The role and use of fortified milk-based products in the diets of older infants and young children

MPI Technical Paper No: 2013/40

Prepared for MPI
By Emily Watson and Dr Anne-Louise Heath
Reviewer: Professor Rosalind Gibson

ISBN No: 978-0-478-42056-2
ISSN No: 2253-3923

October 2013

Disclaimer

While every effort has been made to ensure the information in this publication is accurate, the Ministry for Primary Industries does not accept any responsibility or liability for error of fact, omission, interpretation or opinion that may be present, nor for the consequences of any decisions based on this information.

Requests for further copies should be directed to:
Publications Logistics Officer
Ministry for Primary Industries
PO Box 2526
WELLINGTON 6140

Email: brand@mpi.govt.nz

Telephone: 0800 00 83 33
Facsimile: 04-894 0300

This publication is also available on the Ministry for Primary Industries website at
http://www.mpi.govt.nz/news-resources/publications.aspx

Background

The purpose of the literature review is to evaluate available evidence on the use and role of fortified milk-based products in the diets of older infants and young children, in addition to the efficacy of such products on nutritional and health outcomes. There is a paucity of data available on the usage and role of follow-up formula and fortified milk based products in the diets of infants and young children worldwide. The data gathered in this document will be used to inform the review of the Codex Standard for Follow-up Formula (FUF) (CODEX STAN 156-1987). The Codex Standard for FUF defines the product as "a food intended for use as a liquid part of the weaning diet for the infant from the 6th month on and for young children (12 - 36 months)".

The current Codex Standard for FUF is over 20 years and there have been significant changes in the use and marketing of formulas covered by this standard. This includes the emergence of a new product category of toddler milks otherwise known or marketed as "growing-up milks" or "junior milks" targeted to young children aged 12 to 36 months.

At the 34th CCNFSDU Committee meeting (December 2012) the Committee agreed to a full review of the Standard led by New Zealand, France and Indonesia. An electronic working group was established and contributed scientific papers, and regulatory information relating to use of FUF and toddler milks in their country or region. The information gathered from the electronic working group was used as part of this review.

The specific objectives of this review are to review:

1. The role of cows' milk products and fortified milk-based products (including FUF, growing-up milks, and toddler milks) in the diets of older infants and young children, including:
    - Age of introduction;
    - Quantity and frequency of consumption;
    - Whether they are as supplementary to the diet, or as a replacement for something (i.e. breast milk or animal milk)
    - How their inclusion in the diet affect nutrient intakes; and
    - Parental perceptions of necessity and rationale for use

2. Trials of fortified milk-based products
    - Evidence to their efficacy in improving nutritional imbalances/inadequacies
    - Impact on health outcomes

UNIVERSITY OF OTAGO
DIVISION OF SCIENCES

The role and use of fortified milk-based products in the diets of older infants and young children

Authors: Emily Watson and Dr Anne-Louise Heath
Reviewer: Professor Rosalind Gibson

Department of Human Nutrition

July 2013

Table of Contents

1 Literature search

A search of the electronic database Medline, OvidSP, (from 1946 to June 2013) was conducted using the search strategies in Tables 1-3. Only articles that were available electronically were considered.

Search strategy 1 identified articles relevant to Chapter 2 – Role of fortified products in the diets of infants and young. This initial search identified 239 articles. After searching titles, 27 were kept. After reading abstracts and reviewing reference lists of articles, 9 were used in this review. A call for data by MPI also provided an additional 4 articles, resulting in the total of 13 articles used in this review. Articles were excluded if the study:

- Had no relevance to nutrition issues in the complementary diet of infants and young children
- Was not observational (i.e. included an intervention).

Table 1: Search strategy 1

Step	Search term
1	Infant Formula [MeSH] OR Milk [MeSH]
2	Toddler$ [keyword] OR Child, Preschool [MeSH] OR Infant [MeSH]
3	Nutritional status
4	1 AND 2 AND 3
5	Limit 4 to (english language and full text and humans)

MeSH, Medical Subject Headings;

Search strategy 2 identified articles relevant to Chapter 2.6 – Parental perceptions of necessity and rationale for use of fortified milk products. This initial search identified 65 articles. After searching titles, 15 were kept. After reading abstracts and reviewing reference lists of articles, 12 were used in this review. A call for data by MPI also provided an additional 18 data sources, resulting in the total of 33 articles and data sources used. Articles were excluded if:

- They had no relevance to parental perceptions of necessity and rationale for use of fortified milk-based products
- They referred only to reasons why parents chose to feed formula from birth (as opposed to weaning from breastmilk and introducing formula).

Table 2: Search strategy 2

Step	Search term
1	Infant Formula [MeSH] OR Milk [MeSH]
2	Toddler$ [keyword] OR Child, Preschool [MeSH] OR Infant [MeSH]
3	Perception [MeSH]

4	Health Knowledge, Attitudes, Practice [MeSH]
5	3 OR 4
6	1 AND 2 AND 5
7	Limit 6 to (english language and full text and humans)

MeSH, Medical Subject Headings;

Search strategy 3 identified articles relevant to Chapter 3 – Trials of fortified milk-based products. This initial search identified 1238 articles. After searching titles, 70 articles were kept, and after reading abstracts, 30 articles were kept. Furthermore, a review of reference lists of articles was conducted to identify more studies, resulting in the total of 46 articles used in this review. Articles were excluded if the trial:

- Had no relevance to trials of milk-based products
- Only used products that were not milk-based in the intervention
- Was conducted in infants all of whom were less than six months or greater than 36 months of age during the entire intervention period of the study
- Was not a randomised controlled trial
- Did not use a control group.

Table 3: Search strategy 3

Step	Search term
1	Infant Formula [MeSH] OR Milk [MeSH]
2	Toddler$ [keyword] OR Child, Preschool [MeSH] OR Infant [MeSH]
3	Micronutrients [MeSH]
4	Dietary Carbohydrates [MeSH]
5	Dietary Fats [MeSH]
6	Dietary Proteins [MeSH]
7	Energy Intake [MeSH]
8	3 OR 4 OR 5 OR 6 OR 7
9	1 AND 2 AND 8
10	Limit 9 to (english language and full text and humans)

MeSH, Medical Subject Headings;

2 Role of fortified milk-based products in the diets of infants and young children

Infant formula, as defined by the Codex Alimentarius (CODEX STAN 72-1981) (6), is: 'a breast-milk substitute specially manufactured to satisfy, by itself, the nutritional requirements of infants during the first months of life up to the introduction of appropriate complementary feeding'.

Follow-up formula (FUF), defined by the Codex Alimentarius (CODEX STAN 156-1987) (7), is: 'a food intended for use as a liquid part of the diet from the 6th month on and for young children…the product shall be nutritionally adequate to contribute to normal growth and development when used in accordance with its directions for use'.

Growing-up milk (GUM) is fortified milk intended for children from 12 months of age, however, it is not defined by the Codex Alimentarius Committee.

Infants are defined as 'a person not more than 12 months of age' while **young children** are 'persons from the age of more than 12 months up to the age of three years (36 months)' (7). The term "**toddler**" is used in this review to refer to young children aged from 12 to 36 months.

2.1 Literature

The information used in chapter 2 (summarised in Appendix A, Tables 4.1-4.3) is mainly from two sources, one of which is the data submitted to the Ministry for Primary Industries (MPI) from a call for data. The other source is from a document written by FAO/WHO where a call for data was also made including information on the consumption, frequency, and amount of follow-up formula consumed by toddlers, and consumer attitudes towards follow-up formula (8). Unfortunately a limitation of the data provided by some countries is that it is not always representative of the whole country. For example, the information provided by Guatemala is taken from a survey undertaken by 300 mothers in the capital city. This will not necessarily be representative of mothers and children from other parts of the country. Information on follow-up formula alone was not always available, as often during studies all formula types were grouped together. It is stated in the text when this information is used.

2.2 What ages is follow-up formula introduced?

As mentioned above, the Codex Alimentarius Committee recommend follow-up formula to be used by infants and young children no earlier than six months, and up to 36 months of age (7). This recommendation is followed by the majority of developed countries (six out of 10), however Luxembourg, Malta, and Norway all market follow-up formula to children from four months of age (8). Germany also markets follow-up formula to an earlier age group, children five to seven months. The official recommendations in the United Kingdom (UK) (9) and Germany (10) specifically state that if breastmilk or infant formula is already being consumed, there is no need to change to follow-up formula.

The majority of developing countries market follow-up formula for children from six months. Only one country, Nicaragua, allowed the introduction of follow-up formula before six months, with follow-up formula marketed for children from birth to 60 months (8).

There is some evidence that these recommendations for the minimum age of follow-up formula introduction are not always followed. France, Ireland, Luxembourg, and UK all reported introduction earlier than their country's recommendation, as did the developing countries Ghana and the Philippines. There is a large range in the age at which follow-up formula was first introduced to the child. The earliest follow-up formula introduction reported was at one month by 2% of children in a UK study of 9,416 mothers (8). Even within countries there was a range of ages at which follow-up formula was introduced, such as in Sweden, where 44% of children were introduced to follow-up formula at less than four months old (11), 30.5% at four to six months, and 50% at six months or older. It did however appear that in the majority of studies from developed countries, the highest proportion of children were in age groups less than six months when first introduced to follow-up formula (France (8,12), Ireland (8), Luxembourg (8), Norway (8,13), and Sweden (11,14)). Children from developing countries were more likely to first be offered follow-up formula at six months (Brazil, Guatemala, Korea, and Nicaragua) (8).

Rates of follow-up formula consumption at or before six months of age were reported by eight developed countries and three developing countries. The mean proportion consuming follow-up formula in developed countries was 50% (four to six months), range 11% at five months in Ireland to 90% at six months in Sweden. Developing countries had a mean 18% of children (from birth to six months) consuming follow-up formula, with a range of <10% in Guatemala before six months up to 33% in Ghana at six months.

The proportion of children consuming follow-up formula between six and 12 months of age was reported by seven developed countries and two developing countries. In the developed countries, there was a mean of 51% of children consuming follow-up formula, ranging from 27% in Ireland at 12 months, up to >80% in France between the ages of six to eight months. In the developing countries, there was a mean of 73% of children consuming follow-up formula, range 65% in Guatemala (six to 12 months) up to 78% in Malaysia (nine to 11 months). This age group, six to 12 months, appeared to be the age group at which the largest proportion of children consumed follow-up formula.

Although there were fewer data available than for the other age groups (five from developed countries, two from developing countries), from 12 months onwards, the consumption rates of follow-up formula decreased in all countries (developed countries: mean 21%; developing countries: mean 45%). Included in this age range is also the children consuming growing-up milks, which were not reported to be consumed by any child before 12 months of age.

2.3 How much fortified milk-based product is consumed and how frequently?

The data available on the amount of follow-up formula consumed by children six to 36 months are limited, and particularly the frequency with which it is consumed. For this reason, if data were available for any type of formula or milk and not just follow-up formula

alone, it has been included in the summary below. Data were only available from developed countries, with no developing countries providing data on the amount or frequency of milk consumed.

Three studies reported the mean amount of infant formula, follow-up formula or toddler milk consumed per day when the infants were six months or less. One country (USA) reported 887mL/day of any formula consumed (8), while the two others (Germany (15) and UK (9)) had a mean consumption of 375 g/day (364 mL/day assuming a specific gravity of 1.03 g/mL). Children greater than six months and up to 12 months of age in Germany (10,15), Ireland (16), UK (9), and Norway (17) had a mean intake of 314 g/day (305 mL/day) or in the USA (8) 628 mL/day, while children older than 12 months had a mean fortified milk-based product intake of 353 g/day (343 mL/day) in Germany (10,15), Ireland (16), UK (9), and Norway (18).

Frequency of feeds was only reported by two countries, one of which reported Norwegian six month old children to have 1.6 feeds of follow-up formula per day (13). A longitudinal Swedish study (following children from birth to a mean age of 8.7 months) found the frequency to fluctuate significantly within individuals, with the average frequency ranging from none to twice a day over any 14 day period for an individual (11).

2.4 Are fortified milk-based products consumed in addition to the diet or as a replacement for something?

Data were available from five developed countries on the different types of milks consumed by children six to 36 months old (9,12,16,19,20). No information was available from developing countries.

It appears that as age increased from four or five months up to 12 months, rates of breastfeeding decreased, while rates of follow-up formula consumption increased. For example, in Switzerland (19) at seven to nine months 29% were breastfed which decreased to 18% at 10-12 months. The percentage consuming follow-up formula meanwhile increased from 53% to 63% across the same time period. From 13 months onwards follow-up formula also decreased, although it was still higher than the percentage breastfed at 31-36 months (15% vs. 0%). In the available data summarised in table 1, it appears that follow-up formula may replace breastmilk as the main milk drink in children up to 12 months of age in these Swiss children, however, some children do consume both, and data on infant formula consumption were not available. In the Growing Up in New Zealand Study (21), at nine months of age, although 48% were breastfed, 78% had been fed a milk other than breast milk on at least one occasion. Follow-up formula was the most common breast milk substitute, with 73% of the children having had follow-up formula (compared to 59% having had infant formula), while 5.4% had tried cow's milk (it was not stated whether this was whole or reduced fat, or whether it had been diluted).

From 12 months, rates of both breastfeeding and follow-up formula consumption decreased with growing-up milk becoming the prominent milk choice replacing follow-up formula in some countries (9,12,22). However, by 31 months of age most children (>85%) were no longer drinking any type of fortified milk-based product (12,18,19,22–25).

2.5 How does the inclusion of follow-up formula in the diet affect energy and nutrient intakes?

Only two studies (26,27) assessed the association between drinking follow-up formula or growing-up milk and energy or nutrient intakes. Both were from developed countries.

A cross-sectional study in Sweden of 332 children who were 30 months old, found that in the children with iron-deficiency (ID), 11% drank follow-up formula compared to 43% in the children with iron sufficiency (26). They also found a positive correlation between drinking follow-up formula and serum ferritin levels, whereas a negative correlation was found between cow's milk consumption and serum ferritin, suggesting that the follow-up formula increased iron intakes. Certainly follow-up formula contains considerably more iron than cow's milk, with the iron concentration proposed by Koletzko et al (2013) for follow-up formula 6.6-13.3 mg/L (28), compared to <0.3 mg/L in cow's milk (New Zealand full fat milk) (29).

A study in French children aged 12-24 months found that total energy and macronutrient intakes, except for protein, were not different in children consuming either cow's milk (of which 70% was semi-skimmed milk) or growing-up milk (GUM) as their predominant milk (27). Children consuming cow's milk (CM) had a mean protein intake of 41.6g/day compared to 35.5 g/day in those consuming growing-up milk (French Adequate Intake (AI) = 35.9 g/day). The children drinking growing-up milk were less likely to have low daily intake of alpha-linoleic acid (GUM: 0.51 g/day; CM: 0.33 g/day; AI: 0.43), iron (GUM: 9.4 mg/day; CM: 5.2 mg/day; French Estimated Average Requirement (EAR): 5.4 mg/day), vitamin C (GUM: 82 mg/day; CM: 52 mg/day; EAR: 46 mg/day), or vitamin D (GUM 6.8 µg/day; CM: 0.8 µg/day; EAR: 7.7 µg/day). It was not stated whether the cow's milk was being consumed as purchased, or diluted.

There were no studies from developing countries assessing the effect on energy or nutrient intakes of the inclusion of follow-up formula in the diet of children aged six to 36 months (30). There was, however, a national survey in Indonesia where information was collected on the foods and drinks consumed by children six to 59 months old. The survey found 30% of children from rural areas and 40% from urban slums were drinking a commercial multi-nutrient fortified milk (the concentration of fortificants was not stated). The prevalence of anaemia was 47-56% in children drinking the fortified milk, significantly less than the anaemia prevalence of 60-64% in children not drinking the fortified milk.

2.6 Parental perceptions of necessity and rationale for use of fortified milk-based products

Only one study, the Infant Feeding Survey (IFS) from the UK, reported the reasons why parents used follow-up formula as opposed to infant formula (31). All other studies from developed countries reported: reasons why parents fed formula (32,33) or weaned from breastmilk to formula or cow's milk (11,34); or where parents got their information from with respect to infant feeding (21).

The main reason for parents in the IFS 2010 survey (31) introducing follow-up formula into their child's diet at four to six months of age was that they thought it was better for their

baby or provided more nutrients (18%). This reason was closely followed by advice from a health professional (17%), and then experience with a previous children (13%).

In Norway, the most common reason given for introducing any type of formula was that the child was old enough (23%), or that the child should get used to the bottle and formula (18%) (11). In Canada, 79% of mothers chose to wean from breast milk at eight months as they were either returning to work or due to a personal decision (35). In Luxembourg, many parents reportedly chose to use follow-up formula as they thought it increased satiety in infants, and used it earlier than recommended to increase sleep during the night (8), whereas 62% of Chinese mothers in Singapore thought that certain formulas could improve their baby's Intelligence Quotient (IQ) (33).

In the Growing Up in New Zealand Study (21) information on infant feeding practises was obtained from Plunket (a support service for children under five years) by almost all the mothers in the study (93%), with 77% also asking family and friends for advice. Only 6% used self-knowledge in New Zealand (21), compared to 15% in France (32). In France, 85% of parents asked at least one source (for 77% of parents this was a medical professional) for recommendations on infant feeding.

At four to six months of age, mothers in the UK who had never worked were most likely to have given follow-up formula to their child (19%), whereas mothers in managerial and professional occupations were the least likely (6%). At eight to ten months, however, there were no differences between the mothers' occupations and whether they fed follow-up formula to their child or not (31).

There was no information available on the reasons why follow-up formula was specifically chosen by parents in developing countries, with all four studies looking at the reasons why parents had chosen to feed any type of formula (36–39).

In Malaysia, the main factors influencing which follow-up formula was purchased were quality, brand, and convenience, whereas medical official's advice, advertising and promotions were the least influential factors (37). Unfortunately the reasons for choosing follow-up formula instead of breast milk or infant formula are not reported. In Bangladesh, the majority of parents reported a perceived insufficiency of breast milk (62%) as their reason (38).

Formula advertisements in the Philippines were recalled by 75% of parents, who were then 6.4 times more likely to have stopped breastfeeding before 12 months (and presumably then fed either formula or cow's milk to their child), than parents who could not recall any formula advertisements (39). Fifty-nine percent of mothers recalled formula advertisements that portrayed messages that formula would make their baby healthy, or smarter, and protect against infections.

2.7 Conclusion

There appears to be evidence that the majority of countries (both developed and developing) are following the Codex Standard recommendation by only allowing follow-up formula to be marketed for children above six months of age. Parents, however, often do not follow these recommendations, with the follow-up formula often fed to children before

six months of age in developed countries. Children from developing countries were generally first introduced to follow-up formula from six months. Consumption of follow-up formula was highest in the six to 12 months age group, with consumption decreasing with increasing age after 12 months. It appeared that follow-up formula was drunk instead of infant formula or cow's milk, although it may be drunk as a supplementary food to breast milk in some countries. Information on whether follow-up formula replaced food intake was not available. There were many reasons provided as to why follow-up formula or any type of formula was consumed, such as the belief that it provided more nutrients, or was healthier for the child. Mother returning to work was also a strong motivator for providing formula.

3 Trials of fortified milk-based products

3.1 Iron-fortified milk-based product trials

This literature review has identified 12 trials (13 papers) assessing iron status as an outcome, comparing iron-fortified milk with a control in children aged six to 36 months (Appendix B, Table 5.1). Of these, eight trials are from developed countries (40–48), and four were conducted in developing countries (49–52).

3.1.1 Measuring iron status

The material in this section is from Gibson (53) unless stated otherwise. Iron deficiency occurs in progressive stages, the first of which is iron depletion. The supply of iron to functional tissues is not compromised at this stage, only the iron stores. This is identified by a decrease in serum ferritin concentration (as long as infection is not present). The second stage is iron-deficient erythropoiesis, where the iron stores are exhausted, and the iron supply to erythropoietic cells is reduced. It is not until the third stage of iron deficiency that haemoglobin levels decrease, and iron-deficiency anaemia (IDA) occurs (53). There are multiple indexes which can be used to measure the stages of iron deficiency, however, many have limitations which restrict their use.

A decrease in haemoglobin concentration, although often used to indicate iron deficiency anaemia, is not exclusive to iron deficiency. Other deficiencies of micronutrients such as vitamins A, B_6, B_{12}, and folate also result in anaemia, as do various genetic haemoglobin disorders, malaria, and disease states. Other indices are therefore required in combination with haemoglobin to identify IDA.

Serum ferritin is an extremely useful marker of iron deficiency as it falls only when there is a decrease in the total amount of storage iron. However, acute and chronic infections along with some disease states and vitamin A deficiency may also artificially raise serum ferritin concentrations and lower haemoglobin levels. Consequently, it is necessary to include a marker of infection or inflammation to confirm the accuracy of serum ferritin data. C-reactive protein (CRP) is commonly used to assess acute infections, while serum α-1 acid glycoprotein or α-1 antichymotrypsin are chronic infection markers.

Soluble transferrin receptor (TfR) is elevated as iron deficiency develops. Unlike serum ferritin, it is not significantly affected by inflammation arising from infection. However, it is used less frequently than serum ferritin to measure iron status as it is more expensive to measure, and the reference limits indicative of deficiency vary with the assay method.

It has been recommended that haemoglobin concentration, serum ferritin, serum transferrin receptor, and preferably two biomarkers of infection and inflammation (i.e., CRP and α-1 acid glycoprotein) should be used to assess iron status (54). Using this combination, total body iron (mg/kg) calculated using transferrin receptor and serum ferritin, adjusted for inflammation where appropriate (55), can also be calculated (56,57). Calculated “body iron” provides a measure of the full range of iron status (i.e., iron deficiency, storage iron, iron overload), together with a more accurate reflection of the prevalence and severity of nutritional iron deficiency (57).

Although less commonly used, there are other less specific haematological biomarkers of iron status that can be used. These include haematocrit which, like haemoglobin, only decreases after red blood cell formation is impaired, and mean cell volume (i.e., average red blood cell size) which is also reduced in the presence of microcytic anaemia, the characteristic anaemia of iron deficiency. Biomarkers of iron supply include serum iron and total-iron binding capacity, which are used to calculate transferrin saturation (i.e., the proportion of transferrin bound to iron). The latter is markedly reduced in iron deficient erythropoiesis. In contrast, zinc protoporphyrin, a measure of iron supply to red blood cells, increases in iron-deficient erythropoiesis, because zinc replaces the missing iron during the formation of the protoporphyrin ring (54). Red cell distribution width (a measure of the variation in red blood cell size, which is increased in iron deficiency anaemia) is also used by some researchers (48).

