (80 kCal/100 mL).	
<i>Risk of bias</i>		
Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Insufficient information - only abstract available
Allocation concealment (selection bias)	Unclear risk	Insufficient information - only abstract available
Blinding of participants and personnel (performance bias) All outcomes	Low risk	"Double-blinded"
Blinding of outcome assessment (decision bias) All outcomes	Low risk	"Double-blinded"
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcomes reported for all participants
Selective reporting (reporting bias)	Unclear risk	Protocol not available
Other bias	Unclear risk	Funder: Not stated

Szajewska 2004

Methods	RCT	
Participants	Preterm infants (< 2500 g) with at least one first degree relative with atopy	
Interventions	Extensively (N= 26) or partially hydrolysed whey-casein preterm formula* (N= 32) versus standard preterm formula* (N= 32)	
Outcomes	[Allergic disease in infancy] Feed intolerance	
Notes	<p>Primary aim of trial was to assess effects on allergy and atopic disease.</p> <p>In hospital feed tolerance, growth, or adverse outcomes not reported. We contacted corresponding author to seek these data in December 2016.</p> <p>*Energy content of both formulas = 80 kCal/100 mL.</p> <p>NB. 33% "drop-out" prior to assessment at 4-5 months post term</p>	
<i>Risk of bias</i>		
Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Randomised schedule generated - unspecified how
Allocation concealment (selection bias)	Low risk	Sealed numbered envelopes- not stated if opaque, but codes concealed from investigators until trial completed
Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	"Double-blind" but study and control formulas not identical in texture and smell
Blinding of outcome assessment (decision bias) All outcomes	Unclear risk	"Double-blind" but study and control formulas not identical in texture and smell
Incomplete outcome data (attrition bias) All outcomes	Low risk	Outcomes reported for all participants
Selective reporting (reporting bias)	Unclear risk	Protocol not available
Other bias	Unclear risk	Funded by Ovita Nutricia Research Foundation

20.3 Characteristics of excluded studies

Table 3 Characteristics of excluded studies

Study	Reason for exclusion
Agosti 2003	Not an RCT
Corvaglia 2003	Cross-over RCT with cross-over at each enteral feed
Logarajaha 2015	Cross-over RCT with cross-over at 24 hours
Mihatsch 1999	Cross-over RCT with initial formula allocation for 5 days only
Mihatsch 2001	Cross-over RCT with initial formula allocation for 5 days only
Rigo 1994a	Not an RCT
Rigo 1994b	Five-arm RCT with term infants receiving different types of hydrolysed formula (three different whey hydrolysate formulas, a soy-collagen hydrolysate formula, or a whey-casein hydrolysate formula)

20.4 Characteristics of studies awaiting assessment

Table 4 Characteristics of included studies

Dobryansky 2015

Methods	RCT
Participants	Very low birth weight (< 1500 g) infants
Interventions	Hydrolysed formula (N= 35) vs standard preterm formula (N= 25)
Outcomes	Feed intolerance Time to full enteral feeding Necrotising enterocolitis NB. Nine additional infants (originally randomised) who died were excluded.
Notes	ARTICLE IN UKRANIAN. Awaiting translation.

Luo 2016

Methods	RCT
Participants	"Very/extremely" low birth weight infants
Interventions	Hydrolyzed protein formula vs preterm formula
Outcomes	Feed intolerance Growth rates
Notes	ARTICLE IN CHINESE. Awaiting translation.

20.5 Characteristics of ongoing studies

Table 5 Characteristics of ongoing studies

Baldassarre 2016

Study name	"Tolerance of an extensively hydrolyzed protein infant formula versus a premature infant formula"
Methods	RCT
Participants	Preterm infants 28 to 33 weeks' gestational age, inclusive, at birth
Interventions	Extensively hydrolysed casein infant formula vs standard cow milk-based preterm infant formula (double blind)
Outcomes	Enteral intake (ml/kg/day) during first 14 days after birth
Starting date	2014
Contact information	Mariella Baldassarre, Universita degli Studi di Bari, Italy [Contacted mariellabaldassarre@gmail.com in January 2017 seeking data]
Notes	Funded by Mead Johnson Nutrition

del Moral 2015

Study name	"Protein hydrolyzed formula for very premature infants"
Methods	RCT
Participants	Very preterm newborns admitted to the neonatal intensive care unit with a birth weight 500-1500 g and who survive more than 3 days
Interventions	Empirical use of 100% whey protein partially-hydrolysed vs 60/40 whey:casein ratio standard (non-hydrolysed) preterm formula (fed when breast milk not available)
Outcomes	Time to achieve full enteral feeds
Starting date	2013
Contact information	Teresa del Moral, University of Miami, USA [Contacted tdelmoral@miami.edu in November 2016 seeking data]
Notes	Funded by Nestle