3.1.2 Efficacy of iron-fortified milk-based products for improving iron status in developed countries

Cow's milk was used as the control milk by most trials of the efficacy of iron-fortified milk-based products for improving iron status from developed countries (6 out of 8 (41,42,44–47)), with the other trials using either breast milk (1 out of 8 (43)), or iron supplements (1 out of 8 (48)). The concentration of the iron in the control milks ranged from 0.09 to 0.6 mg/L (mean 0.44 mg/L), providing a mean daily iron intake from the milk of 0.31 mg. These control milks were then compared to either fortified milk or formula, with iron concentrations ranging from 0.9 to 15 mg/L (mean 9.7 mg/L), which provided a mean daily iron intake from the milk of 5.1 mg. Four of the studies reported that the participants were given the study milks *ad libitum*, and asked to replace their regular milk with the study milk (44,46–48). The other studies did not report any information on how much the participants were recommended to drink.

The mean duration of the study intervention periods was 8.4 months, with a range of 3 to 12.3 months. The children in the study had a mean age of 7.9 months at recruitment, with the studies recruiting children from birth up to 20 months. Their mean age at the end of the studies was 17.1 months, range 9 to 25 months.

3.1.2.1 Iron deficiency anaemia

Three trials reported the prevalence of iron deficiency anaemia (IDA) in their participants. The study by Wall et al (2005) recruited hospitalised infants (aged 9-23 months at baseline) with an acute illness and IDA. The iron status data were adjusted for an increased CRP (defined as >6 mg/L) by multiple regression analysis, in an effort to take into consideration the effects of illness on the measures of iron status (48). Infants were given iron-fortified follow-on formula (containing 12.0 mg/L of iron), iron-fortified cow's milk (containing 12.9 mg/L of iron) (interventions), or an iron supplement (3 mg/kg of body weight/day) (control). The iron supplement was used as a positive control in this study, with the objective that the three groups would not differ at the end of the study. The prevalence of IDA (defined as two of the following: serum ferritin concentration <10 mg/L, transferrin saturation <10%, and red cell distribution width >14.5%; in the presence of haemoglobin <110 g/L) was significantly reduced in all three groups. However, the prevalence of IDA in the iron-fortified cow's milk group was reduced by significantly less (66 percentage points (PP)) than in the

iron supplement group (86 PP) or iron follow-on formula (75 PP) (p-value not reported). The iron-fortified cow's milk, although not as efficacious in treating IDA as the iron supplement or iron follow-on formula, still significantly reduced the prevalence of IDA in this group of children. Nevertheless, this study is problematic as the participants were sufficiently unwell to be hospitalised, and certain illnesses, especially those accompanied by inflammation from infection, are known to impair the mobilization and transport of iron to the target tissues, even when iron stores are present. This effect is termed "functional iron deficiency", and in such circumstances iron indices would be expected to return to normal as a result of the resolution of the illness, whether or not there was supplementation or fortification with iron. In this study, after the intervention, haemoglobin and transferrin saturation both increased, and serum ferritin decreased. It is possible these changes were due to the children recovering from their illness rather than the successful treatment of IDA with iron, but because there was no negative control (i.e. a group who received no iron treatment) this cannot be determined.

The two other studies that reported data on IDA were from healthy populations with a prevalence of IDA at baseline (12 to 20 months) of 0% (46,47). As all the participants entered these studies without IDA, it is not surprising that the iron interventions (toddler milk or iron-fortified milk vs. cow's milk) had no effect on the prevalence of IDA, with both studies reporting no change from baseline in any of the groups, nor any differences between intervention or control groups.

3.1.2.2 Serum ferritin

Six out of the eight studies found that serum ferritin concentrations were significantly higher in the intervention group than the control group after the study (40–42,44,46,48). In nearly all the study groups, whether control or intervention, mean serum ferritin levels declined from baseline (40–43,48). Although not all studies reported using log transformation to account for the non-normal distribution of serum ferritin concentration (i.e., by reporting geometric means), both Gill et al (42) and Wall et al (48) reported a decline in serum ferritin using geometric mean serum ferritin. It is not clear why this decline occurs, but the decline in geometric mean serum ferritin concentration in the control group of the Toddler Food Study was accompanied by a significant increase in haemoglobin concentration (46) suggesting that the changes reflect the transfer of stored iron to the erythropoietic tissue to form haemoglobin. In the majority of studies in which there was a decline in serum ferritin concentration in both intervention and control group, the decline was significantly less in the intervention milk group compared to the control milk group (40–42,48), suggesting a positive effect of the iron-fortified milk. Only one study, with children aged 12-20 months, reported an increase in serum ferritin in any of the groups, with a 44% increase in the iron-fortified toddler milk (or "GUM") group (there was a 14% decrease in serum ferritin in the cow's milk group) (46). Two of the eight studies showed no effect of the iron-fortified milk on serum ferritin concentrations (43,47).

There is no evidence that serum ferritin results varied by age of the child at recruitment (the positive studies recruited participants from birth (40) to 20 months of age (46) at baseline, whereas the negative studies recruited three (43) and 12 (47) month old participants). Nor is there evidence that duration of the intervention affected the results (positive studies had durations from three (48) to 12 (40) months, whereas both negative studies had a duration of six months (43, 47)).

It is very important to note, however, that just two of the eight studies accounted for the impact of infection on serum ferritin concentration (i.e., the artificial elevation of values) appropriately: one study collected blood samples from infants who were healthy at baseline and controlled for CRP concentration using a multivariate model (46), and in the other study all the children had normal CRP concentrations (<9 mg/L) (47). Five of the eight studies did not report assessing the impact of infection on serum ferritin concentration (40-44), and one, although controlling for CRP in the analyses, specifically enrolled participants who were unwell at baseline (48). It is very difficult to determine the effect that infection may have had on the results of these studies particularly in this age group that has such high rates of acute illness (a mean of 8.8 episodes a year in toddlers aged 12-24 months with an average duration of 4.5 days per episode (58)). Ultimately, it is best practice to account for the possible effects of infection, and just two studies have done this (46,47). Although Virtanen et al (47) found no effect of an iron-fortified milk on serum ferritin concentration, they had lost 33% of their participants by the end of the six month study, leaving just 20 in their intervention group, and 16 in their control group at age 18 months so did not have sufficient statistical power to detect an effect of the intervention (a sample size calculation for serum ferritin is not reported by Virtanen et al (47), but the Toddler Food Study estimated that a sample size of 45 participants in each group would be necessary to have 80% power and α=0.05 to detect a difference of 42% in geometric mean serum ferritin concentration between their toddler milk group and the control group (46)). The Toddler Food Study (46), therefore, is the only study to have accounted appropriately for infection and to have maintained a sample size large enough to detect an effect of an iron-fortified milk on serum ferritin concentration, were one present. The study found that consumption of an iron-fortified toddler milk *ad libitum* for five months in New Zealand toddlers aged 12-20 months resulted in a significant and substantial increase in serum ferritin concentration.

Three of the studies reported the effects of a fortified-milk intervention on the prevalence of low serum ferritin, using cutoffs of <10 μg/L (42,43), and <8 μg/L (45). Two of these studies found the prevalence of low serum ferritin was significantly less in the intervention group compared to the control group (37 PP (42) and 13.5 PP (45) less), with the other study finding no difference between the groups after the intervention period, although in that study fewer than 3% of the participants had low serum ferritin at baseline (43). However, none of these three studies accounted for the effects of infection on serum ferritin concentration, so the results should be treated with caution.

3.1.2.3 Body iron

Only one study, the Toddler Food Study (46), has reported the effects of an iron-fortified milk on body iron. Body iron was significantly higher (1.9 mg/kg) in the toddler milk group than the cow's milk group at the end of the 20 week intervention.

3.1.2.4 Anaemia and haemoglobin concentration

The prevalence of anaemia (haemoglobin <110 g/L) rather than IDA was reported in four of the studies (41–44). Two of these studies found a significantly higher prevalence of anaemia in the control cow's milk group post-intervention compared to the intervention group (iron-fortified follow-on formula) (41,42). The study by Daly et al (41) recruited infants at six months who were already consuming cow's milk and randomised them to either continue to consume cow's milk, or to consume iron-fortified follow-on formula (12 mg of iron/L which contributed 5.8 mg of iron/day) until 18 months of age when all children were switched to

cow's milk (41). The prevalence of anaemia was significantly lower in the intervention group not only at 12 and 18 months, but also at 24 months (i.e., six months after consumption of the iron-fortified milk had ceased), at which time 0% of the original intervention group had anaemia compared to 26% for the cow's milk group. However, paediatric groups recommend that unmodified (i.e., non-formula) cow's milk is not consumed as the main source of milk for infants before 12 months of age because cow's milk may cause gastro-intestinal bleeding, has a very low iron content, and is likely to displace other milks that are richer in iron (26). Hence, it is unclear the extent to which the protection against anaemia in the intervention group is the result of removal of a negative effect (i.e., cow's milk consumption before 12 months of age), and the extent to which it is due to consumption of the follow-on formula. It is unlikely to be due to an effect of folate or vitamin B_{12} intake (deficiency of these nutrients causes anaemia) as the milks had similar concentrations of these nutrients. The study by Gill et al. (42) reported significantly lower rates of anaemia at 15 months (following 11 months of intervention) in their follow-on formula group, but unfortunately did not randomise participants to the intervention and control groups. Rather, participants who were consuming infant formula at six months of age (baseline) were randomised to either the follow-on formula or a low-iron formula, but the control group were participants who were already drinking cow's milk and were asked to continue doing so. At baseline those in the cow's milk group already had significantly lower haemoglobin concentrations, and these continued to decrease during the study, again suggesting that at least part of the reported intervention effect was a result of a negative effect of early cow's milk introduction on iron status in the control group.

Two studies reported no difference in the prevalence of anaemia post-intervention. One study reported zero prevalence across all groups at nine months (43). The other study found that after a nine month intervention, 5% of children were anaemic in the iron-fortified formula group, 11% in the unfortified formula group, and 32% in the cow's milk group at age 18 months, although the latter was not found to be significantly different to the iron-fortified group presumably due to the considerable reduction in the sample size post-intervention (data were only available for 20% of the participants at the end of the intervention because of unforeseen technical difficulties) (44).

One study only reported the mean haemoglobin concentration and not the prevalence of anaemia or IDA (40). No differences were found at six or 12 months of age in mean haemoglobin concentrations among these infants receiving, from the first week of life, a low iron-fortified formula (7.4 mg iron/L), a high iron-fortified formula (12.7 mg iron/L), or breast milk. There were no data on haemoglobin concentration at baseline.

Although anaemia (i.e., a decreased haemoglobin concentration) is not specific to iron deficiency, if an improvement is seen in anaemia with iron-fortification, particularly with a corresponding increase in serum ferritin concentration (and appropriate measures taken to address inflammation as a result of infection), it can be assumed that the low haemoglobin concentration was caused by iron deficiency. In the five trials discussed in this section that did not report IDA (i.e., they reported anaemia and haemoglobin (41–44) or just haemoglobin concentration (40)), three studies found that haemoglobin concentrations had risen in their intervention groups (mean increase of 6.7 g/L over nine to 18 months) compared to baseline concentrations (99 to 119 g/L) (40–44). In all three of these studies there was also a corresponding increase in serum ferritin. It is unfortunate, however, that

these three studies that measured both haemoglobin and serum ferritin did not combine the indexes to specifically identify IDA. Two studies found no difference between the intervention and control groups for haemoglobin concentrations at the end of the study (40,43). Only one of these studies reported baseline haemoglobin concentrations, which suggested that participants did not have anaemia (118-121 g/L) (43), so would not be expected to show a haemoglobin response to additional iron. The other study did not report baseline haemoglobin concentrations but did see an increase in serum ferritin concentration in response to the high-iron formula suggesting, alongside the absence of a haemoglobin effect, that the participants did not have IDA at baseline.

Although the two studies that did not find an intervention effect on either anaemia or mean haemoglobin concentration had recruited children who were younger than those in the positive studies (aged three months or younger (40,43), compared to six (42) or nine (44) months), the low rates of anaemia at baseline are more likely to explain the lack of response to the intervention in these studies. The duration of the studies is unlikely to have impacted on the results with all studies intervening for at least six months (negative studies: six (43) and 12 (40) months; positive studies: nine (42,44) and 12.3 months (41)).

3.1.2.5 Discussion

The two trials looking at the efficacy of an iron-fortified milk for decreasing the prevalence of IDA in non-hospitalised infants in developed countries (46,47) suggest that an iron-fortified milk does not result in a lower prevalence IDA than cow's milk. However, it must be noted that one of these studies (46) specifically excluded toddlers with IDA at baseline, and that none of the toddlers in the small study (n=36) by Virtanen et al (47) had IDA at baseline. Although some children in developed countries have IDA (e.g., in South Island New Zealand infants: 7% in 6-11.9 month olds, 3% in 12-24 month olds (59), and these infants (many of whom would not usually be identified) may possibly benefit from iron-fortified milk, this has not been tested, and could not be ethically tested (it would not be appropriate to randomise an infant identified as having IDA to a non-treatment cow's milk group).

The studies investigating the efficacy of an iron-fortified milk for increasing iron stores (measured as an increase in serum ferritin concentration) have largely failed to account for the effects of illness (leading to inflammation) on serum ferritin concentration. This is a particular concern because of the high rates of acute illness in this age group (58). Only the Toddler Food Study (46) is able to provide information on the efficacy of an iron-fortified milk in toddlers in a study with sufficient power to detect an effect of an iron-fortified milk on serum ferritin concentration. The study found that consumption of an iron-fortified toddler milk *ad libitum* for five months in New Zealand toddlers aged 12-20 months resulted in a significant and substantial increase in serum ferritin concentration. Although problematic (largely because of their failure to account for infection), five of the seven other studies identified confirm this finding.

The Toddler Food Study (46) also found a significant increase in body iron in its toddler milk group. This is not surprising, however, given the large increase in serum ferritin concentration, since serum ferritin concentration is a key component of the body iron calculation.

Three of the five studies that reported the prevalence of anaemia (or haemoglobin concentration alone) but not IDA reported an increase in haemoglobin concentration as well as serum ferritin concentration, suggesting that the iron-fortified milk was efficacious (41,42,44). However, all three of these studies (41,42,44) had control groups who were drinking cow's milk before 12 months of age which would be expected to result in poorer iron status, thereby inflating the efficacy of the iron-fortified intervention milk.

Overall, of the eight studies identified, six reported a decrease in the prevalence of IDA or anaemia, or an increase in serum ferritin concentration, when an iron-fortified milk was consumed in place of cow's milk (40–42,44,46,48). The two studies finding no changes had recruited apparently healthy children, either by excluding those with a haemoglobin less than 100 g/L (43), or by only including children who had been consuming iron-fortified follow-on formula for the past six months (47). Hence, the children in these studies had an adequate iron status at the beginning of the study, and were at low risk for developing iron deficiency.

The majority of studies specifically recruited full term normal birthweight (40,43,44,47) or full term (41,42) infants. However, two of the studies did not list prematurity or low birthweight in their exclusion criteria (46,48). In theory, any effect of prematurity or low birthweight should be accounted for by the design of a randomised controlled trial in that randomisation should ensure these characteristics are present to the same extent in each of the study groups, and therefore do not impact on the analysis (which should compare the outcome in the intervention with the outcome in the control group).

3.1.3 Efficacy of iron-fortified milk-based products for improving iron status in developing countries

There were four trials investigating the efficacy of iron-fortified milk-based products for improving iron status in developing countries (Mexico (49,51), Chile (50), and India (52)). Two of these studies used a multi-nutrient milk (49,52), one study an iron-fortified formula (50), and one study an iron-fortified cow's milk (51) as the intervention. The range of mean iron concentration in these milks was 9.6 to 13.2 mg/L, with mean daily iron intake ranging from 5.3 to 8.3 mg. These were compared against non-fortified formula (49), low-iron formula (50), or cow's milk (51,52) with a range of mean iron concentration in the milks of 0 to 0.5 mg/L, and mean daily iron intake from the milks ranging from 0 to 0.4 mg. The two Mexican studies asked the participants to drink 400 mL per day (49,51), while the Indian study supplied the participants with 21 sachets per week, the volume of milk these sachets made was not reported (52). The Chilean study did not report any instructions (50).

Two of the studies had an intervention of six months (50,51), the other two of 12 months (49,52). The children in the study had a mean age of 19.4 months at recruitment, with the studies recruiting children from six to 30 months of age. The mean age of the children at the end of the studies was 26 months, ranging from 12 to 48 months.

3.1.3.1 Iron deficiency anaemia

Rates of iron deficiency anaemia were reported in two studies, one of which found after consuming the iron-fortified milk for 12 months, 13.3% had IDA compared to 55.2% in the control group (52). The other study found no difference in the prevalence of IDA after the intervention when the children were 12 months old: 3.8% in the control group and 2.8% in

the intervention group (50). The low prevalence of IDA in both the control and intervention groups post-intervention (i.e., after six months) may be due to both the low (2.3 mg of iron/L corresponding to 1.4 mg/day) and high (12.7 mg of iron/L corresponding to 7.9 mg/day) iron-fortified cow's milk) iron formulas providing enough iron to the participants. Unfortunately, baseline IDA rates for the infants at aged six months were not determined. However, another study conducted soon after by the same researchers, using similar recruitment criteria in the same population, found the prevalence of IDA in children 12-months-old drinking unfortified cow's milk was 21% (50).

3.1.3.2 Serum ferritin

Two studies reported the prevalence of low serum ferritin (<12 μg/L), both finding the intervention milk decreased the prevalence at the end of the study compared to the control milk (49,50). After an intervention of six months, the prevalence of low serum ferritin in the iron-fortified milk groups was 23 PP lower (49), and after 12 months, 11 PP (49) and 20 PP (50) less than in the control milk groups.

Arithmetic or geometric mean serum ferritin concentrations were reported by three of the studies (51,52). Again, all reported a positive intervention effect. One of the studies, with children aged 10-20 months at baseline, reported that geometric mean serum ferritin increased by 6.3 μg/L in the iron-fortified milk group after six months of intervention, compared to no change in the control group (51). The other two studies who recruited younger (six months (50)), and older (12-36 months (52)) children reported the intervention group values to be significantly higher than the control group post-intervention by 3.9 μg/L and 5.5 μg/L respectively. Two of the three studies accounted for infection appropriately – one by delaying blood testing for two weeks after an infection (50), and the other by controlling for CRP concentration using a multivariate model (51).

3.1.3.3 Anaemia and haemoglobin concentration

Two studies reported the prevalence of anaemia (haemoglobin <110 g/L) instead of IDA. In both of these studies, there was a larger decline in anaemia in the iron-fortified milk group than in the control group. The decline in prevalence attributable to the intervention was 24 PP over six months (51) and 7 PP over 12 months (49) in these studies. In both of these studies, there was also a corresponding significant improvement in serum ferritin status, implying that the anaemia was due to iron deficiency.

3.1.4 Conclusion

It would appear from this review of the literature that healthy children from developed countries, 6-36 months old, are not in need of added iron in their milk to prevent IDA. Milk fortified with iron, however, does result in higher body iron stores, thus potentially preventing iron deficiency later on in childhood. Children with a higher risk of iron deficiency, particularly those consuming unmodified cow's milk before the age of 12 months, would benefit from iron-fortified milk. Children from developing countries, who are more likely to have IDA, also appeared to benefit significantly from consuming iron-fortified milks from six months of age. It is essential, however, to determine that the population has high rates of IDA rather than anaemia due to other causes (e.g. haemoglobin disorders, vitamin A, B_{12} or folate deficiency (53)), as iron-fortified milk-based products will obviously not improve anaemia rates unless iron deficiency is present. Iron-fortified milk-based products will always be preferable to the consumption of unmodified cow's milk before 12

months of age. However, data are not available from randomised controlled trials on the effects of iron-fortified milk-based products replacing breastfeeding. Two studies compared iron-fortified milk against a breastfed group, one of which found no differences in iron indices between the groups (43), while the other found serum ferritin to be higher in the iron-fortified milk group than in the breastfed children (40). These results, however, may not show the true effect of replacing breast milk as the participants could not be randomised into the groups due to ethical reasons, instead being included based on their current choice of milk.

3.2 Zinc-fortified milk-based product trials

This literature review has identified five studies that have reported the impact of zinc-fortified milks on the zinc status of children six to 36 months old (Appendix B, Table 5.2). Three of these studies were conducted in developed countries (40,60,61) and two in developing countries (51,52).

3.2.1 Measuring zinc status

The material in this section is from Gibson (53) unless stated otherwise. Serum zinc is the recommended (62) and most commonly used biomarker of zinc status at the population level. Unfortunately, it is not very sensitive to varying degrees of zinc deficiency as it is homeostatically controlled. Several factors affect serum zinc concentrations, including infection and inflammation which reduce serum zinc as a result of the metabolic redistribution of zinc induced by pro-inflammatory cytokines (63), fasting status, and time of the last meal. Diurnal variation also occurs, with higher serum zinc levels in the morning irrespective of fasting state, compared to the afternoon.

Hair zinc can reflect chronically inadequate intakes of zinc, with lower hair zinc concentrations in deficient children. Assuming a normal hair growth rate, 1-2 cm lengths cut closest to the scalp reflect zinc uptake by the hair follicles 4-8 weeks prior to collection.

3.2.2 Efficacy of zinc-fortified milk-based products on zinc status in developed countries

The trials of the efficacy of zinc-fortified milk-based products on zinc status in developed countries used breast milk (40), breast milk and unfortified infant formula (1.1 mg of zinc/L) (61), or cow's milk (2.8 mg zinc/L) (60) as the control milks. These control milks were compared with fortified milks with, on average, a zinc concentration of 7.5 mg/L (range 4 to 13.2 mg/L). The duration of the studies ranged from five to 12 months, with the mean age of the children at recruitment being 7.4 months (range 0 to 20 months). Post-intervention, the children ranged from nine to 25 months of age.

Only one study reported total dietary zinc intake (60) which increased significantly from baseline in the zinc-fortified milk groups, with an intervention effect of 1.2 mg of zinc/day compared to the control milk. This resulted in a mean daily zinc intake of 5.6 mg compared to 4.8 mg in the control milk group. This increase in dietary zinc intake, however, did not result in an increase in serum or hair zinc concentrations (60). In fact, none of the studies showed an increase in serum zinc (38,52,53) or hair zinc when measured (52), as a result of the consumption of zinc-fortified milk-based products (40,61).

In the five month intervention with children aged 12 to 20 months at baseline, consumption of the zinc-fortified toddler milk significantly increased the serum zinc concentrations from baseline (9% higher), however when compared to the change in the control group, the difference was not significant (60). A study in Finland in which children were recruited from birth to 3.5 months of age, after six months of intervention, the zinc-fortified infant formula group had significantly higher serum zinc compared to the breastfed and unfortified infant formula group (13 vs 9.9 µmol/L). However, once the children began eating foods and were no longer exclusively fed milk, the serum zinc concentration in the zinc-fortified infant

formula group decreased and by nine months of age was the same as that of the control group (61).

3.2.3 Efficacy of zinc-fortified milk-based products for improving zinc status in developing countries

There have been two studies which have examined the effect of zinc-fortified milk-based products on zinc status in children from developing countries (India (52) and Mexico (51)). The control milks (unfortified cow's milk) provided 1.5 (64) and 1.9 mg Zn/day (51), while the intervention milks (fortified cow's milk), provided 8.2 (64) and 13.2 mg Zn/day (51). In both studies the children were provided with a set amount (3 sachets providing an unstated volume (64)) or 400 mL (51)) per day of milk and encouraged to drink all the milk supplied. At recruitment, the age of the children ranged from 10 to 36 months for an intervention which lasted for either six (51) or 12 (64) months. At the end of the study, the children were 16 to 48 months old.

The results were similar to those found in the developed countries, with the intervention milks having no significant effect on serum zinc concentrations compared to the control milks. The Indian study found neither a change from baseline nor a difference after the 12 month intervention between the intervention and control milk groups (52). The Mexican study did not report baseline levels of serum zinc, however there was no difference between the intervention group or control milk group after the six month intervention period (51).

3.2.4 Conclusion

It would appear from these studies that zinc status based on serum zinc concentrations, is not influenced by an increase in zinc intake from zinc-fortified milk-based products fortified at the levels of 4.0 (61) to 13.2 (51) mg/L in this age group over a period of five to 12 months. However, the low level of the current recommended upper limit (UL) (i.e., 7 mg (65)), restricts the amount of zinc that can be used as a fortificant. Consequently, in some of these trials there were only small differences between the amount of zinc contributed by the intervention and control milks (e.g. 2.8 compared with 4.7 mg/L (60)). Moreover, studies to date have not controlled well for diurnal variation and fasting status. Ideally, all blood samples should be collected in the morning, preferably fasting. Alternatively non-fasting morning samples should be taken at a standardized time (i.e. one hour) following the consumption of a snack (53) in order to minimize any variation in serum zinc concentrations arising from these confounding factors, and thus improve the ability to identify any changes in serum zinc concentrations among the study groups.

3.3 Docosahexaenoic acid (DHA) fortified milk-based product trials

This literature review has identified seven studies, all of which are from developed countries, assessing DHA status as an outcome of trials comparing DHA-fortified milks with a control milk in children aged six to 36 months (Appendix B, Table 5.3).

3.3.1 Measuring DHA status

Docosahexaenoic acid (DHA: 22:6n-3) is a long-chain polyunsaturated fatty acid that is part of the omega-3 family of fatty acids. DHA in erythrocytes and plasma phospholipids have been shown to be acceptable markers of DHA status (66).

3.3.2 Efficacy of DHA-fortified milk-based products for improving DHA status in developed countries

There are seven trials which have assessed the effect of DHA-fortified milk-based products on changes in DHA blood levels of children aged 6-36 months in developed countries: one from Australia (67), and the other six from the United States of America (USA) (68–73). Two studies recruited infants after weaning from breastmilk: one when the infants were aged six weeks (72), and one at four to six months of age (73)). The remaining five studies recruited infants within the first week of birth. All infants drank the study milks until they were 12 months old. In one of the studies, children were followed-up until they were 39 months old (69).

All of the studies used infant formula containing no DHA as the control milk, with three studies including breastfed infants as a second control group (67–69). The infant formulas for the intervention groups contained added DHA with a mean concentration of 0.36% of total fatty acids (range 0.12-0.96%). Three out of the eight studies also added arachidonic acid (ARA) to their intervention milks, with a mean concentration of 0.50% of total fatty acids (range 0.34-0.72%) (67–69). All of the studies provided the study milk *ad libitum*, with the six American studies also instructing the participants that the study milk was to be the sole source of dietary intake until the children were at least four months of age (68–73).

3.3.2.1 DHA in total red blood cell lipids

Four studies reported the change in concentration of DHA in total red blood cell (RBC) lipids during the study (70–73). All reported a decrease in DHA in RBC lipids in the control formula group from baseline to the end of the study, and an increase in DHA concentrations in the DHA-fortified milk group. The concentration of DHA was therefore significantly higher in the fortified formula groups than in the control formula groups when the children were either 10 months (71) or 12 months of age (70,72,73). Mean DHA concentration in RBC lipids at 10-12 months was 79 mg/L in the formulas with a DHA concentration of 0.32-0.36% of total fatty acids, compared to 30.5 mg/L in the control formula groups (70–73). The one study which compared three different levels of DHA supplemented-formula (DHA concentrations in the formulas of 0.32-0.96% of total fatty acids) found a dose-response with DHA in the RBC lipids increasing as DHA concentration in the intervention milk increased (70).

3.3.2.2 DHA in red blood cell phosphatidycholine and phosphatidylethanolamine

Two studies did not report the concentration of DHA in the total RBCs, instead reporting the results as DHA in red blood cell phosphatidycholine (RBC-PC) and red blood cell phosphatidylethanolamine (RBC-PE) (68,69,74). These studies both had similar results to the other DHA studies, in that after the 12 month intervention the DHA fortified formula group had higher RBC DHA, for both indexes measured, than the control formula group. Breastfed infants had RBC DHA similar to that of the fortified formulas, and 40% higher than those in the control formula groups (74). However, one of the studies conducted follow-up measurements, and found none of the differences between the three groups (DHA-fortified formula, DHA and ARA-fortified formula, and the breastfed group) persisted when the children were 39 months old (69).

3.3.2.3 DHA in plasma phospholipids

Two studies reported the change in concentration of DHA in plasma phospholipids from four months to either 8.5 months (67) or 12 months (72) of age. Both studies found significantly higher concentrations of DHA in the fortified formula groups compared to the control formula group after the study. The study by Birch et al (72) found the DHA concentration in plasma phospholipids was 43.3 mg/L in the fortified formula group (DHA 0.35% of total fatty acids) compared to 13.1 mg/L in the control formula group (no DHA). Makrides et al (67) found that after 8.5 months of intervention, DHA plasma phospholipid concentration was highest in the group consuming the formulas containing DHA at 0.34% and 0.35% of total fatty acids (DHA 5.3-5.8% of total plasma phospholipids). This was followed by the breastfed group (DHA 4.3% of total plasma phospholipids); the placebo formula group had the lowest plasma phospholipid DHA concentration (1.6% of total plasma phsopholipids) (67).

3.3.3 Efficacy of DHA-fortified milk-based products for improving DHA status in developing countries

This literature review found no trials of DHA-fortified milk-based products investigating DHA status in children six to 36 months old from developing countries.

3.3.4 Conclusion

All of these studies showed an increase in the level of DHA (RBC or plasma) in the children consuming the DHA fortified formula. This effect appeared to be dose-dependent, with higher DHA milk concentrations producing higher DHA levels in the children. All of these studies supported the theory that fortifying the milk with DHA does increase DHA levels in children 6-36 months old. However, in the four studies that had a breastfed group, the DHA levels of the breastfed children were consistently higher than those drinking a “placebo” formula (67,68,74,75). One study found no difference between a formula fortified with DHA 0.12% of total fatty acids and the breastfed group (74), whereas in all of the other studies, DHA levels were less in the breastfed children than those consuming a fortified formula. As there currently is no reference limit indicative of a low concentration of DHA in RBCs or plasma available in children, it is difficult to know whether fortification is necessary, however see comments in sections 6.5 and 6.6 on health outcomes.

3.4 Other micronutrient fortified milk-based product trials

This literature search has identified five studies where a fortified milk-based product was compared with a control to assess the effect of nutrients other than iron, zinc and DHA on the nutrient status of children six to 36 months of age (Appendix B, Table 5.4). There are four studies conducted in developed countries assessing the following micronutrients: copper (76), iodine (77), selenium (78), and vitamin D (79). One study in Mexican children, a developing country, assessed the efficacy of fortifying a milk-based product with vitamin A (80).

3.4.1 Efficacy of fortified milk-based products for improving micronutrient status in developed countries

3.4.1.1 Copper

Copper-fortified milk (0.5 mg/L) had no effect on plasma copper or ceruloplasmin concentrations of children nine months of age consuming the milk from 2-3.5 months of age (76). Plasma copper concentrations at two months of age were 9.9-11.2 µmol/L, and at nine months were 14.3-16.8. Three months after the intervention, when the children were 12 months of age and had all been drinking cow's milk for the past three months, plasma copper concentrations had increased in both the control and fortified milk group to 17.8-20.6 µmol/L.

3.4.1.2 Vitamin D

The effect of fortifying milk with vitamin D was assessed in the Toddler Food Study from New Zealand (79). Data from two milk groups consuming 6.3 µg cholecalciferol/100g powder (fortified 'Toddler Milk') and 6.0 µg cholecalciferol/100 g powder (fortified cow's milk), providing a mean daily intake of vitamin D of 25.2 µg and 24.0 µg were compared to a red meat intervention without fortified milk. The baseline prevalence of serum 25-hydroxycholecalciferol (25(OH)D) <75 nmol/L was 78%, and of serum 25(OH)D <30 nmol/L was 11%. The prevalence of serum 25(OH)D < 75 nmol/L did not decrease during the study, however the prevalence of serum 25(OH)D < 30 nmol/L decreased significantly after the five month intervention to 3%. The control group (increased red meat intake) had no change. The intervention effect was estimated as: for every 1 µg of vitamin D consumed in winter, serum 25(OH)D concentration would increase by 9%.

3.4.1.3 Iodine

The Toddler Food Study from New Zealand compared iodine-fortified milk (138.5 µg of iodine/100 g powder) with a non-fortified cow's milk (40.5 µg of iodine/100 g powder) in children 12-20 months of age (77). At baseline, median urinary iodine concentrations (UIC) were 43 and 55 µg/L in the two groups, and indicative of iodine deficiency (UIC<100 µg/L). After the five month intervention, median UIC increased significantly ($p<0.01$) from 43 ug/L to 91 µg/L in the fortified milk group compared to a non-significant change from 55 to 49 µg/L in the control group.The prevalence of UIC <100 µg/L and UIC <50 µg/L also decreased in the fortified milk group from 86% to 50%, and 66% to 29%, respectively, from baseline to the end of the intervention, whereas the urinary iodine status of the unfortified milk group did not change.

3.4.1.4 Selenium

One study assessed serum selenium in children 12 months old after consuming either selenium-fortified formula (20 µg selenium/L), infant formula (<5 µg selenium/L), or breast milk from weaning until 9 months of age, at which point the formulas were exchanged for cow's milk (78). The study found that the selenium-fortified formula increased serum selenium until six months of age, after which it remained stable to 12 months of age. Serum selenium in the unfortified infant formula group and breastfed group increased from six months with the introduction of solid foods, and there were no differences among the groups at 12 months of age.

This apparent increase in serum selenium concentrations that corresponded with the introduction of solid food implies that the children were obtaining adequate selenium from food sources other than the study milks. Dietary intake of selenium varies considerably worldwide due to the variation in soil selenium concentrations, which impact on selenium concentrations in plant-based foods (81). These results therefore need to be interpreted with caution, and more studies in populations in settings with varying soil selenium concentrations are required.

3.4.2 Efficacy of fortified milk-based products for improving micronutrient status in developing countries

3.4.2.1 Vitamin A

One study in Mexico compared a group consuming a vitamin A-fortified milk (784 retinol equivalents/L) with a control group who continued eating and drinking their 'typical' diet. Children between the ages of 36-72 months were recruited, and completed the study three months later when they were aged 39-75 months. At the end of the intervention, the participants from the fortified milk group had increased total body vitamin A stores (estimated using deuterated retinol dilution), 40% above baseline levels. Liver vitamin A stores (also estimated using deuterated retinol dilution) were also significantly increased, 28% higher than baseline. The control group had no changes in vitamin A status over this period.

3.4.3 Conclusion

One study per micronutrient is not sufficient evidence to draw conclusive recommendations. Hence the results must be interpreted with caution. The evidence presented, based on the two biomarkers of copper status used, does not support the fortification of milk with copper to increase copper status. However, the biomarkers used are only reliable in cases of severe copper deficiency, not marginal copper status. The vitamin D study was conducted in an area with minimal sun exposure, particularly during the winter months. The results support the fortification of milk with vitamin D for young children living in at risk areas, as do the studies on iodine fortification, and vitamin A fortification, both of which were also conducted in populations at higher risk of deficiency. Results of the selenium-fortified milk study did not support selenium fortification, however the study was in a population with access to sufficient food sources of selenium, and further research is needed among at-risk populations living in regions where soil selenium concentrations are low.

Only two studies compared a fortified milk group with a breastfed group (76,78). The trial fortifying milk with copper found no differences between any groups, irrespective of whether they received breast milk, or formula fortified with copper (76). In the other trial of a selenium fortified formula, serum selenium concentrations for the breast fed group reached a concentration equal to that of the fortified formula group only after the introduction of solid food at six months of age. However, as breast milk selenium concentrations are dependent on maternal dietary selenium intake (82), more studies are needed before concluding that universal fortification of formula with selenium is warranted These studies are also ethically unable to randomise the participants into breastfed or intervention milk groups, and this combined with only having one trial available for each of two nutrients, means there are insufficient data to conclude what the effects on nutrient intake would be of replacing breast milk with milk fortified with copper, vitamin D, iodine, selenium or vitamin A.

3.5 Fortified milk-based product trials assessing morbidity and growth

This literature review has identified thirteen trials assessing morbidity and growth in children 6-36 months old participating in fortified milk-based trials, twelve of which were from developed countries (40–42,45,46,61,68–70,74,75,83–85) and one from a developing country (India (64)) (Appendix B, Table 5.5).

3.5.1 Efficacy of fortified milk-based products for improving rates of morbidity and growth in developed countries

The ten trials assessing the efficacy of fortified milk-based products on morbidity and growth from developed countries were all conducted to investigate the clinical safety of the fortified milk products, as opposed to assessing any improvements in morbidity or growth compared to the control group.

Infant formula, follow-up formula, or toddler milk comprised the intervention milk in all of the studies, fortified with: iron (40), iron and vitamin C (41,42,45), iron, vitamin C, and zinc (46), vitamin A and vitamin D (46), DHA (70,74), DHA and ARA (68,74,75), zinc (61), or protein (83,84). The outcomes of children consuming these milks were then compared to children who were either breastfed (40,61,68,74,75,83), or given unfortified infant formula (68,70,74,75,83), or cow's milk (41,42,45) to drink, or to children continuing with their usual milk intake (breast milk or cow's milk) (46). In seven of the studies (45,68,70,74,75,83,84), the children were provided with the study milk *ad libitum.* In three of these, the investigators requested that the milks were the sole source of nutrition until the children were four months of age, after which they were to be the only milk drunk (68,70,74). Two studies asked that the study milks were the predominant milk drunk (46,75). Two studies provided no information on how the milks were to be consumed by the children (42,61).

The duration of the interventions ranged from five months to 12 months (mean 8.9 months). Four studies had follow-ups post-intervention (41,61,69,84). The children were recruited from birth through to 20 months of age (mean age 4.7 months). After the intervention period the children ranged in age from 5.5 to 25 months (mean age 13.5 months).

3.5.1.1 Morbidity

Three studies assessed morbidity following the consumption of milk-based products fortified with iron and vitamin C (45), DHA (69), DHA and ARA (69), vitamin A and D (46), multi-nutrient fortified toddler milk (46). All of the studies found the intervention milks to have no effect, positive or negative, on any morbidity-related outcomes measured at the end of the intervention period (45,46), or at a follow-up visit 27 months later (69). Outcomes measured included: infection, gastrointestinal problems, general morbidity (45); gastric effects that the parents associated with the study milks (46); three or more prescriptions since birth, use of pressure equalization tubes for chronic otitis media, or hospitalisation since birth (69). Outcomes were determined by maternal reports at each follow-up visit (at 9, 12, 15, and 18 months of age (45)), at the end of the intervention (46), and during the follow-up visit 27 months after completion of the intervention (69).

3.5.1.2 Growth

Twelve studies assessed growth outcomes following consumption of milk-based products fortified with DHA (69,70,75), DHA and ARA (68,69,75), iron (40), iron and vitamin C (41,42,45), zinc (61), vitamin A and D (46), protein (83,84), or multi-nutrient fortified toddler milk (46). All but one study found no effect on the child's length or weight, positive or negative, attributable to the fortified milks. The one study which found a difference between study groups compared breastfed children against a standard formula group (protein 7.1% of energy) and a follow-on formula group (protein 11.7% of energy) (84). Children consumed the formulas from two months of age up to 12 months, with a follow-up assessment at 24 months old. At the six and 12 month-old assessments, weight, weight-for-length, and BMI were all significantly higher in the follow-up formula group than the standard formula group. At 24 months, there was no longer any difference between formula groups for either weight or length, although weight-for-length z-scores were 0.20 standard deviations (SD) greater in the follow-up formula group compared to the standard formula group. Weight, length, weight-for-length, and BMI z-scores were all significantly higher (0.18-0.27 SD higher) in the follow-up formula group than the breastfed group. Weight and length were both higher, by 0.16 and 0.29 SD respectively, in the standard formula group than in the breastfed group, while there was no difference between these two groups for weight-for-length or BMI z-scores.

Six studies also examined head circumference (42,45,68,69,75,83), two assessed skinfold thicknesses (45,61), and one lower leg strength (83), all of which found no effects.

3.5.2 Efficacy of fortified milk-based products for improving rates of morbidity and growth in developing countries

A single trial investigated the effect of fortified milk-based products on rates of morbidity and growth from a developing country. This trial was undertaken in India (52,64).

In this study, a multi-nutrient fortified milk was compared with an unfortified milk which contained less vitamin A, E, and C, iron, zinc, selenium, and copper than the fortified milk. The children were recruited when they were between 12 and 36 months old, and consumed the study milks for the next 12 months. At baseline, mean haemoglobin was 89 and 91 g/L, mean (arithmetic or geometric not stated) serum ferritin 8.6 and 9.3 µg/L, and mean plasma zinc 61 and 63 µg/dL in the multi-nutrient fortified milk group and control group respectively All three of these indices are low, signifying poor iron and zinc status in the recruited children.

3.5.2.1 Morbidity

In the study from India, the incidence of diarrhoea was 4.5 episodes per year in the multi-nutrient milk group and 5.4 in the control milk group (64). The intervention was associated with a significant 18% reduction in risk of developing diarrhoea. The incidence of acute lower respiratory tract infection was 26% lower in the intervention group than in the control milk group, and in the sub-group of children less than 12 months of age, the incidence of acute lower respiratory tract infection was 47% lower than the control milk group. Days with severe illness were 15% less, and the use of antibiotics 4% less in the multi-nutrient fortified milk group than the control group. Morbidity data were collected at twice-weekly home interviews when researchers asked the parent for information on illnesses in the previous 3-4 days.

3.5.2.2 Growth

In the study by Sazawal et al (52), the multi-nutrient intervention milk was associated with an improved weight gain of 0.21 kg/year and improved height gain of 0.51 cm/year compared to the control milk. After the 12 month intervention, there was a significant decrease in the proportion of children with weight-for-age less than -2 z-scores.

3.5.3 Discussion

Four studies from developed countries did not report any sample size calculation (40,42,61,69) and a further three did not justify their sample size for either growth or morbidity outcomes (41,45,46). One study, of milk fortified with DHA, reported the sample size for an outcome other than growth and morbidity, but also reported that they had recruited more subjects than required (56-65 recruited per group), and indicated that there was enough statistical power for the other outcomes investigated (70). One study, comparing milks with different concentrations of protein, reported the calculation used to estimate the required sample size but did not report the result of their calculation (i.e, the number of participants required) (83). Three studies reported the sample size required to identify a difference in growth between groups (68,75,84), but none based their sample size on morbidity outcomes. The three studies reporting a sample size calculation reported: 54 infants (recruited at birth) required per group to identify a difference of 1-SD in "growth" (length, weight, and head circumference were measured, but which one was considered to reflect "growth" was not specified) at 12 months of age between groups drinking DHA and ARA fortified milk or unfortified cow's milk (68); 296 infants (recruited at two months of age) required per group to identify 0.8 cm difference in length at 12 months of age between groups consuming milk with different protein levels (84); 20 infants (recruited at 1 week of age) per group to identify a difference in weight of 700g at 12 months of age between groups consuming milk fortified with different levels of DHA and ARA (75). Using these guidelines, it would appear all of the studies fortifying milk with either DHA or ARA (all of which recruited children within one week of birth) for 12 months had enough participants in each group to detect a difference in weight (68,70,74,75) with two of these also recruiting enough to detect a difference in length (68,70). One of the two protein studies had the required sample size of greater than 296 to detect a difference in length (84), the other only had 20-22 in each group (83). It is difficult to know whether the other studies fortifying milk with different nutrients and in different age groups had the required sample sizes. However, two of these seven studies had more than 54 in each group (age at recruitment: birth (40) and nine (45) months), and an additional five studies had more than 20 (ages at recruitment: 5.7-8.6 (41), 6 (42), 0-3.5 (61), 12-20 (46) months) in each group.

The duration that the study milks were consumed by the children in developed countries, combined with the fortification levels in the intervention milks, appears to be acceptable in all of the studies to emulate what would occur if they were to be consumed in a real-life situation. The four studies using iron-fortified milk all had levels equal to or above 12 mg/L which is at the high end of the range proposed by Koletzko et al (28) for the iron concentration in follow-up formula (6.6-13.3 mg/L). Trials fortifying with DHA had levels ranging from 0.12-0.96% of total fatty acids, which compares with the levels found in breast milk worldwide (0.06-1.4% of fatty acids (86)). The two trials comparing different levels of protein in milks used concentrations similar to those of standard infant formula and follow-on formula. The proposed range for zinc in follow-up formula is 3-10.5 mg/L compared to

zinc concentrations of 4-6 mg/L in the milks used in the trials, which are at the lower end of the proposed range.

The one study from a developing country did not report a sample size calculation, however it can be assumed that as differences were found between groups for both growth and morbidity outcomes, the sample size, duration of the intervention, and fortification levels of the milks were appropriate.

3.5.4 Conclusion

All but one study assessing the clinical safety of fortified formula in children from developed countries found no effects, positive or negative, on morbidity or growth outcomes.

A systematic review concluded that iron supplementation given to healthy children can reduce linear growth, the magnitude of the effect increasing with a longer duration of supplementation (87). There were four studies that included formula or toddler milk containing additional iron, yet none of these reported any negative effects associated with the fortified milk-based product.