Terrin 2013

Study name	"Effects of a new hydrolyzed powdered formula on feeding tolerance in preterm neonates: a randomised placebo-controlled study"
Methods	RCT
Participants	Newborns with birth weight <1500 g (N = 60)
Interventions	Powdered hydrolyzed formula vs standard preterm formula
Outcomes	Time to reach full enteral feeding (120 kCal/kg/day)
Starting date	2013
Contact information	Prof Gianluca Terrin, University of Rome "La Sapienza", Italy
Notes	Trial has not proceeded due to lack of funding (personal communication from Prof Terrin)

Yin 2015

Study name	"Extensively hydrolyzed milk protein formula in preterm children"
Methods	RCT
Participants	Preterm infants of gestational age less than 34 weeks' meeting the inclusion criteria who cannot be breastfed (N = 370)
Interventions	Extensively hydrolysed (100% whey protein) formula (66 kCal/100 mL) vs preterm formula (80 kCal/100 mL) fed until discharge from the neonatal intensive care unit
Outcomes	Incidence of feed intolerance and time to achieve full enteral nutrition
Starting date	2016
Contact information	Zhongda Hospital Southeast University, Nanjing, China [Contacted lipingyin_zd@163.com in November 2016 seeking data]
Notes	Registered with the Chinese Clinical Trial Registry (ChiCTR-IOR-14005696) in 2014

20.6 Electronic search strategy

Database: Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) <1946 to May 5 2017>

- 1 exp Infant, Newborn/ (534737)
- 2 Premature Birth/ (8667)
- 3 (neonat\$ or neo nat\$).ti,ab. (216537)
- 4 (newborn\$ or new born\$ or newly born\$).ti,ab. (142561)
- 5 (preterm or preterms or pre term or pre terms).ti,ab. (55189)
- 6 (preemie\$ or premie or premies).ti,ab. (135)
- 7 (prematu\$ adj3 (birth\$ or born or deliver\$)).ti,ab. (12958)
- 8 (low adj3 (birthweight\$ or birth weight\$)).ti,ab. (28475)
- 9 (lbw or vlbw or elbw).ti,ab. (6471)
- 10 infan\$.ti,ab. (365104)
- 11 (baby or babies).ti,ab. (57831)
- 12 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 (901232)
- 13 Infant Formula/ (3194)
- 14 (infant\$ adj2 formula\$).ti,ab. (5615)
- 15 (pediatric adj2 formula\$).ti,ab. (366)
- 16 (paediatric adj2 formula\$).ti,ab. (158)
- 17 ((baby or babies) adj2 formula\$).ti,ab. (228)

- 18 (formula\$ adj2 milk).ti,ab. (2830)
- 19 (hydrolysed adj2 (formula\$ or milk or protein\$ or whey)).ti,ab. (399)
- 20 (hypoallergen\$ adj2 (formula\$ or milk or protein\$ or whey)).ti,ab. (231)
- 21 (Nutramigen or Nutriprem or Pregestamil or Profylac or Nan or Aptamil Pepti or Pepti-Junior or Pepdite or Infatrini or Similac or Gold Prem Pro or Alimentum).ti,ab. (1722)
- 22 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 (11381)
- 23 randomized controlled trial.pt. (416680)
- 24 controlled clinical trial.pt. (90725)
- 25 randomized.ab. (353269)
- 26 placebo.ab. (172037)
- 27 drug therapy.fs. (1857871)
- 28 randomly.ab. (253354)
- 29 trial.ab. (365448)
- 30 groups.ab. (1580905)
- 31 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 (3779279)
- 32 exp animals/ not humans.sh. (4243660)
- 33 31 not 32 (3256479)
- 34 12 and 22 and 33 (2440)
- 35 13 or 14 or 15 or 16 or 17 or 18 (9413)
- 36 19 or 20 or 21 (2307)
- 37 35 and 36 (339)

38 12 and 33 and 37 (130)

39 19 or 20 or 21 (2307)

40 12 and 33 and 39 (203)

Database: OVID Embase <1974 to May 5 2017>

1 exp Infant, Newborn/ (508714)

2 Premature Birth/ (46718)

3 (neonat\$ or neo nat\$).ti,ab. (271413)