The sample sizes, duration of the intervention, and fortification levels of the study milks were all considered adequate for the detection of differences in growth and morbidity outcomes, if present, between groups, except for the zinc studies.

It would appear that formulas fortified with micronutrients are safe for use in developed countries. The one study that did find an adverse effect on growth was designed to compare protein levels in formulas (84). The higher protein level of follow-up formula, compared to that of standard infant formula or breast milk, appeared to result in increased growth, which is not necessarily advantageous. Of particular concern are the increased weight-for-length and BMI z-scores as rapid weight gain in infancy is associated with an increased risk of obesity later in life (88,89).

Although the single study in a developing country (India) suggests a beneficial effect on weight, height and morbidity when 1-3 year olds received a multi-micronutrient fortified milk for 12 months, the wider implications of advocating a micronutrient fortified formula in such a setting need to be considered carefully, particularly because the control group was also consuming a reconstituted milk powder. There was no breastfed control group so it is not possible to determine what the morbidity, or growth, outcomes would have been had a reconstituted milk powder not been used.

3.6 Fortified milk-based trials assessing neurodevelopment outcomes

This literature review has identified nine fortified milk trials (12 articles) assessing neurodevelopment outcomes in children aged 6-36 months, all of which are from developed countries (Appendix B, Table 5.6).

3.6.1 Efficacy of fortified milk-based products for improving neurodevelopment outcomes in developed countries

Infant formula was used as the intervention milk in all of the trials investigating the efficacy of fortified milk-based products on neurodevelopment outcomes in developed countries.

Three of the nine trials (six papers) used a milk fortified with DHA (67,69,70,90–92). Two of these trials (three papers) had a second intervention milk fortified with both DHA and ARA (67,69,90). Four of the nine trials (four papers) only used milk fortified with both DHA and ARA as their intervention (68,71–73). The mean concentration of DHA in the formulas from the seven trials using a milk containing DHA was 0.36% of total fatty acids, and the mean concentration of ARA from the six trials using a milk containing ARA was 0.55% of total fatty acids. In four of the trials (six papers) the DHA (and ARA) fortified milk was based on a commercial infant formula which contained added iron (concentration not reported) (Enfamil with Iron or Enfamil LIPIL with Iron; Mead Johnson Nutrition (70–73,91,92). This same iron formula with no added DHA or ARA was used as the "placebo" infant formula in these trials.

Two of the nine trials fortified the study milks with iron, but not DHA or ARA, one containing iron concentrations of 0.9 mg/L and 1.2 mg/L in its two intervention milks (44), and the second containing an iron concentration of 12 mg/L plus 100 mg of vitamin C/L (34).

In these nine trials (12 papers), children consuming the intervention milks were compared with children consuming: infant formula containing neither DHA nor ARA (67–73,90–92), cow's milk (34,45), or breast milk (67–69,90). The majority of studies reported providing the study milk *ad libitum* (34,44), with four of these trials (seven papers) also requiring the milk to be the sole source of nutrition for the first four months of life (68–70,73,90–92).

The duration of the interventions was 12 months or less in the trials using DHA fortified milks (mean: 9.4 months; range: 6-12 months). Five of the trials (eight papers) in which milk was fortified with DHA recruited children up to one week of age (67–71,90–92), with the other two recruiting children up to six months of age (72,73). At the end of these studies all children were 12 months of age, although one study also completed a follow up interview 27 months later (when the children were 39 months of age) (69).

The two iron studies had intervention durations of nine months (45), and 16-18 months (34). They recruited children aged six to eight months (34) and nine months (44), with the children aged 18 (45) and 24 months (34) post-intervention.

3.6.1.1 Visual acuity

Visual acuity cannot be assessed using an eye chart in young infants. Instead, subjective tests are used, either psychophysical methods where the infant's attention and looking behaviour are assessed (Teller Acuity Cards), or electrophysiologic methods, where the

electrical activity of the visual cortex is assessed (visual evoked potential (VEP)). Both tests are reported to be reliable, based on the test-retest reliability of the methods (93). Results of the tests, however, were not found to be strongly correlated, implying that they may each be assessing a different aspect of vision.

3.6.1.1.1 Visual Evoked Potential acuity

Five studies assessed VEP acuity, all using DHA, or DHA and ARA, fortified milks (67,70–73). Four of these five studies found infants consuming the control formula (containing no DHA or ARA) had significantly lower VEP acuity than those groups consuming the DHA or ARA-fortified formula at age 4.5-6 months (71,72), 9-10 months (70,71), and 12 months (70–73). Two of these studies also reported an interaction between plasma or RBC DHA and VEP acuity (72,73). In both studies, a positive correlation between RBC or plasma DHA concentrations and VEP acuity was found when the children were 12 months old. One study did not find an improvement in VEP acuity in infants consuming the fortified formula, when compared to an unfortified infant formula (67). However, at 8.5 months of age, the breastfed group was reported to have better VEP acuity than the fortified formula groups (67).

Three of these trials (all of which were conducted at the same research centre with the same study protocols (71–73)) have been combined by Morale et al (94) and compared with an additional trial (in infants less than six months (95)) and two groups of breastfed children. This pooled analysis found that consuming a dietary source of DHA or ARA (irrespective of the milk source i.e. breast milk or formula) for a longer duration (up to at least 12 months) was associated with improved VEP acuity compared to consuming a “placebo” formula. Infants who received any DHA for the first 12 months of life, had on average VEP acuity of 0.14 logMAR better than infants receiving no DHA in the first 12 months of life (from a milk source). This difference corresponds to about 1.5 lines on an eye chart. This study also found that the improvement in VEP acuity did not plateau in the breastfed groups, the benefit continuing to at least 12 months.

3.6.1.1.2 Teller Acuity Card Procedure

The two studies by Auestad et al (68,69) measured visual acuity using the Teller Acuity Card Procedure, whereby the infant is shown cards containing black and white stripes with varying width (68,69). In the first study (68), children who were recruited up to three months of age (at the time of weaning from breast milk) were randomised to receive DHA and ARA fortified infant formulas or unfortified formula until 12 months of age. At the post-intervention assessment, the visual acuity of all the infant groups was found to be in the normal range, with no differences within or between the DHA and ARA fortified formula groups and the breastfed group. In the later study by Auestad et al (69), visual acuity was measured at a follow-up assessment (69), 27 months after completion of the intervention study (90), although not at the end of the intervention when the children were 12 months old. At this 27 month follow-up assessment when the children were aged 39 months, there was no difference in visual acuity between the fortified formula groups, the unfortified formula group, or the breastfed group.

3.6.1.2 General development scores

3.6.1.2.1 Bayley's Indexes

Four studies assessed Bayley's Mental Development Index (MDI) and Psychomotor Development Index (PDI). In one study, infants consumed an iron-fortified formula (1.2 mg/L) (44), whereas in the other three, DHA (0.12-0.35% of total fatty acids) and ARA (0.34-0.46% of total fatty acids) were used as the fortificants in the intervention milks (67,68,90). Only one of these studies (67) found a difference in scores, however this was only at 24 months (which was 12 months after the intervention finished) and only comparing the breastfed and formula fed infants, with the breastfed infants having significantly higher MDI scores than any of the three formula groups (i.e., DHA and ARA fortified, DHA fortified, or unfortified formulas). PDI scores did not differ between any of the groups in any of the studies.

3.6.1.2.2 Griffiths general quotient

Griffiths general quotient is an overall score of development assessed by five subscales: locomotor, personal and social, hearing and speech, eye and hand coordination, and performance (manipulation and precision). Williams et al (1999), in a study of children aged 6-8 months at recruitment, and reported a decrease in the general quotient across all groups from enrolment to 24 months of age, although only by 9.3 points in the iron-fortified milk group compared with 14.7 points in the cow's milk group (34). The only subquotient score that differed significantly from enrolment to 24 months was that of personal and social skills which had a significantly greater decrease in the cow's milk group than in the iron-fortified formula group. The participants who had both a haemoglobin concentration >120 g/L and general quotient score >100 at 24 months, were more likely to be from the iron-fortified milk group (13 out of 16), whereas the participants with a haemoglobin concentration <120 g/L and general quotient score < 100, were more likely to be in the cow's milk group (20 out of 24).

3.6.1.2.3 The Bracken Basic Concept Scale-Revised (BBCS-R)

The Bracken Basic Concept Scale-Revised (BBCS-R) consists of 11 components (colours, letters, numbers/counting, sizes, shapes, comparisons, quantity, direction/position, textures/materials, time/sequence, and self/social awareness), with the first six comprising the School Readiness Composite (SRC). This was used by Drover et al (92) in their study of children recruited at birth to consume milk fortified with three levels of DHA (0.32%, 0.64%, and 0.96% of total fatty acids) or an unfortified formula. They found no difference in scores for any of the 11 components after the 12 month intervention.

3.6.1.3 Intelligence Scores

Two studies assessed intelligence using the Stanford-Binet Intelligence Scale Form L-M (69) and The Fagan Test of Infant Intelligence (68). In one study, there was no difference found between the groups consuming DHA and ARA fortified infant formulas, unfortified formula, or breast milk, from three months until 12 months of age (68). In the other study the efficacy of DHA and ARA fortified infant formula, DHA fortified infant formula, unfortified formula, and breast milk were compared in an intervention from birth until 12 months of age (69). The only measures reported were at 39 months (i.e. 17 months after the intervention finished), at which time there was no difference between groups (69).

3.6.1.4 Vocabulary development and recognition

3.6.1.4.1 MacArthur Communicative Development Inventories

Two studies used the MacArthur Communicative Development Inventories to assess language development at 14 months (68,90). This evaluates vocabulary comprehension (words the child understands), expressive vocabulary (words the child says), and gestural communication. One of these studies found the group consuming formula fortified with only DHA had lower scores in the vocabulary comprehension section than the breastfed group, and lower expressive vocabulary scores than the control formula group at 14 months (90). This difference was not present at the 39 month follow-up assessment (69). In addition, the scores for the group consuming the formula fortified with both DHA and ARA were not different from those for the breastfed or control formula groups at any age. The other study found scores for all of the groups to be in the normal ranges for vocabulary comprehension and expression, with no differences between the scores for those consuming fortified formulas and either the control formula or breast milk (68).

3.6.1.4.2 The Peabody Picture Vocabulary Test-Third Edition (PPVT-III)

The Peabody Picture Vocabulary Test–Third Edition (PPVT-III) assesses language comprehension and was used in one study when the children were 24 and 42 months old (92). The control formula group had higher scores than both the 0.32% and 0.96% DHA formula groups at 24 months of age. However, at 42 months there were no differences present between any of the groups.

3.6.1.5 Behaviour

The Infant Behaviour Questionnaire measures activity levels, distress to novel stimuli, distress to limitations, soothability, smiling and laughter, and duration of orienting. Auestad et al (2001) found no differences in the performance of infants consuming DHA and ARA-fortified formulas, control formula, or breast milk for five out of the six dimensions of this questionnaire (68). The smiling and laughing score was the only dimension with a significant difference between groups, with those infants consuming the control formula having higher scores than those in the egg derived triglyceride DHA and ARA formula group. There was no such association in the fish oil or fungal oil derived DHA and ARA formula group despite it having the same concentration of DHA and ARA as the egg derived triglyceride DHA and ARA formula.

3.6.2 Efficacy of fortified milk-based products for improving neurodevelopment outcomes in developing countries

This literature review found no trials of DHA-fortified milk-based products from developing countries investigating neurodevelopment outcomes in children six to 36 months old.

3.6.3 Discussion

Six of the seven trials using milk fortified with DHA or ARA provided sample size calculations. A sample size of 15-37 per group of children recruited at birth was estimated to detect a difference of 0.1-0.2 logMAR in VEP acuity between groups at 12 months of age (70,75); 21 in each group of children recruited either at birth or at four to six months to detect a 1- SD difference in VEP acuity at 12 months of age (71,73); 47 in each group of children recruited at birth to detect a 0.75-SD difference in vocabulary scores at 12 months of age (68). All of these studies recruited the sample size that was calculated to be required. The one study

that did not report a sample size calculation had 43-63 subjects in each group recruited from birth, with an intervention until 12 months of age. Based on the other studies' estimations, this sample size would have been adequate to detect a difference between groups for VEP acuity and the MacArthur vocabulary score (69,90). No studies fortifying milk with DHA or ARA reported sample size estimations for the PPVT-III test, Infant Behaviour Questionnaire, Intelligence scores, Bayley's Indexes, BBCS-R, or visual acuity assessed by the Teller Card Procedure. It is therefore difficult to establish whether the sample sizes used for these studies were large enough to detect any potential differences between groups for these specific neuro-developmental assessments.

Two trials used milks fortified with iron in their intervention. One of these studies reported that their sample size of 133-136 per group was adequate to detect a five point difference in Bayley MDI and PDI scores (44). The other study did not report a sample size calculation. Their sample size, however, of 41-46 per group was sufficient to detect a significant difference in the Griffiths General Quotient score between groups, but only in one of the subquotients (34). It is possible that with more participants in each group, differences in other subquotient scores may have reached statistical significance.

The concentrations of DHA and ARA in the intervention milks ranged from 0.12-0.96% and 0.34-0.72%, respectively. These ranges are similar to the concentrations found in breast milk (i.e., DHA 0.06-1.4%, and ARA 0.24-1.0% (86)). Two studies used iron as a fortificant, albeit at differing concentrations: 12 mg Fe/L (34) and 1.2 mg Fe/L (44). Koletzko et al (28) have proposed an iron concentration in follow-up formula of 6.6-13.3 mg/L, so the infant formula containing only 1.2 mg Fe/L was below the recommended range, and may have been too low to provide any benefit on neurodevelopment outcomes (44). The study using an iron-fortified milk with a concentration of 12 mg iron/L was within the recommended range and did find a difference in the Griffiths general quotient score between groups (34).

3.6.4 Conclusion

The studies assessing the effect of fortified milk-based products on neurodevelopment outcomes had varying results. It does appear, however, that for VEP acuity a dose-dependent relationship exists, whereby the longer the duration of DHA consumption, the better the VEP acuity. The response is independent of the source of DHA (i.e., from formula or breast milk); with both fortified formula and breast milk resulting in VEP acuity results that are better than those observed when infants are consuming "placebo" infant formula. Comparable differences have not been found when the Teller Acuity Card Procedure has been used to assess visual acuity in children at 12 or 39 months.

The results for other neurodevelopment outcomes were inconsistent. Four out of six studies found no differences between groups for general development scores. In contrast, in the study by Williams et al (34) of children aged six to eight months given iron-fortified formula (12 mg iron/L) until age 24 months, the overall Griffiths general quotient and the subquotient "personal and social skills" were significantly higher in the iron-fortified formula group compared to the cow's milk group after 16-17 months of intervention, however the values declined from baseline in all groups (34). In the study by Makrides et al (67), Bayley's MDI scores were significantly higher in a breastfed group at 24 months than the DHA and ARA-fortified formula group (67). Surprisingly, vocabulary comprehension (90,92) and expression (90) were reduced in children consuming DHA-fortified formulas compared to

those who were either breastfed or consuming “placebo” formula, although none of these differences were present at follow-up assessments (i.e., at 39 or 42 months of age). There were no significant differences found between groups for intelligence scores in two trials. The only behavioural outcome (out of six measured) to be significantly different was a higher score (for “smiling and laughing”) achieved for the infants consuming the non-DHA and ARA-fortified control formula than those consuming the egg-derived triglyceride DHA and ARA-fortified formula, however this difference was not detected for fish oil and fungal oil derived DHA and ARA-fortified formula, even thought it had the same DHA and ARA concentrations (68).

In the absence of sample size calculations for the PPVT-III test, Infant Behaviour Questionnaire, Intelligence scores, Bayley’s Indexes, BBCS-R, or visual acuity assessed by the Teller Card Procedure, it is is difficult to establish whether the sample sizes used by the DHA and ARA fortified milk studies looking at these outcomes were large enough to detect any potential differences between groups for these specific neuro-developmental assessments.

Ultimately, however, only one study had participants starting the intervention milks at four to six months (73) (most interventions began from birth), and none used follow-up formula (all used infant formula). Moreover, the findings for VEP, Bayley’s MDI, intelligence scores and vocabulary all suggest no additional benefit of infant formula over breastfeeding, and in some cases, poorer performance, confirming the important role of breastfeeding in human development.

4 Appendices

Appendix A

Table 4.1: Summary of follow-up formula consumption in children six to 36 months old

Ref	Author (year)	Study details	Age group for which FUF is marketed or recommended	Intake of follow-up formula		
				Proportion of infants consuming FUF, amount consumed per day, and frequency		
				≤6 months	>6 <12 months	≥12 months
Follow-up formula consumption in developed countries						
(8)	FAO/WHO report	Austria	NA	10% at 3 months 60.4% at 6 months consume FUF	48.9% at 12 m consume FUF	NA
(108)	Garriguet et al (2008)	Canada 2004 Canadian Community Health Survey – Nutrition 1-18 years n=14,493	NA	NA	NA	12-36 months: 87% boys drink mean 459 g, 88% girls drink mean 450 g of any milk.
(109)	Coleman et al (2006)	Canada From birth n=1781	NA	NA	12.7% <9 months drink cow's milk as the primary source of milk.	NA
(8)	FAO/WHO report	Estonia NA	2 age groups: 0-12 m 6-12 m	NA	NA	NA
(12)	Turberg-Romain et al (2008)	France 1-36 months n=713	6 months and older	Exchanged infant formula for FUF at 4-5 months. At 5 months 75% drank FUF	At 6-8 months >80% drank FUF	Toddler milk instead of FUF at 13-18 months. At:

Ref	Author (year)	Study details	Age group for which FUF is marketed or recommended	Intake of follow-up formula		
				Proportion of infants consuming FUF, amount consumed per day, and frequency		
				≤6 months	>6 <12 months	≥12 months
						13-18 months, 52%; 19-24 months, 27%; 25-30 months, 26%; 31-36 months, 8% Drank toddler milk. Cow's milk 19-24 months onwards.
(97) (24)	Fantino et al (2008)	France 1-36 months n=706 Not breastfed	NA	NA	10-12 months, 25% do not consume any type of formula	31-36 months, <10% consumed any type of formula
(10) (15)	Kersting and Dulon (2002) VELS Study	Germany n=1717	Feeding of FUF is dispensable as is GUM, however if wish to used them, should not do so before the complementary feeding period of 5-7 months. Recommend 300 mg at 12 months, 330 mg at 12-36 months of milk and milk	At 6 months 38% drinking FUF	At 9 months 46% drinking FUF. At 6-12 months, boys consumed mean 187g, girls 242g infant formula/FUF/GUM. At 12 months 39% drinking FUF, Boys consumed 44g, girls 81.5 g of infant formula/FUF/GUM.	At 24 months, boys consumed mean 19.5 g, girls 13g of infant formula/FUF/GUM. At 36 months, boys consumed mean 16g, girls 0.1 g infant formula/FUF/GUM.

Ref	Author (year)	Study details	Age group for which FUF is marketed or recommended	Intake of follow-up formula		
				Proportion of infants consuming FUF, amount consumed per day, and frequency		
				≤6 months	>6 <12 months	≥12 months
			products. Packet recommendations are often up to 500 mL per day.			
(15)	MPI call for data	Germany DONALD study. Details not available.	NA	Mean consumption of infant formula, FUF, and GUM at 6 months was 264 g.	Mean consumption of infant formula, FUF, and GUM at: 9 months: 264 g. 12 months: 146 g	Mean consumption of infant formula, FUF, and GUM at: 18 months: 41 g; 24 months: 20 g; 36 months: 13 g;
(8)	FAO/WHO report	Ireland	6 m	11% of infants at 5 months have consumed FUF Average age of introduction: 5.5 months	NA	NA
(19) (16)	MPI call for data	Ireland National Pre-school Nutrition Survey (2012 1-4 years n=500	NA	NA	At 12 months: 3% infant formula, mean 257 g; 2% FUF, mean 553 g; 25% GUM, mean 360 g;	At 24 months: 2% infant formula, mean 406 g; 3% FUF, mean 258 g; 14% GUM, mean 298 g; At 3 years: 0% FUF; 6% GUM, mean 225 g;
(8)	FAO/WHO report	Luxembourg Details not provided	4 m and older	42% at 4 months exclusively breastfed 58% fed any formula, majority FUF	NA	NA

Ref	Author (year)	Study details	Age group for which FUF is marketed or recommended	Intake of follow-up formula		
				Proportion of infants consuming FUF, amount consumed per day, and frequency		
				≤6 months	>6 <12 months	≥12 months
				May be as young as 2 months when first introduced		
(8)	FAO/WHO report	Malta Details not provided	4 m forward	NA	NA	NA
(8)	FAO/WHO report	New Zealand Details not provided	6-12 months	NA	NA	NA
(20)	Szymlek-Gay at al (2010)	New Zealand 12-24 months n=188 South Island non-breastfed children Data collected 1998-1999	NA	Median age of any milk introduced is 3 months (mean 3.5 months)	At 9 months: 47% breastfed, but 78% had been given other milk: 73% FUF; 59% infant formula; 5.4% cow's milk;	NA
(21)	Morton et al (2012)	New Zealand Growing up in New Zealand Study Followed from birth to 24 months. n=6822 North Island children	NA	NA	At 9 months: 46% breastfed, of which 75% were fed 3-4 times per day 78% had been given a formula product on at least one occasion, of which 73% were given FUF, 5.4% cow's milk.	NA
(8)	FAO/WHO report	Norway Details not provided	4 months	FUF introduced as early as 4 months	NA	NA

Ref	Author (year)	Study details	Age group for which FUF is marketed or recommended	Intake of follow-up formula		
				Proportion of infants consuming FUF, amount consumed per day, and frequency		
				≤6 months	>6 <12 months	≥12 months
(13,17,18)	MPI call for data	Norway 6-months n=1986 12 months n=1635 24-months n=1674	NA	Infant formula or FUF introduced as either milk or in food: 22% <3 months 6% 3-3.5 months 8% 4-4.5 months 5% 5-5.5 months 2% 6 months At 6 months: 25% drank FUF, 1.6 times per day	At 12 months: 29% drinking FUF, mean 197 g	At 24 months: 6% drank a milk substitute other than infant formula
(14)	Persson et al (1984)	Sweden From birth n=312	NA	At 4-5 months FUF replaced infant formula At 6 months 90% of infants drank FUF	NA	NA
(11)	Hornell et al (2001)	Sweden From birth to mean 8.7 months n=506 Total of 85% given any formula. Amount drunk varied significantly within individuals, average frequency fluctuated 0-	NA	Age of any formula introduced: 44% < 4 months 30.5% 4-6 months 25.4% ≥ 6 months	NA	From 15 months, 55% were given formula consistently