4 (newborn\$ or new born\$ or newly born\$).ti,ab. (172205)

5 (preterm or preterms or pre term or pre terms).ti,ab. (73103)

6 (preemie\$ or premie or premies).ti,ab. (178)

7 (prematu\$ adj3 (birth\$ or born or deliver\$)).ti,ab. (17047)

8 (low adj3 (birthweight\$ or birth weight\$)).ti,ab. (34278)

9 (lbw or vlbw or elbw).ti,ab. (8306)

10 infan\$.ti,ab. (431407)

11 (baby or babies).ti,ab. (76516)

12 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 (1007803)

13 (hydroly\$ adj3 (formula\$ or milk or protein\$ or whey)).ti,ab. (8452)

- 14 (hypoallergen\$ adj3 (formula\$ or milk or protein\$ or whey)).ti,ab. (396)
- 15 (Nutramigen or Nutriprem or Pregestamil or Profylac or Nan or Aptamil Pepti or Pepti-Junior or Pepdite or Infatrini or Similac or Gold Prem Pro or Alimentum).ti,ab. (1633)
- 16 13 or 14 or 15 (10219)
- 17 clinical trial/ (861853)
- 18 randomized controlled trial/ (403031)
- 19 randomization/ (70301)
- 20 single blind procedure/ (22044)
- 21 double blind procedure/ (130612)
- 22 crossover procedure/ (46975)
- 23 placebo/ (286950)
- 24 randomi?ed controlled trial\$.tw. (134914)
- 25 rct.tw. (20175)
- 26 random allocation.tw. (1559)
- 27 randomly allocated.tw. (24807)
- 28 allocated randomly.tw. (2124)
- 29 (allocated adj2 random).tw. (834)
- 30 single blind\$.tw. (17551)
- 31 double blind\$.tw. (167855)
- 32 ((treble or triple) adj blind\$).tw. (571)
- 33 placebo\$.tw. (237305)

34 prospective study/ (332345)

35 or/17-34 (1586794)

36 case study/ (37722)

37 case report.tw. (314194)

38 abstract report/ or letter/ (976941)

39 or/36-38 (1321873)

40 35 not 39 (1545375)

41 12 and 16 and 40 (385)

Database: OVID Maternity & Infant Care Database (MIDIRS) <1971 to May 2017>

Search date: May 20 2016

1 (neonat\$ or neo nat\$).ti,ab. (35102)

2 (newborn\$ or new born\$ or newly born\$).ti,ab. (16236)

3 (preterm or preterms or pre term or pre terms).ti,ab. (20374)

4 (preemie\$ or premie or premies).ti,ab. (45)

5 (prematu\$ adj3 (birth\$ or born or deliver\$)).ti,ab. (3362)

6 (low adj3 (birthweight\$ or birth weight\$)).ti,ab. (9112)

7 (lbw or vlbw or elbw).ti,ab. (2452)

8 infan\$.ti,ab. (52277)

9 (baby or babies).ti,ab. (25039)

10 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 (97194)

11 (hydroly\$ adj3 (formula\$ or milk or protein\$ or whey)).ti,ab. (135)

12 (hypoallergen\$ adj3 (formula\$ or milk or protein\$ or whey)).ti,ab. (28)

13 (Nutramigen or Nutriprem or Pregestamil or Profylac or Nan or Aptamil Pepti or Pepti-Junior or Pepdite or Infatrini or Similac or Gold Prem Pro or Alimentum).ti,ab. (32)

14 11 or 12 or 13 (178)

15 10 and 14 (168)

16 limit 15 to randomised controlled trial (19)

Database: CINAHL

Search date: May 5 2017

Search Terms	Search Options	Actions	
S6	S3 AND S4	Limiters - Clinical Queries: Therapy - High Sensitivity Search modes - Boolean/Phrase	View Results (85) View Details
S5	S3 AND S4	Search modes - Boolean/Phrase	View Results (198)

			View Details Edit
S4	TX ((hydroly* NEAR/3 (formula* or milk or protein* or whey))) OR TX ((hypoallergen* NEAR/3 (formula* or milk or protein* or whey))) OR TX ((Nutramigen or Nutriprem or Pregestamil or Profylac or Nan or Aptamil Pepti or Pepti-Junior or Pepdite or Infatrini or Similac or Gold Prem Pro or Alimentum))	Search modes - Boolean/Phrase	View Results (2,841) View Details Edit
S3	S1 OR S2	Search modes - Boolean/Phrase	View Results (355,439) View Details Edit
S2	TX ((neonat* or neo nat*)) OR TX ((newborn* or new born* or newly born*)) OR TX ((preterm or preterms or pre term or pre terms)) OR TX ((preemie\$ or premie or premies)) OR TX ((prematu* NEAR/3 (birth* or born or deliver*))) OR TX ((low NEAR/3 (birthweight* or birth weight*))) OR TX ((lbw or vlbw or elbw)) OR TX infan* OR TX ((baby or babies))	Search modes - Boolean/Phrase	View Results (355,439) View Details Edit
S1	(MH "Infant, Newborn+")	Search modes - Boolean/Phrase	View Results (94,408) View Deta