Ref	Author (year)	Study details	Age group for which FUF is marketed or recommended	Intake of follow-up formula		
				Proportion of infants consuming FUF, amount consumed per day, and frequency		
				≤6 months	>6 <12 months	≥12 months
		twice daily over any 14 day period.				
(8)	FAO/WHO report	Switzerland Details not provided	After 6 months	NA	NA	NA
(19)	MPI call for data	Switzerland MOSEB Study 2008-2012 0-36 months n=493 mothers of infants	NA	NA	At 7-9 months: 29% breastmilk; 53% FUF; At 10-12 months: 18% breastmilk; 63% FUF;	At 13-18 months: 7% breastmilk; 32% FUF; At 19-24 months: 2% breastmilk; 18% FUF; At 25-30 months: 1% breastmilk; 14% FUF; At 31-36 months: 0% breastmilk; 15% FUF;
(9)	Lennox et al (2011)	United Kingdom (UK) Diet and Nutrition Survey in Infants and Young Children 4-18 months n=2,683	FUF can be used after 6 months, but not recommended to change from either breastmilk or infant formula	FUF introduced at: 3 months, 7% 5 months, 16% 6 months, 50% At 4-6 months 32% drinking mean 485g FUF; 0% GUM	At 7-9 months: 56% drinking mean 475 g FUF; 0% GUM At 10-11 months: 59% drinking mean 475 g FUF; 3% drinking mean 397 g	At 12-18 months: 16% drinking mean 323 g FUF; 18% drinking mean 342 g GUM;

Ref	Author (year)	Study details	Age group for which FUF is marketed or recommended	Intake of follow-up formula		
				Proportion of infants consuming FUF, amount consumed per day, and frequency		
				≤6 months	>6 <12 months	≥12 months
					GUM;	
(31)	McAndrew et al (2010)	UK Infant Feeding Study, 2010 4-18 months n=10,768	Not before 6 months	4-6 months: 88% of breastfed children also given infant formula 9% given FUF 1% cow's milk	At 8-10 months: 57% of breastfed children drinking FUF and 35% infant formula At 8-10 months: 69% of all mothers, had given their child FUF 42% had given cow's milk (29% with food, 24% as a drink)	
(8)	FAO/WHO report	UK n=9,416 mothers	NA	2% of mothers said they had fed FUF by 4 weeks 34% had given their baby FUF by 6 months	51% had given their baby FUF by 9 months	NA
(8)	FAO/WHO report	UK Telephone survey of 1,000 new mothers and pregnant women. 272 had fed FUF to their infants.	NA	17% of all mothers who fed FUF started at 3 months 74% had given their baby FUF by 6 months	23% who fed FUF started between 7 and 12 months	NA
(25)	Siega-Riz et al (2010)	USA 4-23.9 months n=1596	NA	At 4-5.9 months 65% drank any formula	At: 6-8.9 months 75%; 9-11.9 months 64%; drank any formula	At: 12-14.9 months 24%; 15-17.9 months 7%; 18-20.9 months 1%; 21-23.9 months 1%; drank any

Ref	Author (year)	Study details	Age group for which FUF is marketed or recommended	Intake of follow-up formula		
				Proportion of infants consuming FUF, amount consumed per day, and frequency		
				≤6 months	>6 <12 months	≥12 months
						formula
(110)	Krebs et al (2006)	USA n=88 Exclusively breastfed at 4 months	NA	Any formula: At 4 months, 1/88 drank 120mL At 5 months, 0% At 6 months, 2/79 drank 30-88 mL	At 9-12 months, 28-72 drank 240-270 mL of any formula	NA
(8)	FAO/WHO report	USA Infant Feeding Practices Study II Collected 2005-2007 6-12 months old n=5,468	NA	Any formula type at: 6 months: 67.3%, median 887 mL/day	Any formula type at: 7 months: 68.9%, 813 mL/day; 9 months: 70.8%, 732 mL/day; 10 months: 70.9%, 665 mL/day; 12 months: 36.4%, 486 mL/day;	NA
Follow-up formula consumption in developing countries						
(8)	FAO/WHO report	Argentina Details not provided	2 age groups: Infants: 6-12 months Young children: 12-36	NA	NA	NA

Ref	Author (year)	Study details	Age group for which FUF is marketed or recommended	Intake of follow-up formula		
				Proportion of infants consuming FUF, amount consumed per day, and frequency		
				≤6 months	>6 <12 months	≥12 months
			months			
(8)	FAO/WHO report	Brazil Details not provided	2 age groups: Infants: 6-12 months Young children: 12-36 months	FUF first introduced at 6 months (as established by national legislation)	NA	NA
(8)	FAO/WHO report	Ghana 60 working mothers at the ministry 28% used only infant formula, 12% only FUF and 38% used both.	6 months and older	3.3% fed their child FUF at 4 months 33.3% who fed their child FUF started at or before 6 months	16.7% who fed their child FUF started between 7 and 12 months	1.7% who fed their child FUF started at 14 months
(8)	FAO/WHO report	Guatemala 300 mothers in Guatemala city	6-36 months	<10% of infants <6 months in urban areas. Very few <6 months from rural areas consume FUF	65% 6-12 months consume FUF	20% of children >1 year consume FUF
(8)	FAO/WHO report	Korea (The Rep of) Details not provided	6 m and older	Introduced from 6 months	NA	NA
(36)	MPI call for data	Malaysia The Third National Health and Morbidity Survey (2006) 6-23 months	Breastfeeding recommended up to 2 years, complementary foods introduced at 6 months.	Consuming any formula/powdered milk: <2 months: 51.8% 2-3 months: 65.4% 4-5 months: 66.5%	Infant/follow-up/growing up formula: 6-8 months: 75.6% 9-11 months: 77.6%	Infant/follow-up/growing up formula: 12-17 months: 81.1% 18-23 months: 81%
(8)	FAO/WHO report	Nicaragua	0-60 months	Introduced from 6 months	NA	67% <2 years old are breastfed

Ref	Author (year)	Study details	Age group for which FUF is marketed or recommended	Intake of follow-up formula		
				Proportion of infants consuming FUF, amount consumed per day, and frequency		
				≤6 months	>6 <12 months	≥12 months
(8)	FAO/WHO report	Philippines Details not provided	6-36 months	FUF first introduced at a mean age of 4.4 months 10.9% were consuming FUF at 0-5 months old	14% were consuming FUF at 6-11 months old	18% were consuming FUF at 12-23 months old
(36)	MPI call for data	Philippines Details not provided	NA	Between 0 and 5 months: 78.8% breastfed 90.6% drinking infant formula/FUF/GUM	6-11 months old: 62.7% breastfed 73.2% drinking infant formula/FUF/GUM	12-23 months old: 51.6% drinking infant formula/FUF/GUM

Abbreviations: NA, Not available; FUF, Follow-up formula; GUM, Growing-up milk;

Table 4.2: The effect on nutrient and energy intakes with the inclusion of follow-up formula in the diet of children six to 36 months old

Reference	Study details	Effect on nutrient intakes
Studies from developed countries		
Bramhagen (1999) (26)	Sweden 30 months n=332	11% of ID children drank FUF 43% of Fe-sufficient children drank FUF Positive correlation between amount of FUF drunk and SF and serum-Fe Mean daily intake of cow's milk: 382 mL in ID children 257 mL in Fe-sufficient children Negative correlation between amount of cow's milk drunk and SF, MCV, MCHC. Children with SF < 12 ug/L: significantly higher intake of cow's milk compared with children with SF $\geq$ 12 ug/L.
Fantino et al (2008) (24,97)	France 1-36 months n=706 Not breastfed	Greater consumption of infant milk does not significantly increase the total energy intake Greater consumption of infant milk is associated with significantly higher ALA, vitamin C, vitamin E, and iron. Also associated with lower sodium intake up to age 9 months, and lower protein intake up to age of 24 months old. Iron intake is significantly higher in children fed infant formula. > than 1/3 French children over 1 yr old are at risk of deficient iron intake Premature introduction of cow's milk appears to be the main determinant of deficient iron status. In 2005, milk and other infant milk products provided 54% of total energy intake at 8-9 months old. At 18 months, consumption of growing up milk falls and represents only 6% of energy intake between 19 and 30 months. Almost 0% after 30 months.
Ghisolfi et al (2012) (27)	France 12-24 months n=63 drinking cow's milk n=55 drinking growing-up milk	Total energy and macronutrient intakes were similar in the two groups, except protein intake of cow's milk group, which was much higher than the RDA and sig higher than growing-up milk. Cow's milk: 51% had linoleic acid, 84% alpha-linoleic below the lower limit of AI. Iron (59%), vitamin C (49%) and vit D (100%) were below the EAR. Cow's milk (>250ml/d) entails the risk of insufficiency in alpha-linoleic acid, Fe, vitamin C, vitamin D. Use of growing-up milk (>250ml/d) sig reduces the risk of insufficiencies in these nutrients
Opinion	Germany	Toddler milk products are not suited to the specific nutritional needs of children up to the age of three.

Reference	Study details	Effect on nutrient intakes
036/2011 of the Federal Institute for Risk Analysis – Nutrients in toddler milk (From MPI) (111)	12 months	Toddler milk instead of cow's milk, as part of a varied diet, unlikely to significantly reduce protein intake. Insufficient evidence that high protein intake in toddlers increases the risk of overweight and obesity in later childhood. Fat content of toddler milk is higher than the recommended reduced fat milk. This may cause uncontrolled intake of nutrients as no need for them. It does appear good nutritional sense to supplement calcium, iron, iodine, and folate only, yet toddler milk has less calcium than cow's milk.
Webb et al (2008) (112)	Australia 16-24 months n=429	Milk supplies over 1/3rd of the energy and a large portion of the total quantity of foods/beverages consumed in this age group. Mean energy intake exceeded the EERs by 10%, 8.3% of children had an intake below EAR for calcium,23.3% iron, and 13.8% for vitamin C from the whole diet.
Studies from developing countries		
Semba et al (2010) (30)	Indonesia 6-59 months n=81,885 rural n=26,653 urban slums	Milk fortified with vitamins A, C, D, E, K, B_{12}, thiamin, and riboflavin. Drinking multi-nutrient fortified milk: 30.1% rural, 40.1% urban Prevalence of anaemia among children 6-59 months was 56% in rural areas and 61% urban slums. Less likely to be anaemic if drinking fortified milk: Drinking fortified milk: 47.4% (rural), 56.1% (urban) with anaemia Not drinking fortified milk: 59.7% (rural), 64.2%, (urban) with anaemia

Abbreviations: ID, Iron-deficiency; FUF, Follow-up formula; RDA, Recommended Daily Allowance; Fe, Iron; ALA, alpha-linolenic acid;

Table 4.3: Summary of parental perceptions of necessity and rationale for use of follow-up formula

Ref	Author (year)	Study details	Parental perceptions of necessity and rationale for using follow-up formula
Studies from developed countries			
(31)	McAndrew et al (2010) Infant Feeding Survey 2010	United Kingdom 4-18 months n=10,768	At 4-6 months: Mothers from routine and manual occupations and mothers who had never worked were more likely than average to have given follow-on formula at an earlier age (18% and 27% respectively) compared to managerial and professional occupations (12%). At 4-6 months: Mothers who had never worked were the most likely to have given FUF (19%), mothers from managerial and professional occupations least likely (6%). At 8-10 months no differences by occupation. Reasons for introducing FUF at 4-6 months: 18%: Thought it was better for the baby/provide more nutrients 17%: Advised to do so by a doctor, health visitor or other health professional 13%: Experience with other children 9%: Advised by friend/relative 9%: Baby still hungry after feeding 8%: Read leaflets/seen information 8%: part of weaning 6%: baby not gaining enough weight 68% said they knew the difference between follow-on and infant formula when their child was 4-6 months. 31% did not know the difference. Both just as likely to have reported using it. First-time mothers were more likely to use FUF because they thought it provided more nutrients/was better for their baby (20% vs. 15%), more influenced by friends or relatives (12%) and leaflets/information (11%) than mothers of second or later babies (5% for each). Primary reason for mothers of multiple children was experience with previous children (27%).
(11)	Hornell et al (2001)	Norway From birth n=506	Reasons for introducing formula at 6-10 months: 23%: Infant old enough 18%: Infant should get used to bottle and/or formula 15%: Mother is planning to stop breastfeeding

Ref	Author (year)	Study details	Parental perceptions of necessity and rationale for using follow-up formula
(32)	Le Heuzey et al (2008)	France 1-36 months n=706	15% use on experience with regards to feeding their child 85% use at least one source; mainly medical professional (77%). Other sources: paediatricians and general practitioners, midwives, pharmacists.
(21)	Morton et al (2010)	New Zealand Parents of 9 month old children n=6384	Information about infant feeding was sourced from: 93%: Plunket 76.9%: Family or friends 43.9%: Books 29.8%: Internet 33.8%: Magazines 23.9%: General practitioner 14%: Midwife 6.6%: Public health 5.9% Self-knowledge 4.6% Other 3%: Maori/Pacific health provider 2.6% Pharmacist
(33)	Poon et al (2007)	Singapore Chinese mothers – child any age n=93	62% thought certain formulas could improve their baby's IQ
(35)	Williams et al (1999)	Canada > 6 months n=167	Decision to wean from breast milk to either cow's milk of formula at 6 months or greater was: 79%: Returning to work or personal decision 33%: Cost 32%: Books 31%: Availability 28%, Experience 28%, Friends advice 27%, Prenatal class 26%, Physician 21%, Hospital support

Ref	Author (year)	Study details	Parental perceptions of necessity and rationale for using follow-up formula
			18%, Family advice 14%, Public health
Studies from developing countries			
(36)	Philippines research (From MPI)	Philippines 6-23 months	Reason for choosing the milk currently giving to child (0-23 months): 40.3%, Affordable 13.9%, Nutritious 10.6%, Child's preference 9.9%, As per advice by doctor/health professional 8.2%, As per advice of relatives/friends 7.4%, To make child healthy/active 5.3%, Always available 2.2%, To make child intelligent 1.3%, As per benefits shown by media 1%, Others (mother's own decision)
(38)	Roy et al (2002)	Bangladesh 6-12 months n=252	Reason for introducing breastmilk substitute: 62%, Perceived insufficiency of breastmilk 9.9%, Baby needed extra milk 9.3%, Influenced by relatives 8%, Disinterest of their children in suckling milk 5.6%, Illness of mothers 4.3%, Working mother/student 2.5%, Own choice 2.4%, Other
(39)	Sobel et al (2011)	Philippines 0-24 months n=345	75% recalled formula advertising, 59% recalled the content: 'make babies healthy', 'make children smart', 'protects against infections'. Significantly more likely to formula feed if recalled an advertising message - excluding those who never breastfed, infants were 6.4 times more likely to have stopped breastfeeding before 12 months of age. Feeding decisions influenced by television advertisements, doctors, and milk company reps.
(37)	Yee et al (2007)	Malaysia 0-6 years old n=505	Factors influencing consumer purchase decision of follow-up formula: Quality, brand, convenience, child's reaction (like/dislike), family and friends' opinions, health attributes, price, age and developmental stage of child, medical official's advice, advertising and promotions.

Appendix B

Table 5.1: Trials of iron-fortified milk-based products in children six to 36 months old

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control (n=sample size that completed the study (% of total sample))	Intervention duration	Impact on nutritional imbalances/ inadequacies	Comments
Iron-fortified milk-based trials in developed countries							
(40)	Bradley et al (1993)	USA n=347 From birth Healthy term infants.	1) H-FM group: high Fe-formula Fe: 12.7 mg/L (ferrous sulphate) n=106 from birth n=44 late start 2) L-FM group: low Fe-formula Fe: 7.4 mg/L (ferrous sulphate) n=106 n=40 late start Author did not report amount provided or drunk.	1) BF group: breastfed exclusively for 6 mo, maximum of one feed per day of low-Fe formula from 6-12 mo n=51 If breastfed infants changed to formula at less than 2 mo old, they were randomised into an intervention group into the 'late start' groups.	12 mo Until 12 mo of age	Mean SF (µg/L) sig higher H-FM group: At 12 mo: 23 BF 18 L-FM 31 H-FM No sig differences in Hb, haematocrit, serum Fe, Zn, or Cu among any of the groups at 6 or 12 mo of age.	Author did not report an index of infection. Author did not report a sample size calculation. 172 infants (50%) were included in all analyses.
(41)	Daly et al (1996)	Deprived area of Birmingham	1) FUF group: follow-up formula Fe: 12 mg/L	1) CM group: cow's milk Fe: 0.5 mg/L	Intervention 9.4-12.3 mo, then	Prevalence of anaemia (Hb <110g/L) FUF group sig higher: At 12mo: 3% CM 31% FUF	Author did not report an index of infection.

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control (n=sample size that completed the study (% of total sample))	Intervention duration	Impact on nutritional imbalances/ inadequacies	Comments
		n=100 5.7-8.6 months (mean age 7.8 months) All drinking cow's milk. Excluded if: Hb <90 g/L	=5.8 mg/day VC: 100 mg/L = 48 mg/day n= 41 (82%) (mg/day based off a mean intake of 480 mL of formula/day) Cow's milk from 18-24 months of age Mean intake (mL/day): At 12 mo: 582 At 18 mo: 378 (plus 160mL cow's milk per day)	=0.3 mg/day VC: 10 mg/L =5.6 mg/day n=43 (86%) (mg/day based off a mean intake of 564 mL of formula/day) Mean intake (mL/day): At 12 mo: 592 At 18 mo: 576	additional 12 mo follow up Intervention until 18 mo of age, complete study including follow-up until 30 mo of age.	At 18 mo: 2% CM 33% FUF At 24 mo: 0% CM 26% FUF Mean SF levels (µg/L) FUF group sig higher: At 12 mo: 22.5 CM 30.9 FUF At 18 mo: 15.9 CM 30.5 FUF At 24 mo: 14.9 CM 32.4 FUF	Sample size calculated with respect to identifying a difference in Hb concentration and not growth or morbidity.
(42)	Gill et al (1997)	UK and Ireland n=406 6 mo Health term infants. All drinking either cow's milk or formula.	1) Fe-FUF group: Fe-fortified follow-up formula Fe: 12.3 mg/L VC: 126 mg/L n=192 (73%) Author did not state amount of milk provided or consumed.	1) FUF group: follow-up formula Fe: 1.4 mg/L VC: 130 mg/L n=60 (71%) 2) CM2 group: cow's milk Fe: 0.5 mg/L VC: 10 mg/L n=50 (88%)	9 mo Until 15 mo of age	Prevalence of anaemia (Hb <110g/L) sig higher FM group: At 15 mo: 13% FUF 33% CM 11% Fe-FUF Prevalence of SF <10µg/L sig less in FM group: At 15 mo: 22% FUF 43% CM 6% Fe-FUF	Author did not report an index of infection. Author did not report a sample size calculation.

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control (n=sample size that completed the study (% of total sample))	Intervention duration	Impact on nutritional imbalances/ inadequacies	Comments
		Excluded if: Hb <100g/L and SF <10 µg/L.		Author did not state amount of milk provided or consumed.			
(43)	Haschke et al (1993)	Austria n=59 3 mo Healthy term infants with Hb >100 g/L.	1) Low Fe-fortified infant formula Fe: 3.0 mg/L = 2.7 mg/day aged 6 mo = 2.4 mg/day aged 9 mo n=27 (93%) 2) High Fe-fortified infant formula Fe: 6.0 mg/L = 4.9 mg/day aged 6 mo = 4.3 mg/day aged 9 mo n=24 (80%) Author did not state Fe forms. Author did not state	1) Breastfed n=30 (68%)	6 mo Until aged 9 mo	Hb concentration, haematocrit, MCV, transferrin saturation, and SF were not significantly different between the study groups after 6 mo.	Author did not report an index of infection. Sample size calculated as 21 participants in each group to detect a difference of 7 g/L in Hb concentration between groups. None of the infants on the low Fe-formula met the RDA for Fe, and only 4-8% of the high Fe-formula did.

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control (n=sample size that completed the study (% of total sample))	Intervention duration	Impact on nutritional imbalances/ inadequacies	Comments
			amount of formula provided to participants.				
(44) (45)	Morley et al (1999) Singhal et al (2000)	UK n=493 9 mo All ready drinking cow's milk Healthy full-term infants, birth weight > 2500 g	1) FM group: Fe-fortified formula Fe: 12 mg/L (as ferrous sulphate) VC: 66 mg/L n=133 (82%) 2) NFM group: Non-fortified formula Fe: 0.9 mg/L VC: 66 mg/L n=135 (82%) Unlimited amount of formula provided to participants. Author did not state actual amount of milk consumed.	1) CM group: cow's milk Fe: 0.5 mg/L n=160 (96%) Unlimited amount of formula provided to participants. Actual amount consumed not provided by the author.	9 mo Until aged 18 mo	Mean SF levels (µg/L) sig higher in FM1 group: At 18 mo: FM 21.4 NFM 13.3 CM 14.3	Author did not report an index of infection. Sample size calculated with respect to finding a difference in Bayley MDI and PDI not growth or morbidity.
(46)	Szymlek-Gay et al (2009) The Toddler	New Zealand n=225 12-20 mo (mean: 17.1 mo)	1) TM group: Toddler Milk Fe: 13.1 mg/L = approx. 6g/day (as ferrous sulphate) n=41 (91%)	1) RM group: Encouraged to eat more red meat Fe aim: approx. 2.6 mg/day Actual Fe: 0.91	5 mo Until aged 17-25 mo	No difference in the risk of developing suboptimal Fe status by any of the groups after 5 mo. Adjusted mean SF change: TM 44% increase	All data was controlled for CRP levels. Sample size was restricted by funding, 45 participants did not

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control (n=sample size that completed the study (% of total sample))	Intervention duration	Impact on nutritional imbalances/ inadequacies	Comments
	Food Study	Excluded if: Hb <105 g/L or, Hb <110 g/L and SF <12 µg/L	Asked to replace their regular milk with the study milk. > 400 g/day of fortified milk consumed.	mg/day n=83 (92%) 2) CM group: whole cow's milk Fe: 0.09 mg/L n=81 (90%) Asked to replace their regular milk with the study milk. Actual amount consumed not provided by the author.		RM no change CM 14% decrease Intervention effect of 1.68 µg/L SF in FM group compared to CM group.	have enough power to detect a change from baseline of 30% to 10% of non-anaemic suboptimal iron status, but could detect a difference of 42% in mean SF between fortified milk and control groups. Power of 80%, and 5% significance level. 87% adherence to the milk groups, 10% drank both study milk and non-study milks, 3% drank only study milks.
(47)	Virtanen et al (2001)	Sweden n=54 12 mo Full-term infants, birth weight within 2 SD of the	1) L-FM group: low-Fe fortified cow's milk Fe: 7.0 mg/L = 3.1 mg/day (as ferrous gluconate) VC: 75 mg/L =33.4 mg/day 2) H-FM group: high-	1) LF-CM group: low fat cow's milk Fe: 0.6 mg/L = 0.34 mg/day Fat: 10 g/L = 5.6 g/day 2) HF-CM group: high fat cow's milk	6 mo Until aged 18 mo	No differences were found between L-FM group and H-FM group so combined. Also no differences between LF-CM and HF-CM group so combined. No children had IDA at 12 mo, and only 1 at 18 mo (in the intervention group).	All children had normal CRP (less than 9 mg/L). Sample size of 25 participants in each group to detect a 4 g/L change from baseline in Hb. Power of 80%,

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control (n=sample size that completed the study (% of total sample))	Intervention duration	Impact on nutritional imbalances/ inadequacies	Comments
		Swedish growth chart. All children breast-fed or given iron-fortified formula from birth to 6 mo. From 6 mo to 12 mo given iron-fortified follow-on formula.	Fe fortified cow's milk Fe: 14.9mg/L = 6.6 mg/day (as ferrous lactate) VC: 140 mg/L = 62.3 mg/day n=20 (74%) (both Fe-milks combined) Fed unlimited amount of milk. Mean 445 mL/day in both fortified milk groups.	Fe: 0.6 mg/L = 0.34 mg/day Fat: 35 g/L = 19.7 g/day n=16 (59%) (both control groups) Fed unlimited amount of milk. Mean 562 mL/day in both cow's milk groups.		No difference in changes to Hb, MCV, transferrin Fe saturation or serum Fe between the groups at 18 mo.	5% significance level. Intervention group had significantly higher daily Fe intakes at 15 and 18 mo of age (10.2 vs 5.2 mg/d at 15 mo; 10.9 vs 5.8 mg/d at 18 mo).
(48)	Wall et al (2005)	New Zealand n=234 9-23 mo (mean 13.5 mo) All with IDA and hospitalised with an acute illness	Fe-fortified follow-on formula: Fe: 12mg Fe/L (as ferrous casienate) VC: 124-195 mg/L n=74 (32%) Fe-fortified cow's milk: Fe: 12.9mg/L (as ferrous sulphate) VC: 124-195 mg/L n=76 (33%)	Fe-medicine: Fe: 3 mg/kg of body weight/day (as ferrous gluconate) n=59 (70%) Mean body weight of children not provided, therefore unable to calculate mean daily intake.	3 mo Until aged 12-26 mo	Increase in Hb (mean 15 g/l) and Fe saturation (9%) and decrease in SF (-53µg/l) in all groups. IDA decreased in all groups after 3 mo: Fe-medicine: 93%-7%; Fe follow-on formula: 83%-8%; Fe-milk: 96%-30%.	All values adjusted by CRP. Sample size calculated as 65 participants in each group to correct 75% of IDA cases. Power of 80%, and 5% significance level.

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control (n=sample size that completed the study (% of total sample))	Intervention duration	Impact on nutritional imbalances/ inadequacies	Comments
			Asked to replace usual milk with study milk. Actual amount consumed not provided by the author.				
Iron fortified milk-based trials in developing countries							
(49)	Rivera et al (2010)	Mexico n=795 12-30 mo old Low-income children receiving free milk.	MN group: multi-nutrient fortified milk: Fe: 13.2 mg/L = 8.1 mg/day (Author does not state Fe form) VC: 120 mg/L = 73 mg/day Encouraged to give 400 mL/day, actual mean intake was 611 mL (per day nutrient intake based on actual mean). n=405 (82%) at 6 mo	NFM group: non-fortified formula: Fe: 0.4 mg/L = 0.24 mg/day (Author does not state Fe form) VC: 17 mg/L = 10.4 mg/day Encouraged to give 400mL/day, actual mean intake was 609 mL (per day nutrient intake based on actual mean). n=230 (84%) at 6 mo	12 mo Until aged 24-42 mo	Change in prevalence of anaemia (Hb<110 g/L) after 12 mo intervention: NFM 33.2% decrease MN 40.5% decrease (sig higher) Change in prevalence of SF <12 µg/L after 12 mo intervention: NFM 17.1% decrease MN 23.2% decrease (sig higher)	CRP was only calculated for some participants so was unable to be used. Data on serum transferrin receptor (unaffected by infection) was consistent with SF results so conclusions can be used with some confidence. Author did not report a sample size calculation.

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control (n=sample size that completed the study (% of total sample))	Intervention duration	Impact on nutritional imbalances/ inadequacies	Comments
			n=371 (75%) at 12 mo follow-up	n=213 (78%) at 12 mo follow-up			
(50)	Walter et al (1998)	Chile n=835 6 mo Healthy infants, without IDA (defined as Hb <100 g/L and 2 of 3: mean cell volume <70 fL, erythrocyte protoporphyrin > 100 µg/L, or SF <12 µg/L).	H-FM group: high Fe-fortified formula Fe: 12.7mg/L =7.9 mg/day (Author did not state Fe form) Author did not state amount of milk provided. Mean intake was 620 mL per day.	L-FM group: low-Fe formula Fe: 2.3 mg/L =1.4 mg/day (as ferric chloride) Author did not state amount of milk provided. Mean intake was 620 mL per day.	6 mo Until aged 12 mo	After 12 mo intervention: Prevalence of IDA: H-FM 3.8% L-FM 2.8% - no sig difference Prevalence of ID: H-FM 20% L-FM 39% - sig higher Mean SF (µg/L): H-FM 14.7 L-FM 10.8 – sig lower Mean Hb concentration (g/L): H-FM 123 L-FM 125 – sig lower Mean MCV (fL): H-FM 74.6 L-FM 73.4 – sig lower	Author did not report an index of infection, however, blood tests were postponed if a child was sick or had been febrile in the past 2 weeks. Author did not report a sample size calculation. Bioavailability of the low-Fe formula was 38%, and the high-Fe formula was 20% in adult volunteers.
(52,64)	Sazawal et al (2007), (2010)	India n=633 12-36 mo Breastmilk was	MN group: multi-nutrient fortified milk (per 3 sachets) VC: 48mg = 41 mg/day Fe: 9.6 mg	CM group: cow's milk (per 3 sachets) VC: 7.8 mg = 6.6 mg/day Fe: 0 mg = 0 mg/day	12 mo Until aged 24-48 mo	Prevalence of IDA at baseline: MN 58.2% CM 50.3% MN group had 88% lower risk of IDA after 12 mo	Author did not report an index of infection. Sample size achieved was able to detect a change in Hb level of

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control (n=sample size that completed the study (% of total sample))	Intervention duration	Impact on nutritional imbalances/ inadequacies	Comments
		not the only or predominant milk given. Any children with Hb <70 g/L were given therapeutic iron as well as the study milk.	= 8.3 mg/day (Author did not report nutrient forms) n=316 (100%) Given 21 sachets of milk powder per week, extras for family members if requested to prevent sharing. Actual mean of 2.58 sachets consumed per day.	(Author did not report nutrient forms) n=317 (100%) Given 21 sachets of milk powder per week, extras for family members if requested to prevent sharing. Actual mean of 2.54 sachets consumed per day.		Change after 12 mo intervention: Hb levels: MN 13.6g/L increase SF levels: MN 7.9µg/L increase	5.0 g/L over the baseline level. Power of 90% and 5% significance level. Adherence to the milk feeds was 85.6% in the intervention group, and 86.7% in the control group.
(51)	Villalpando et al (2006)	Mexico n=130 10-30 mo (mean 20.4 mo) Healthy children living in a poor periurban community.	FM group: fortified cow's milk Fe: 13.2mg/L = 5.3mg/day (ferrous gluconate) Zn: 13.2mg/L = 5.3 mg/day (zinc oxide) Vitamin C: 120 mg/L = 48 mg/day (sodium ascorbate) n=57 (92%) at 6 mo Encouraged to drink	CM group: cow's milk Fe: 0.5 mg/L = 0.2 mg/day (ferrous gluconate) Zn: 4.8 mg/L = 1.9 mg/day (zinc oxide) Vitamin C: 17 mg/L = 6.8 mg/day (sodium ascorbate) n=58 (85%) Encouraged to drink 400 mL/day	6 mo Until aged 16-36 mo	Prevalence of anaemia at baseline and 6 mo later: FM 41% to 12% - sig diff CM 30% to 24% - not sig diff Intervention effect of 58% Mean SF (µg/L), baseline and 6 mo later: FM 6.79 to 13.1 – sig different CM – not sig different Difference in the changes of each group were not sig different Intervention effect of 36%	CRP measured and data adjusted by recorded value. A sample size of 77 participants in each group to detect a change of 10 PP in the prevalence of anaemia. Power of 80% and 5% significance level.

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control (n=sample size that completed the study (% of total sample))	Intervention duration	Impact on nutritional imbalances/ inadequacies	Comments
			400 mL/day			reduction in Fe deficiency (SF <12 μg/L)	

Abbreviations: Fe, iron; Mo, months; Hb, haemoglobin; Zn, Zinc; Cu, copper; SF, serum ferritin; sig, significant; VC, vitamin C; MCV, mean cell volume; RDA, recommended dietary allowance; CRP, C-reactive protein; IDA, Iron deficiency anaemia;

Table 5.2: Trials of zinc-fortified milk-based products in children six to 36 months old

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control	Intervention duration	Impact on nutritional imbalances/ inadequacies	Comments
Zinc-fortified milk-based trials in developed countries							
(60)	Morgan et al (2010) The Toddler Food Study	New Zealand n=225 12-20 mo (mean: 17.1 mo) Excluded if: low Hb (<105 g/L) or, low Hb (<110 g/L) and low SF (<12 µg/L).	TM: Toddler Milk Zn: 4.7 mg/L n=35 (78%) Asked to replace their regular milk with the study milk. > 400 g/day of fortified milk consumed.	1) RM: Encouraged to eat more red meat Fe aim: approx. 2.6 mg/day Actual Fe: 0.91 mg/day n=83 (92%) 2) CM: Whole cow's milk VA and VD added. Zn: 2.8 mg/L n=81 (90%) Asked to replace their regular milk with the study milk. Actual amount consumed not provided by the author.	5 mo Until aged 17-25 mo	Baseline: Prevalence of serum Zn <9.9 umol/L: CM 30% RM 48% TM 43% Prevalence of hair Zn <1.07 umol/g (spring/summer) or <1.68 umol/g (autumn/winter): CM 29% RM 31% TM 39% Change is serum Zn concentration baseline to 5 mo: CM 3% RM 3% TM 9% - sig different from baseline - no sig differences between groups Change in hair Zn concentration from baseline to 5 mo: CM 2% RM 3% TM 13% - no sig differences between groups	Sample size achieved had 80% power to detect differences of 1.1 umol/L in mean serum zinc and 0.6 umol/g in mean hair zinc concentrations between the TM group and placebo milk group. 5% significance level. 87% adherence in the milk groups, (10% drank both study milk and non-study milks, 3% drank only study milks). At baseline <4% were below the EAR for dietary Zn intake.

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control	Intervention duration	Impact on nutritional imbalances/ inadequacies	Comments
(61)	Salmenpera et al (1994)	Finland n=200 0-3.5 mo	Randomised into group when weaned: 1) Fortified infant formula: Zn: 4 mg/L ($ZnSO_4$) n=32 aged 3.5 mo 2) Infant formula: Zn: 1.1 mg/L ($ZnSO_4$) n=23 aged 2 mo At 9 mo all formulas exchanged for cow's milk. Zn: 4.0-4.9 mg/kg Author did not state amount of formula provided or drunk.	1) Breastmilk n=84 at age 6 mo n=105 at age 9 mo n=65 at age 12 mo Exclusively breastfed until 6 mo at which time solids were introduced, and then cow's milk products after 9 mo of age.	5.5-9 mo Intervention until aged 9 mo, then cow's milk until aged 12 mo.	Breast-fed and unfortified formula-fed groups, mean serum Zn concentration decreased from birth to 2 mo, then remained stable. Zn-fortified formula group increased from 2mo of age, until 6 mo old (at which age solid foods were introduced), then decreased, by 9 mo was not significantly different to the breastfed or unfortified formula groups. No significant difference in prevalence of low serum Zn (below the 10^{th} percentile of the breastfed infants) between Zn-fortified and unfortified formula groups.	Author did not report a sample size calculation.
(40)	Bradley et al (1993)	USA n=344 From birth	1) H-FM group: high Fe-formula Fe: 12.7 mg/L (ferrous sulphate) Zn: 6 mg/L Fe-Zn ratio = 2.1	BF group: breastfed exclusively for 6 mo Fe-Zn ratio ~ <1 Maximum of one feed per day of low-Fe formula from 6-12 mo.	12 mo Until 12 mo of age	Serum zinc (umol/L): At 6 and 12 months of age: L-FM 11.7 12.0 H-FM 11.8 11.6 LS-L-FM 11.8 11.0 LS-H-FM 11.5 10.9 BF 11.1 11.4	Author did not report a sample size calculation.

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control	Intervention duration	Impact on nutritional imbalances/ inadequacies	Comments
			n=106 from birth n=44 late start (LS) 2) L-FM group: low Fe-formula Fe: 7.4 mg/L (ferrous sulphate) Zn: 6 mg/L Fe-Zn ratio = 1.2 n=106 n=40 late start Author did not report amount provided or drunk.	If breastfed infants changed to formula at less than 2 mo old, they were randomised into an intervention group into the 'late start' groups.		No sig differences.	
Zinc-fortified milk-based trials in developing countries							
(52, 64)	Sazawal et al (2007), (2010)	India n=633 12-36 mo	Multi-nutrient (MN) fortified milk (per 3 sachets): Fe: 9.6 mg = 8.3 mg/day Zn: 9.6 = 8.3 mg/day (Author did not report nutrient forms) n=316 (100%) Given 21 sachets of	Control milk (per 3 sachets): Fe: 0 mg = 0 mg/day Zn: 1.8 mg = 1.5 mg/day (Author did not report nutrient forms) n=317 (100%) Given 21 sachets of milk powder per week,	12 mo Until aged 24-48 mo	Serum zinc (µg/L) at baseline and after 12 mo intervention: MN 60.7 61.4 CM 62.6 63.4 No differences significant.	Required sample size highest for morbidity outcomes. Assumed MN drink would decrease diarrhoea incidence by 15%, episodes of pneumonia by 25%. 90% power, 5% level of significance. This could also detect a change of 0.5 Z-score over baseline Z-scores.

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control	Intervention duration	Impact on nutritional imbalances/ inadequacies	Comments
			milk powder per week, extras for family members if requested to prevent sharing. Actual mean of 2.58 sachets consumed per day.	extras for family members if requested to prevent sharing. Actual mean of 2.54 sachets consumed per day.			
(51)	Villalpando et al (2006)	Mexico n=130 10-30 mo (mean 20.4 mo)	FM group: fortified cow's milk Fe: 13.2mg/L = 5.3mg/day (ferrous gluconate) Zn: 13.2mg/L = 5.3 mg/day (zinc oxide) n=57 (92%) at 6 mo Encouraged to drink 400 mL/day	CM group: non-fortified cow's milk Fe: 0.5 mg/L = 0.2 mg/day (ferrous gluconate) Zn: 4.8 mg/L = 1.9 mg/day (zinc oxide) n=58 (85%) Encouraged to drink 400 mL/day	6 mo Until aged 16-36 mo	Serum zinc (umol/L) after 6 months intervention: CM 13.07 FM 12.61 Not significantly different.	Sample size not calculated for a change in zinc status.

Abbreviations: Mo, months; Hb, haemoglobin; SF, serum ferritin; Zn, zinc; Fe, iron; VA, vitamin A; VD, vitamin D; EAR, estimated average requirements; MN, multi-nutrient; sig, significantly;

Table 5.3: Trials of DHA-fortified milk-based products in children six to 36 months old

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control	Intervention duration	Impact on nutritional imbalances/ inadequacies	Comments
DHA-fortified milk-based trials in developed countries							
(68)	Auestad et al (2001)	Missouri, Arkansas, Pennsylvania,& Arizona, USA n=404 From birth Breastfed from birth, then mothers choosing to formula feed from age three mo included into intervention group.	Infant formula with added: 1) Fish oil DHA: 0.13% of total FA ARA: 0.46% of total FA 2) Egg derived triglyceride (egg-DTG) DHA: 0.14% of total FA ARA: 0.45% of total FA Formulas fed exclusively, *ad libitum*, until 4 mo, then as the exclusive milk beverage to 12 mo.	1) Control formula DHA: 0 ARA: 0 2) Breastfed DHA: 0.12% of total FA ARA: 0.51% of total FA Infants were breastfed until 3 mo then randomised to one of the three formula groups if they wished to stop breastfeeding. These were analysed as separate groups (ie 'Breastfed/ Control formula' group) in the analysis.	3 -12 mo Until aged 12 mo	Fortified-formula groups (irrespective of type) had significantly higher levels of ARA and DHA in RBC phospholipids than the control formula group at 12 mo. Breastfed group not different at 4 mo compared to the fortified formula groups, but at 12 mo, DHA was 40% less in the control.	Sample size estimated 27 infants in each group required to detect 1 SD difference, 90% power, in levels of DHA and ARA in blood samples. n=294 (73%) completed the study
(69) (74)	Auestad et al (1997, 2003)	Seattle, Portland and Kansas City, USA n=197 From birth	Infant formula supplemented with: 1) DHA 0.12% total FA ARA 0.43% of total FA (from egg-phospholipid) n=46	1) Breastfed exclusively for minimum of 3 mo n=63 2) Infant formula, no DHA or ARA n=45	Intervention 12 mo, then additional 27 month follow-up Intervention until 12 mo of	DHA RBC-PC and DHA RBC-PE at 12 mo: Control formula: 40% lower than breastfed group. AA + DHA formula: not different from the breastfed group, but higher than the control formula	Author did not report sample size calculation.

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control	Intervention duration	Impact on nutritional imbalances/ inadequacies	Comments
			2) DHA 0.23% of total FA (from fish oil) n=43 Formulas provided *ad libitum* as the sole source of nutrition for a minimum of 4 mo. Author did not report amount of formula drunk.		age, complete study including follow-up until 39 mo of age. Follow-up study results at 39 mo n=157 (80%)	group. DHA formula: higher levels of DHA (20-55% higher) than infants in the breastfed and other formula groups (200% higher). Also lower levels of ARA than the other groups. At 39 mo: DHA and ARA levels in RBC FA phospholipids did not differ between any of the groups.	
(72)	Birch et al (2002)	USA n=65 6 weeks	Infant formula (contained Fe) supplemented with: DHA 0.36% ARA 0.72% of total FA n=30 (94%) Author did not report amount of formula provided or drunk.	Infant formula (contained Fe) – contained no DHA or ARA n=28 (85%) Author did not report amount of formula provided or drunk.	10.5 mo Until 12 mo of age	DHA in total plasma lipids at 12 mo: Control formula: 75.5 mg/L in Fortified formula: 109 mg/L (significantly different) DHA in total RBC lipids at 12 mo: Control formula: 23.7 mg/L Fortified formula: 73.5 mg/L (significantly different)	Sample size estimated to require 21 infants in each group to detect a <1% difference in the DHA or ARA FA composition of RBCs. 80% power, 5% level of significance.
(71)	Birch et al (2005)	USA n=103	Infant formula (contained Fe) supplemented with: DHA 0.35%	Infant formula (contained Fe) – contained no DHA or	12 mo Until 12 mo of	RBC total lipids at 9.8 mo: DHA 21% higher in the fortified formula group	Sample size estimated to require 21 infants to detect a mean

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control	Intervention duration	Impact on nutritional imbalances/ inadequacies	Comments
		From birth	ARA 0.72% of total FA n=42 (82%) Author did not report amount of formula provided or drunk.	ARA n=44 (85%) Author did not report amount of formula provided or drunk.	age	than in the control group ARA 15-18% higher in the fortified formula than in the control group.	difference of ≥1.0 SD in VEP acuity between diet groups. 80% power, 5% level of significance.
(70)	Birch et al (2010) DIAMOND Study	Dallas and Kansas City, USA n=343 From birth	Infant formula (contained Fe) with DHA added at: 1) 0.32% of total FA n=64 (77%) 2) 0.64% of total FA n=59 (70%) 3) 0.96% of total FA n=65 (75%) Formulas provided *ad libitum* as the sole source of nutrition for a minimum of 4 mo.	Infant formula (contained Fe) - 0% DHA n=56 (66%) Formulas provided *ad libitum* as the sole source of nutrition for a minimum of 4 mo.	12 mo Until 12 mo of age	Total RBC DHA at 4 mo and 12 mo: Control formula: 46.9, 40.5 0.32% DHA: 113.4, 100.7 0.64% DHA: 139.4, 123.3 0.96% DHA: 152.4, 144.1	Sample size estimated to require 37 participants per group to detect a difference of 0.1 logMAR. 0.08% level of significance, 80% power. Larger sample size was recruited to allow enough statistical power for other analyses. Mean formula intake: At 6 mo: 985-1053mL At 9 mo:875-961mL At 12 mo: 441-772mL (range refers to the two study sites)
(73)	Hoffman et al (2003)	USA n=61 4-6 mo (at weaning)	Infant formula (contained Fe) supplemented with: DHA 0.36% ARA 0.72% of total FA n=30 (86%) Formulas provided *ad*	Infant formula (contained Fe) n=31 (94%) Formulas provided *ad libitum* as the sole source of nutrition for a	6-8 mo Until 12 mo of age	DHA in total RBC (mg/L): At baseline 4.5 mg/L At 12 mo: Control formula: 2.4 Fortified formula: 5.9 mg/L (significantly different)	Sample size estimated to require 21 infants per group to detect a <1% difference in DHA or ARA composition of RBCs between groups. 90% power, 5% level of

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control	Intervention duration	Impact on nutritional imbalances/ inadequacies	Comments
			libitum as the sole source of nutrition for a minimum of 4 mo.	minimum of 4 mo.			significance.
(75)	Makrides et al (1999)	Australia n=146 from 1 week	1) Fortified infant formula: DHA 0.35% of total FA (from tuna oil) n=23 (85%) 2) Fortified infant formula: DHA 0.34% and ARA 0.34% of total FA (from an egg phospholipid fraction) n=24 (86%) Mothers asked to provide the study milk *ad* libitum as the only milk drunk until 12 mo.	1) Infant formula n=21 (75%) 2) Breastfed n=23 (36%) at 8.5 mo Mothers asked to provide the study milk *ad* libitum as the only milk drunk until 12 mo.	12 mo Until aged 12 mo	Control formula group had plasma DHA levels 42% of those consumed in the breastfed group, and 34% of DHA-fortified formula groups. Breastfed infants' plasma DHA remained similar to baseline. DHA-fortified formula groups increased plasma DHA from baseline, control formula, plasma DHA decreased from baseline.	Sample size calculated to require 25 infants per formula-feeding group to detect a mean difference of weight of 700g. 80% power, 5% significance level. Sample size calculate to require 15 infants per formula group to detect a mean difference of 0.2 logMAR in VEP acuity. 90% power, 5% significance level.
DHA-fortified milk-based trials in developing countries							