20.7 PRISMA flowchart

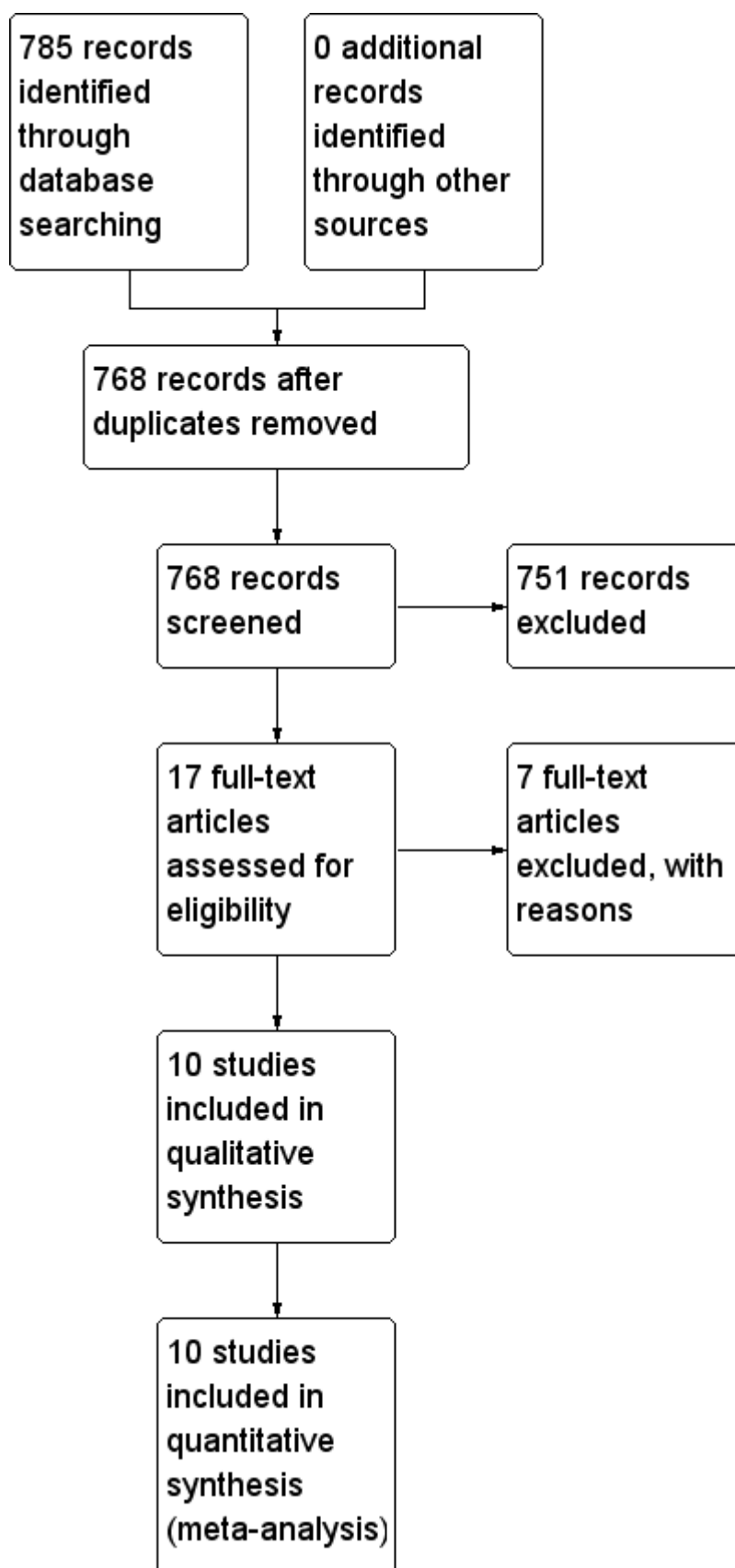


Figure 6 PRISMA flow chart