Abbreviations: Mo, months; DHA, Docosahexaenoic Acid; FA, Fatty acids; ARA, Arachidonic Acid; RBC, Red Blood Cells; RBC-PC, red blood cell phosphatidycholine; RBC-PE, red blood cell phosphatidylethanolamine;

Table 5.4: Trials of other nutrient-fortified milk-based products in children six to 36 months old

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control	Intervention duration	Impact on nutritional imbalances/ inadequacies	Comments
Nutrient-fortified milk-based trials in developed countries							
(76)	Salmenpera et al (1989)	Finland n=200 2-3.5 mo	Randomised into group when weaned: 1) Fortified formula: Cu: 0.5mg Cu/L (as $CuSO_{4)}$ n=32 aged 3.5 mo 2) Infant formula: n=23 aged 2 mo At 9 mo all formulas exchanged for cow's milk. Author did not state amount of formula provided or drunk.	1) Breastmilk n=101 at age 6 mo n=30 (30%) at age 9 mo n=7 (6.9%) at age 12 mo	5.5-7 mo Intervention until aged 9 mo, then cow's milk until aged 12 mo.	Plasma Cu and ceruloplasmin concentrations increased steadily in all three groups from baseline (14.3-15.28 umol/L) to age 12 mo (17.8-20.6 umol/L) (no significant differences between groups though).	Author did not provide a sample size calculation.
(79)	Houghton et al (2011) The Toddler Food Study	New Zealand n=225 12-20 mo (mean: 17 mo)	1) Toddler Milk: VD: 6.3 µg cholecalciferol/100g powder Mean intake of VD: 25.2 µg/day n=43 (78%)	Encouraged to eat more red meat Fe aim: approx. 2.6 mg/day Actual Fe: 0.91 mg/day n=83 (92%)	5 months Until 17-25 months of age	At baseline prevalence of serum 25(OH)D: < 75 nmol/L: 79% <50 nmol/L: 45% <30 nmol/L: 11% Mean serum 25(OH)D concentrations increased in the two milk groups (no difference between these two groups)	Sample size was calculated to detect a change in Fe status but not VD. Only 2 participants consumed >10 µg/day (the EAR),

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control	Intervention duration	Impact on nutritional imbalances/ inadequacies	Comments
		Excluded if: Hb <105 g/L, or, Hb <110 g/L and SF <12 µg/L	> 400 g/day of milk consumed. 2) VD-fortified cow's milk: VD: 6.0 µg cholecalciferol/100g powder. Mean intake of VD: 24 µg/day n=81 (90%) Asked to replace their regular milk with the study milk.	Asked to replace their regular milk with the study milk.		(difference in change relative to meat group: VD-fortified milk, 24.1%; toddler milk, 32.5%), but not the meat group. No difference in the change of PTH between all 3 groups. Change in prevalence of low serum 25(OH)D concentration from baseline to 5 mo: <75 nmol/L: did not differ between groups. <50 nmo/L: no change in meat group, decreased in both milk groups (VD-fortified milk: 44% to 15%; Follow-on formula: 49% to 12%) . <30 nmol/L: meat group no change, milk groups decreased significantly (to total 3%). Serum 25(OH)D concentrations were 9% higher for every 1 µg of VD consumed in winter.	and no participants had intakes that met the RDA of 16 µg/day. 87% adherence to the milk groups (10% drank both study milk and non-study milks, 3% drank only study milks).
(77)	Szymlek-Gay et al (2011) The Toddler Food Study	New Zealand n=135 12-20 mo (mean age 16.8 mo)	Iodine-fortified powdered cow's milk I: 138.5 µg/100 g powder = 103.3 µg/day n=45 Mean intake: 74.6 g/day	Powdered cow's milk I: 40.5 µg/100 g Powder = 26.7 µg/day n=90 Mean intake: 66.5 g/day	5 mo	UIC at baseline, 5mo: Cow's milk: 55, 49 µg/L I-fortified milk: 42, 91 µg/L No significant different at baseline, but significantly different at 5 mo. Proportion of children with UIC less than 50 µg/L decreased from 66% at baseline to 29% at 5 mo, and UIC<100 µg/L decreased from 86% to 53% in the fortified milk group. No change in control group.	Sample size was calculated to detect a change in Fe status but not VD.

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control	Intervention duration	Impact on nutritional imbalances/ inadequacies	Comments
(78)	Kumpulainen et al (1987)	Finland n=200 2-12 mo	Randomised into group when weaned (ages at baseline): Fortified formula: Se: 20 µg Se/L (as Na_2SeO_3) Zn: 4 mg Zn/L (as $ZnSO_{4)}$ Cu: 0.5mg Cu/L (as $CuSO_{4)}$ n=32 aged 4-12 mo At 9 mo formula exchanged for cow's milk. Author did not state amount of formula provided or drunk.	1) Breastmilk (ages at baseline) n=58 aged 2 mo n=41 aged 6 mo n=12 aged 9 mo n=3 aged 12 mo 2) Infant formula: Se: < 5 µg/L n=23 aged 2 mo At 9 mo formula exchanged for cow's milk.	Author not clear on length of intervention.	Serum-Se increased in the breastfed group following the introduction of solid foods at 6 mo, by 9mo was similar to both formula groups. Serum-Se increased in the fortified formula group, between the ages of 2 mo and 6 mo, after which it remained stable until 9 mo after which it declined but not significantly. Serum-Se in unfortified milk group decreased during first 2 mo and remained constant until 6 mo, then increased gradually thereafter. At age 12 mo, no significant differences between any of the groups.	Author did not report a sample size calculation. Note outcomes are measured when the child is 2, 6, 9 and 12 mo old. Unclear what is measured in the children 12 mo old at the beginning of the study.
Nutrient-fortified milk-based trials in developing countries							
(80)	Lopez-Teros et al (2013)	Mexico n=27 36-72 mo (mean 66 mo)	Micronutrient fortified milk: VA: 196 retinol equivalents/day n=11 (79%)	No drink provided, advised to continue their typical diet. n=13 (100%)	3 mo Until aged 39-75 mo	After 3 months: Median change in serum retinol was 0.14 umol/L in intervention group (significantly different) vs -0.21 in control group (not significantly different). Total body VA (TBVA) stores increased	Sample size calculated to require 16 in each group to detect a difference between groups of 0.54 mmol in TBVA.

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control	Intervention duration	Impact on nutritional imbalances/ inadequacies	Comments
		Mild to moderately vitamin A deficient	Received 7x250mL pouches every week.			significantly by a median of 40%. Liver VA stores increased significantly by 28%. Control did not change. TBVA stores were significantly, positively, associated with dietary VA intake.	80% power, 5% significance level.

Mo, months; Cu, copper; Hb, haemoglobin; SF, serum ferritin; VD, vitamin D; Fe, iron; 25(OH)D, ; EAR, estimated average requirement; RDI, recommended daily intake; PTH, ; UIC, urinary iodine concentration; I, iodine; Se, selenium; Zn, zinc; VA, vitamin A; TBVA, total body vitamin A;

Table 5.5: Trials of fortified milk-based products assessing morbidity and growth in children six to 36 months old

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control	Intervention duration	Impact on nutritional imbalances or inadequacies	Impact on health outcomes	Comments
Nutrient-fortified milk-based products and morbidity and growth in developed countries								
(83)	Akeson et al (1998)	Sweden n=71 3 mo Healthy infants, exclusively breastfed at 3mo, study formula gradually introduced when weaned from breastmilk.	Infant formula containing Fe and: 1) 13 g of protein n=21 (84%) Mean intake 406 ml/day at 12 mo 2) 18g of protein n=20 (87%) Mean intake 498 ml/day at 12 mo. Formulas provided *ad libitum* but required to drink at least 125 mL/day to be included.	Infant formula containing Fe and: 1) 15 g of protein (standard formula) n=22 (85%) Mean intake not reported. 2) (BF) breastfed group n=10 at 8 mo n=4 at 10 mo n=3 at 12 mo Formulas provided *ad libitum* but required to drink at least 125 mL/day to be included.	9 mo Until 12 mo of age	Author did not report this.	All infants grew within ±2 SD of the Swedish and American standards for weight, length, and head circumference. No differences between groups for: Weight, length, head or arm circumference, or lower leg strength.	Sample size calculated to detect a difference of at least 1-SD in weight or length. 80% power, 5% significance level. Total protein intake not difference between formula groups at: 8 mo (2.0-2.3 g/kg/day); 10 mo (2.5-2.8 g/kg/day); 12 mo (2.5-2.7 g/kg/day). Total energy intake not different between formula groups at any age.

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control	Intervention duration	Impact on nutritional imbalances or inadequacies	Impact on health outcomes	Comments
(68)	Auestad et al (2001)	Missouri, Arkansas, Pennsylvania, & Arizona, USA n=404 From birth Breastfed from birth, then mothers choosing to formula feed from age three mo included into intervention group.	Infant formula with added: 1) Fish oil DHA: 0.13% of total FA ARA: 0.46% of total FA 2) Egg derived triglyceride (egg-DTG) DHA: 0.14% of total FA ARA: 0.45% of total FA Formulas fed exclusively, *ad libitum*, until 4 mo, then as the exclusive milk beverage to 12 mo.	1) Control formula DHA: 0 ARA: 0 2) Breastfed DHA: 0.12% of total FA ARA: 0.51% of total FA Infants were breastfed until 3 mo then randomised to one of the three formula groups if they wished to stop breastfeeding. These were analysed as separate groups (ie 'Breastfed/ Control formula' group) in the analysis.	3 -12 mo Until aged 12 mo	Fortified-formula groups (irrespective of type) had significantly higher levels of ARA and DHA in RBC phospholipids than the control formula group at 12 mo. Breastfed group not different at 4 mo compared to the fortified formula groups, but at 12 mo, DHA was 40% less in the control.	No differences between groups in weight, length, or head circumference at 12 months.	Sample size estimated 54 infants in each 3 formula groups to detect a 1 SD difference in growth with 90% power. n=294 (73%) completed the study.
(69) (74)	Auestad et al (1997, 2003)	Seattle, Portland and Kansas City, USA n=197	Infant formula supplemented with: 1) DHA 0.12% total FA ARA 0.43% of total	1) Breastfed exclusively for minimum of 3 mo n=63 2) Infant formula, no	Intervention 12 mo, then additional 27 month follow-up	DHA RBC-PC and DHA RBC-PE at 12 mo: Placebo formula: 40% lower than breastfed group. AA + DHA formula: not	At 12 mo of age: No difference between any groups for formula tolerance based on: occurrence of spit-	Author did not report sample size calculation.

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control	Intervention duration	Impact on nutritional imbalances or inadequacies	Impact on health outcomes	Comments
		From birth	FA (from egg-phospholipid) n=46 2) DHA 0.23% of total FA (from fish oil) n=43 Formulas provided *ad libitum* as the sole source of nutrition for a minimum of 4 mo. Author did not report amount of formula provided or drunk.	DHA or ARA n=45	Intervention until 12 mo of age, complete study including follow-up until 39 mo of age. Follow-up study results at 39 mo n=157 (80%)	different from the breastfed group, but higher than the placebo formula group. DHA formula: higher levels of DHA (20-55% higher) than infants in the breastfed and other formula groups (200% higher). Also lower levels of ARA than the other groups. At 39 mo: DHA and ARA levels in RBC FA phospholipids did not differ between any of the groups.	up, vomiting, or stool consistency At 39 mo of age no significant differences between the groups for: • weight, length, head circumference; • IQ, receptive and expressive language, visual-motor function, visual acuity; • 3 or more prescriptions for antibiotics since birth, use of pressure equalization tubes for chronic otitis media, hospitalisation since birth;	
(70)	Birch et al (2010) DIAMO	Dallas and Kansas City, USA n=343	Infant formula with DHA added at: 1) 0.32% of total FA n=64 (77%)	Infant formula - 0% DHA n=56 (66%)	12 mo Until 12 mo of age	Total RBC DHA at 4 mo and 12 mo: Control formula: 46.9, 40.5 0.32% DHA: 113.4, 100.7	Control formula group had significantly poorer VEP at 4, 9, and 12 mo.	Sample size estimated to require 37 participants per group to detect a

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control	Intervention duration	Impact on nutritional imbalances or inadequacies	Impact on health outcomes	Comments
	ND Study	From birth	2) 0.64% of total FA n=59 (70%) 3) 0.96% of total FA n=65 (75%) Formulas provided *ad libitum* as the sole source of nutrition for a minimum of 4 mo.			0.64% DHA: 139.4, 123.3 0.96% DHA: 152.4, 144.1	No significant differences between the intervention groups for VEP at any age. Formula had no significant effect on weight or length z-scores at any age.	difference of 0.1 logMAR. 0.08% level of significance, 80% power. Larger sample size was recruited to allow enough statistical power for other analyses. Mean formula intake: At 6 mo: 985-1053mL At 9 mo:875-961mL At 12 mo: 441-772mL (range refers to the two study sites)
(40)	Bradley et al (1993)	USA n=347 From birth Healthy term infants.	1) H-FM group: high Fe-formula Fe: 12.7 mg/L (ferrous sulphate) Zn: 6 mg/L n=106 from birth n=44 late start	1) BF group: breastfed exclusively for 6 mo, maximum of one feed per day of low-Fe formula from 6-12 mo n=51	12 mo Until 12 mo of age	Mean SF (µg/L) sig higher H-FM group: At 12 mo: 23 BF 18 L-FM 31 H-FM No sig differences in Hb, haematocrit, serum Fe,	No significant differences in growth (length or weight) between the formula groups, except, breastfed group had significantly lower mean length change	Author did not report a sample size calculation. 172 infants (50%) were included in all analyses.

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control	Intervention duration	Impact on nutritional imbalances or inadequacies	Impact on health outcomes	Comments
			2) L-FM group: low Fe-formula Fe: 7.4 mg/L (ferrous sulphate) Zn: 6 mg/L n=106 n=40 late start Author did not report amount provided or drunk.	If breastfed infants changed to formula at less than 2 mo old, they were randomised into an intervention group into the 'late start' groups.		Zn, or Cu among any of the groups at 6 or 12 mo of age.	than the late-start low-Fe group from 6 to 12 mo.	
(41)	Daly et al (1996)	Deprived area of Birmingham n=100 5.7-8.6 months (mean age 7.8 months) All drinking cow's milk. Excluded if: Hb <90 g/L	1) FUF group: follow-up formula Fe: 12 mg/L =5.8 mg/day VC: 100 mg/L = 48 mg/day n= 41 (82%) (mg/day based off a mean intake of 480 mL of formula/day) Cow's milk from 18-24 months of age Mean intake (mL/day): At 12 mo: 582 At 18 mo: 378 (plus	1) CM group: cow's milk Fe: 0.5 mg/L =0.3 mg/day VC: 10 mg/L =5.6 mg/day n=43 (86%) (mg/day based off a mean intake of 564 mL of formula/day) Mean intake (mL/day): At 12 mo: 592 At 18 mo: 576	Intervention 9.4-12.3 mo, then additional 12 mo follow up Intervention until 18 mo of age, complete study including follow-up until 30 mo of age.	Prevalence of anaemia (Hb <110g/L) FUF group sig higher: At 12mo: 3% CM 31% FUF At 18 mo: 2% CM 33% FUF At 24 mo: 0% CM 26% FUF Mean SF levels (µg/L) FUF group sig higher: At 12 mo: 22.5 CM 30.9 FUF At 18 mo: 15.9 CM 30.5 FUF At 24 mo: 14.9 CM 32.4 FUF	No differences in weight-for-age, height-for-age, or weight-for-height z-scores between the two groups at any age.	Sample size calculated as 47 participants in each group to detect a difference of 7.5 g/L in Hb concentration between groups. Power of 95% and significance level 5%.

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control	Intervention duration	Impact on nutritional imbalances or inadequacies	Impact on health outcomes	Comments
			160mL cow's milk per day)					
(42)	Gill et al (1997)	UK and Ireland n=406 6 mo All drinking either cow's milk or formula. Excluded if: Hb <100g/L and SF <10 µg/L.	1) Fe-FUF group: Fe-fortified follow-up formula Fe: 12.3 mg/L VC: 126 mg/L n=192 (73%) Author did not state amount of milk provided or consumed.	1) FUF group: follow-up formula Fe: 1.4 mg/L VC: 130 mg/L n=60 (71%) 2) CM2 group: cow's milk Fe: 0.5 mg/L VC: 10 mg/L n=50 (88%) Author did not state amount of milk provided or consumed.	9 mo Until 15 mo of age	Prevalence of anaemia (Hb <110g/L) sig higher FM group: At 15 mo: 13% FUF 33% CM 11% Fe-FUF Prevalence of SF <10µg/L sig less in FM group: At 15 mo: 22% FUF 43% CM 6% Fe-FUF	No differences between the groups for increases in weight, head circumference, or length.	Author did not report a sample size calculation.
(84)	Koletzko et al (2009)	Europe: Belgium, Germany, Italy, Poland, Spain. n=1,138 2 mo	Follow-on formula – higher protein content: 11.7% of energy n=323 (59%) Formulas provided *ad libitum*. Actual amount consumed not provided.	1) Standard formula – lower protein content: 7.1% of energy Identical energy content to the follow-on formula due to adaption of fat content. n=313 (58%) at 12 mo	Intervention for 10 mo, follow-up 10 mo later. Intervention until 12 mo of age, follow-up at 24 mo of age.	Author did not report.	At 6 and 12 mo Z-scores for: Weight, weight-for-length, BMI, significantly higher in the high-protein formula group than the low-protein formula group. At 24 mo Z-scores for:	Sample size calculated 296 in each group to detect a difference in length of 0.8 cm at 24 mo between the 2 formula groups. 90% power, 5% significance level.

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control	Intervention duration	Impact on nutritional imbalances or inadequacies	Impact on health outcomes	Comments
				Formulas provided *ad libitum*. Actual amount consumed not provided. 2) Breastfed children n=298 (51%) at 24 mo			• Length and weight not different between formula groups. • Weight-for-length 0.20 greater in high-protein formula group than low-protein formula group. • Weight (0.20 SD), length (0.27 SD), weight-for-length (0.18 SD), BMI (0.20 SD) higher in high-protein formula compared with breastfed group • No difference between low-protein formula and breastfed group for weight-for-length or BMI • Weight (0.16 SD) and length (0.29 SD) higher in low-protein formula group than	No difference in energy intake between formula groups at 12 or 24 mo.

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control	Intervention duration	Impact on nutritional imbalances or inadequacies	Impact on health outcomes	Comments
							breastfed group	
(75)	Makrides et al (1999)	Australia n=146 from 1 week	1) Fortified infant formula: DHA 0.35% of total FA (from tuna oil) n=23 (85%) 2) Fortified infant formula: DHA 0.34% and ARA 0.34% of total FA (from an egg phospholipid fraction) n=24 (86%) Mothers asked to provide the study milk *ad* libitum as the only milk drunk until 12 mo.	1) Infant formula n=21 (75%) 2) Breastfed n=23 (36%) at 8.5 mo Mothers asked to provide the study milk *ad* libitum as the only milk drunk until 12 mo.	12 mo Until aged 12 mo	Control formula group had plasma DHA levels 42% of those consumed in the breastfed group, and 34% of DHA-fortified formula groups. Breastfed infants' plasma DHA remained similar to baseline. DHA-fortified formula groups increased plasma DHA from baseline, control formula, plasma DHA decreased from baseline.	Growth: No difference in weight, length, or head circumference between the formula groups at any age.	Sample size calculated to require 25 infants per formula-feeding group to detect a mean difference of weight of 700g. 80% power, 5% significance level.
(44) (45)	Morley et al (1999) Singhal et al (2000)	UK n=493 9 mo All ready drinking cow's milk	1) Fe-FM group: Fe-fortified formula Fe: 12 mg/L (as ferrous sulphate) VC: 66 mg/L n=133 (82%) 2) FM group: Un-fortified formula	1) CM group: cow's milk Fe: 0.5 mg/L n=160 (96%) Unlimited amount of formula provided to participants. Actual amount	9 mo Until aged 18 mo	Mean SF levels (µg/L) sig higher in FM1 group: At 18 mo: Fe-FM 21.4 FM 13.3 CM 14.3	Bayley MDI and PDI tests showed no significant difference between the groups when aged 18 mo. Boys fed cow's milk (n=91) had significantly higher	Sample size calculated as 144 in each group to detect a difference of 1 infection and a 5 point difference in Bayley MDI and PDI. Power of

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control	Intervention duration	Impact on nutritional imbalances or inadequacies	Impact on health outcomes	Comments
			Fe: 0.9 mg/L VC: 66 mg/L n=135 (82%) Unlimited amount of formula provided to participants. Author did not state actual amount of milk consumed.	consumed not provided by the author.			Bayley's MDI than formula fed boys (n=135) (mean 95.0 vs 91.6), but when only boys fed the diet for 6 mo or more included, the difference was no longer significant. No significant differences between any groups after 6 or 9 mo for: length, weight, head circumference, mid-upper arm circumference, or triceps and subscapular skinfolds; Incidence of infection, gastrointestinal problems, general morbidity;	80%, and significance level 5%.
(61)	Salmenpera et al (1994)	Finland n=200	Randomised into group when weaned:	1) Breastmilk n=84 at age 6 mo n=105 at age 9 mo	5.5-9 mo Intervention	Breast-fed and unfortified formula-fed groups, mean serum Zn	No significant differences in growth velocity between	Author did not report a sample size calculation.

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control	Intervention duration	Impact on nutritional imbalances or inadequacies	Impact on health outcomes	Comments
		0-3.5 mo	1) Fortified infant formula: Zn: 4 mg/L ($ZnSO_4$) n=32 aged 3.5 mo 2) Infant formula: Zn: 1.1 mg/L ($ZnSO_4$) n=23 aged 2 mo At 9 mo all formulas exchanged for cow's milk. Zn: 4.0-4.9 mg/kg Author did not state amount of formula provided or drunk.	n=65 at age 12 mo Exclusively breastfed until 6 mo at which time solids were introduced, and then cow's milk products after 9 mo of age.	until aged 9 mo, then cow's milk until aged 12 mo.	concentration decreased from birth to 2 mo, then remained stable. Zn-fortified formula group increased from 2mo of age, until 6 mo old (at which age solid foods were introduced), then decreased, by 9 mo was not significantly different to the breastfed or unfortified formula groups. No significant difference in prevalence of low serum Zn (below the 10th percentile of the breastfed infants) between Zn-fortified and unfortified formula groups.	groups. Length velocity was faster in the unfortified formula group than in the Zn-fortified formula group from 3 to 6 months (boys: 0.84 vs 0.71 mm/day; girls: 0.70 vs 0.65 mm/day). No significant differences in mean skinfold thicknesses or weight-for-length indexes between the two formula groups.	
(46)	Szymlek-Gay et al (2009) The Toddler	New Zealand n=225 12-20 mo (mean: 17.1 mo)	1) TM group: Toddler Milk Fe: 13.1 mg/L = approx. 6g/day (as ferrous sulphate) Zn: 4.7 mg/L I: 138.5 µg/100 g	1) RM group: Encouraged to eat more red meat Fe aim: approx. 2.6 mg/day Actual Fe: 0.91 mg/day	5 mo Until aged 17-25 mo	No difference in the risk of developing suboptimal Fe status by any of the groups after 5 mo. Adjusted mean SF change:	Adverse gastric effects that parents associated with study milks: 2 (2.4%) CM group 1 (2.3%) TM group	Sample size was not calculated for growth or morbidity outcomes. All data was

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control	Intervention duration	Impact on nutritional imbalances or inadequacies	Impact on health outcomes	Comments
	Food Study	Excluded if: Drinking iron-fortified formula, or Hb <105 g/L or, Hb <110 g/L and SF <12 µg/L	powder = 103.3 µg/day n=41 (91%) 2) CM group: Whole cow's milk with: VD: 6.0 µg cholecalciferol/100g powder. I: 40.5 µg/100 g powder Fe: 0.09 mg/L Zn: 2.8 mg/L n=81 (90%) Asked to replace their regular milk with the study milk. > 400 g/day of fortified milk consumed.	n=83 (92%) Asked to replace their regular milk with the study milk. Actual amount consumed not provided by the author.		TM 44% increase RM no change CM 14% decrease Intervention effect of 1.68 µg/L SF in FM group compared to CM group.	No adverse effects on children's growth.	controlled for CRP levels.
Nutrient-fortified milk-based products and morbidity and growth in developing countries								
(12, 13)	Sazawal et al (2007),	India n=633	MN group: multi-nutrient fortified milk (per 3 sachets)	CM group: unfortified milk (per 3 sachets)	12 mo Until aged 24-	Prevalence of IDA at baseline: MN 58.2%	At baseline, 68% had growth faltering. Improved weight	Adherence to the milk feeds was 85.6% in the

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control	Intervention duration	Impact on nutritional imbalances or inadequacies	Impact on health outcomes	Comments
	(2010)	12-36 mo Breastmilk was not the only or predominant milk given. Any children with Hb <70 g/L were given therapeutic iron as well as the study milk.	VC: 48mg = 41 mg/day Fe: 9.6 mg = 8.3 mg/day (Author did not report nutrient forms) n=316 (100%) Given 21 sachets of milk powder per week, extras for family members if requested to prevent sharing. Actual mean of 2.58 sachets consumed per day.	VC: 7.8 mg = 6.6 mg/day Fe: 0 mg = 0 mg/day (Author did not report nutrient forms) n=317 (100%) Given 21 sachets of milk powder per week, extras for family members if requested to prevent sharing. Actual mean of 2.54 sachets consumed per day.	48 mo	CM 50.3% MN group had 88% lower risk of IDA after 12 mo Change after 12 mo intervention: Hb levels: MN 13.6g/L increase SF levels: MN 7.9µg/L increase	gain (difference mean 0.21 kg/year), height gain (difference mean 0.51cm/year) in intervention group vs control. After 12 mo, significant decrease (OR: 0.63) in children with weight-for-age < -2 z-scores. Mean diarrhoea episodes per year was 4.46 in the intervention group, and 5.36 in the control group. Acute lower respiratory tract infection was 0.62 compared to 0.83, intervention vs control group. Intervention reduced odds for days of severe illness by 15%, incidence of	intervention group, and 86.7% in the control group.

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control	Intervention duration	Impact on nutritional imbalances or inadequacies	Impact on health outcomes	Comments
							diarrhoea by 18%, incidence of acute lower respiratory illness by 26% compared to the control group.	

Abbreviations: Mo, months; DHA, Docosahexaenoic Acid; FA, Fatty acids; ARA, Arachidonic Acid; RBC, Red Blood Cells; RBC-PC, red blood cell phosphatidycholine; RBC-PE, red blood cell phosphatidylethanolamine; Fe, iron; Zn, zinc; VC, vitamin C; MN, multi-nutrient; Hb, haemoglobin; LCP, long-chain polyunsaturated; VEP, visual evoked potential; BF, breastfed; CM, cow's milk; SD, standard deviation; MDI, Mental Development Index; PDI, Psychomotor Development Index; sig, significant;

Table 5.6: Trials of fortified milk-based products assessing neurodevelopment in children six to 36 months old

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control	Intervention duration	Impact on nutritional imbalances or inadequacies	Impact on health outcomes	Comments
Nutrient-fortified milk-based products and neurodevelopment outcomes in developed countries								
(90) (69)	Scott (1998) Auestad et al (2003) Follow-up study at 39 mo	Seattle, Portland and Kansas City, USA n=197 From birth	Infant formula supplemented with: 1) DHA 0.12% total FA ARA 0.43% of total FA (from egg-phospholipid) n=46 2) DHA 0.23% of total FA (from fish oil) n=43 Formulas provided *ad libitum* as the sole source of nutrition for a minimum of 4 mo. Author did not report amount of formula drunk.	1) Breastfed exclusively for minimum of 3 mo n=63 2) Infant formula, no DHA or ARA n=45	Intervention 12 mo, then additional 27 month follow-up Intervention until 12 mo of age, complete study including follow-up until 39 mo of age. Follow-up study results at 39 mo n=157 (80%)	Author did not report.	At 12 mo of age: No significant differences in either Bayley Mental Index or Bayley Motor Index between all 4 groups. At 14 mo of age: MacArthur Vocabulary Comprehension (P=0.017) and the Vocabulary Production (P=0.052) was lower in the DHA group than in the breastfed group. Vocabulary Production scores were lower in the DHA group than in the control formula group (P=0.027). At 39 mo of age: No significant differences between the groups for weight, length, or head circumference. No significant differences among the formula groups or between breastfed and formula groups for IQ, receptive and expressive language, visual-motor function, or visual acuity.	Author did not report sample size calculation.

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control	Intervention duration	Impact on nutritional imbalances or inadequacies	Impact on health outcomes	Comments
							No significant difference in health status between the groups for: 3 or more prescriptions for antibiotics since birth, use of pressure equalization tubes for chronic otitis media, hospitalisation since birth.	
(68)	Auestad et al (2001)	Missouri, Arkansas, Pennsylvania, Arizona, USA n=404 From birth Breastfed from birth, then mothers choosing to formula feed from age three mo included into intervention group.	Infant formula with added: 1) Fish oil DHA: 0.13% of total FA ARA: 0.46% of total FA 2) Egg derived triglyceride (egg-DTG) DHA: 0.14% of total FA ARA: 0.45% of total FA Formulas fed exclusively, *ad libitum*, until 4 mo, then as the exclusive milk beverage to 12 mo.	1) Control formula DHA: 0 ARA: 0 2) Breastfed DHA: 0.12% of total FA ARA: 0.51% of total FA Infants were breastfed until 3 mo then randomised to one of the three formula groups if they wished to stop breastfeeding. These were analysed as separate groups (ie 'Breastfed/Control formula' group) in the analysis.	9-12 mo Until aged 12 mo	Fortified-formula groups (irrespective of type) had significantly higher levels of ARA and DHA in RBC phospholipids than the control formula group. Breastfed group not different at 4 mo, but at 12 mo, DHA 40% less than the fortified formula groups.	No difference in weight, length, or head circumference among groups at 12 mo. Visual acuity was not different between any of the groups. No difference between any of the groups at 12 mo for information processing or Bayley scales. At 14 mo, the group drinking formula with added fish oil had a slightly, but significantly higher vocabulary expression score than the formula group fortified with egg-DTG. However neither were significantly different from the control formula or breastfed group.	Sample size calculation estimated 47 infants per group to detect a 0.75 SD difference in vocabulary scores with 90% power. n=294 (73%) completed the study.

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control	Intervention duration	Impact on nutritional imbalances or inadequacies	Impact on health outcomes	Comments
(72)	Birch et al (2002)	USA n=65 6 weeks	Infant formula (contained Fe) supplemented with: DHA: 0.36% of total FA ARA: 0.72% of total FA n=30 (94%) Author did not report amount of formula provided or drunk.	Infant formula (contained Fe) – contained no DHA or ARA n=28 (85%) Author did not report amount of formula provided or drunk.	10.5 mo Until 12 mo of age	Plasma lipids at 12 mo: DHA 75.5 mg/L in control formula group, 109 mg/L fortified-formula group (significantly different). Total RBC lipids at 12 mo: DHA 23.7 mg/L in control group, 73.5 mg/L in fortified formula group (significantly different).	Random dot stereoacuity: No differences at 39 or 52 weeks of age. VEP acuity. No differences at 6 weeks, but at 17, 26, and 52 weeks of age, visual acuity in the control-formula group was significantly poorer than in the LCP-supplemented group. Higher plasma DHA and ARA was associated with better VEP acuity at 17 and 52 weeks. At 52 weeks, acuity associated with higher concentration of RBC DHA and ARA.	Sample size estimated to require 21 infants in each group to detect a 1-SD difference between groups in random dot stereoacuity.
(71)	Birch et al (2005)	USA n=103 From birth	Infant formula (contained Fe) supplemented with: DHA: 0.35% of total FA ARA: 0.72% of total FA n=42 (82%)	Infant formula (contained Fe) – contained no DHA or ARA n=44 (85%) Author did not report amount of	12 mo Until 12 mo of age	At 39 weeks: DHA concentration in RBC total lipids was 21% higher in the fortified formula group than in the control group.	LCP-supplemented groups had significantly higher VEP acuity than the control group at 6, 17, 39 and 52 weeks. Approx 0.12 logMAR poorer that the supplemented group – slightly more than 1 line on the eye chart.	Sample size estimated to require 21 infants to detect a mean difference of $\geq$1.0 SD in VEP acuity

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control	Intervention duration	Impact on nutritional imbalances or inadequacies	Impact on health outcomes	Comments
			Author did not report amount of formula provided or drunk.	formula provided or drunk.		At 39 weeks: ARA 15-18% higher in the fortified formula than in the control group.	LCP-supplemented group had significantly better stereoacuity than did the control group at 17 weeks, but not at 39 or 52 wks.	between diet groups. 80% power, 5% level of significance.
(73)	Hoffman et al (2003)	USA n=61 4-6 mo (at weaning from breast milk)	Infant formula (contained Fe) supplemented with: DHA 0.36% ARA 0.72% of total FA n=30 (86%) Formulas provided *ad libitum* as the sole source of nutrition for a minimum of 4 mo.	Infant formula (contained Fe) n=31 (94%) Formulas provided *ad libitum* as the sole source of nutrition for a minimum of 4 mo.	6-8 mo Until 12 mo of age	mg DHA/L RBC: At baseline 4.5 mg/L At 12 mo: control formula 2.4 mg/L, fortified formula 5.9 mg/L (significantly different). Control formula reduced from baseline levels by 50%, fortified formula group remained at levels similar to baseline.	Stereoacuity not different between groups. At 12 months, LCP-supplemented group had significantly better VEP acuity than infant formula group. Difference of 0.103 logMAR – about 1 line of the eye chart. Higher RBC-DHA corresponded to higher acuity at 12 months. RBC-DHA explained 18% of variability.	Sample size estimated to require 21 infants per group to detect a difference of 1-SD in VEP acuity and 1-SD difference in random dot stereoacuity. 90% power, 5% level of significance.
(94)	Morale et al (2005)	USA n=243 From birth to 6 mo	Infant formula (contains iron): DHA: 0.36% total FA ARA: 0.72% total FA 1) Infant formula from birth to 4 mo n=38	Infant formula (contains iron, no DHA or ARA) 1) Infant formula from birth to 4 mo n=20	4 to 12 mo Until aged 4 mo or 12 months	Author did not assess nutritional indices.	A longer duration of dietary LCPUFA intake, regardless of whether obtained from human milk or formula, resulted in better visual acuity at 12 mo. Infants who received LCPUFAs for 9 mo, on average, are 0.1 logMAR better than infants who receive no	Combination of four trials (71,72,95,113) plus two datasets.

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control	Intervention duration	Impact on nutritional imbalances or inadequacies	Impact on health outcomes	Comments
			2) Infant formula from birth to 12 mo n=42 3) Breastfed until 1.5 mo then infant formula until 12 mo of age n=28 4) Breastfed until 4 mo then infant formula until 12 mo of age n=23 5) Breastfed until 6 mo then infant formula until 12 mo of age n=7 Total n=138	2) Infant formula from birth to 12 mo n=44 3) Breastfed until 1.5 mo then infant formula until 12 mo of age n=30 4) Breastfed until 4 mo then infant formula until 12 mo of age n=16 5) Breastfed until 6 mo then infant formula until 12 mo of age n=15 6) Breastfed until mean age 9.3 months of age n=19 7) Breastfed until 12 mo of age n=14			LCPUFAs in their diet (ie visual acuity is better by one full line on the eye chart). Receiving LCPUFAs for 12 mo results in visual acuity 1.5 lines better than no LCPUFAs.	

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control	Intervention duration	Impact on nutritional imbalances or inadequacies	Impact on health outcomes	Comments
				Total n=158				
(70)	Birch et al (2010) DIAMOND Study	Dallas and Kansas City, USA n=343 From birth	Control infant formula (contained Fe) with DHA added at: 1) 0.32% of total FA n=64 (77%) 2) 0.64% of total FA n=59 (70%) 3) 0.96% of total FA n=65 (75%) Formulas provided *ad libitum* as the sole source of nutrition for a minimum of 4 mo.	Infant formula (contained Fe) with 0% DHA n=56 (66%) Formulas provided *ad libitum* as the sole source of nutrition for a minimum of 4 mo.	12 mo Until 12 mo of age	RBC DHA concentrations were significantly different between all groups at both 4 and 12 months of age, and increased as the % of DHA in the formula increased.	Control formula infants had significantly poorer VEP at 4, 9, and 12 months. No significant differences between the intervention groups for VEP at any age. Formula had no significant effect on weight or length z-scores at any age.	Sample size estimated to require 37 participants per group to detect a difference of 0.1 logMAR. 0.08% level of significance, 80% power. Larger sample size was recruited to allow enough statistical power for other analyses. Mean formula intake: At 6 mo: 985-1053mL At 9 mo:875-961mL

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control	Intervention duration	Impact on nutritional imbalances or inadequacies	Impact on health outcomes	Comments
								At 12 mo: 441-772mL (range refers to the two study sites)
(91)	Colombo et al (2011) DIAMOND Study	Dallas and Kansas City, USA n=343 From birth	Infant formula (contains iron) with DHA added at: 1) 0.32% of total FA n=64 (77%) 2) 0.64% of total FA n=59 (70%) 3) 0.96% of total FA n=65 (75%) Study milks fed *ad libitum*. Author did not report actual amount consumed.	Infant formula (contains iron) with 0% DHA n=56 (66%) Study milks fed *ad libitum*. Author did not report actual amount consumed.	12 mo Until 12 mo of age	Reported previously (Birch et al (2010))	No significant difference between groups for peak look duration. Sustained attention: Declined with age but was significantly higher in the 0.32 and 0.64% DHA formula groups than the controls. The 0.96% DHA formula was not significantly different to the control. All supplement groups had a lower heart rate than the controls (5 to 9 beats per minute less).	Author did not report sample size calculation. Mean formula intake: At 6 mo: 985-1053mL At 9 mo:875-961mL At 12 mo: 441-772mL (Range refers to the two study sites)
(92)	Drover et al (2012) DIAMOND Study	Dallas, USA n=131 Recruited at birth	Infant formula (contained Fe) with ARA 0.64% of total FAs, and added DHA at: 1) 0.32% of total FAs n=24 (73%) at 24 mo	Infant formula (contained Fe): 0% DHA and ARA n=20 (66%) at 24 mo n=19 (63%) at 30 & 42 mo	12 mo Until 12 mo of age Follow-up assessment at 24, 30 and 42	Reported previously (Birch et al (2010))	School Readiness Composite (SRC) from the first six scales from the Bracken Basic Concept Scale – Revised (BBCS-R) at 2.5 years (71% of participants). Language comprehension assessed at 2 (in 76% participants) and 3.5	Sample size calculation required 37 per group to detect a 0.1 logMAR difference in VEP acuity.

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control	Intervention duration	Impact on nutritional imbalances or inadequacies	Impact on health outcomes	Comments
			n=23 (70%) at 30 & 42 mo 2) 0.64% of total FAs n=29 (85%) at 24 mo n=27 (79%) at 30 mo n=24 (71%) at 42 mo 3) 0.96% of total FAs n=26 (76%) at 24 mo n=24 (71%) at 30 mo n=22 (65%) at 42 mo Study milks fed *ad libitum*. Author did not report actual amount consumed.	Study milks fed *ad libitum*. Author did not report actual amount consumed.	mo of age.		(67% of participants) years using the Peabody Picture Vocabulary Test – Third Edition (PPVT-III) No difference in results adjusted for maternal education and sex, across any of the groups for any of the tests.	80% power.
(67)	Makrides et al (2000)	Australia n=146 from 1 week	1) Fortified infant formula: 0.35% DHA as total FA (from tuna oil) n=23 (85%) 2) Fortified infant formula: 0.34% DHA and 0.34% ARA as total FA (from an egg phospholipid fraction)	1) Infant formula n=21 (75%) 2) Breastfed n=23 (36%) at 8.5 mo	12 mo Until aged 12 mo	Plasma DHA highest in the DHA-formula group (5.8) compared to lowest in placebo formula (1.6).	No differences in VEP acuity between any of the formula groups at 4 or 8.5 mo of age. Breastfed infants had better VEP acuity at 8.5 months of age compared to the formula groups Bayley's MDI and PDI values were not different in the formula groups, and not different between the breast-fed and formula fed at 1 year, but was a significant	Sample size calculate to require 15 infants per formula group to detect a mean difference of 0.2 logMAR in VEP acuity. 90% power, 5% significance

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control	Intervention duration	Impact on nutritional imbalances or inadequacies	Impact on health outcomes	Comments
			n=24 (86%) Author does not state amount of formula drunk.				difference at 2 years with the breastfed children achieving higher MDI scores. No difference in PDI scores.	level.
(44)	Morley et al (1999)	UK n=493 9 mo	1) Fe-fortified formula Fe: 1.2mg/L (as ferrous sulphate) n=133 (82%) 2) Formula: Fe: 0.9mg/L n=135 (82%) Study milks fed *ad libitum*. Author did not report actual amount consumed.	1) Cow's milk Fe: 0.05 mg/L n=160 (96%) Study milks fed *ad libitum*. Author did not report actual amount consumed.	9 mo Until aged 18 mo	Fe-fortified formula group had significantly higher plasma ferritin and mean Hb concentrations than unfortified formula or cow's milk groups.	Bayley MDI and PDI tests showed no significant difference between the groups when aged 18 mo. Boys fed cow's milk (n=91) had significantly higher Bayley MDI than formula fed boys (n=135) (mean 95.0 vs 91.6), but when only boys fed the diet for 6 mo or more included, the difference was no longer significant. No significant differences in length, weight, head circumference, mid-upper arm circumference, or triceps and subscapular skinfolds, between any of the groups after 6 or 9 mo of intervention.	Sample size designed to detect an overall 5 point difference in Bayley MDI and PDI between groups. 80% power, 5% level of significance.
(34)	Williams et al (1999) Same	England n=92 6-8 mo (mean age	Fe-fortified milk Fe: 12 mg/L = 7.4 mg/day (Author did not state Fe form)	Cow's milk Fe: 0.5 mg/L = 0.31 mg/day VC: 10 mg/L	16-18 mo Until aged 24 mo	At 18 mo: 33% anaemic in cow's milk group vs 2% in intervention	At 24 mos: Griffiths general quotient score decreased in both groups, less in intervention group; mean decrease 14.7 and 9.3 respectively.	Sample size calculated for Hb concentration and not

Ref	Author (year)	Study population (age at baseline)	Intervention (n=sample size that completed the study (% of total sample))	Control	Intervention duration	Impact on nutritional imbalances or inadequacies	Impact on health outcomes	Comments
	trial as Daly et al (1996) (41)	7.8 mo) socioeconomically deprived.	VC: 100 mg/L = 62 mg/day n=46 (100%) at 18 mo n=41 (89%) at 24 mo At 18 mo of age went back to drinking cow's milk. Study milks fed *ad libitum*. Average intake of formula was 620 ± 181 mL/day.	= 6.2 mg/day n=46 (100%) at 18 mo n=44 (96%) at 24 mo Given money to buy milk equal to 500 mL per day. Actual mean intake was 620 ± 181 mL/day.		group. At 24 months: 26% of cow's milk group anaemic, 0% of intervention group anaemic (Note: intervention group had been drinking cow's milk since age 18 mo).	At 24 mo: Mean subquotient scores lower in cow's milk group (personal and social skills significantly lower) Conclusion: Fe reduced the decline in psychomotor performance.	neurodevelopment outcomes.
Effect of nutrient-fortified milk-based products on neurodevelopment outcomes in developing countries								

Abbreviations: Mo, months; DHA, Docosahexaenoic Acid; FA, Fatty acids; ARA, Arachidonic Acid; RBC, Red Blood Cells; RBC-PC, red blood cell phosphatidycholine; RBC-PE, red blood cell phosphatidylethanolamine; Fe, iron; Zn, zinc; VC, vitamin C; MN, multi-nutrient; Hb, haemoglobin; LCP, long-chain polyunsaturated; VEP, visual evoked potential; BF, breastfed; CM, cow's milk; SD, standard deviation; MDI, Mental Development Index; PDI, Psychomotor Development Index; sig, significant;

5 References

1. Nestle NAN [Internet]. Available from: http://www.nestlebaby.com/nz

2. Heinz Nurture [Internet]. Available from: http://www.forbaby.co.nz/

3. Nutricia Karicare [Internet]. Available from: http://www.nutriciababy.co.nz/

4. Bellamy's Organic [Internet]. Available from: http://bellamysorganic.com.au/

5. S-26 [Internet]. Available from: https://www.s-26.com.au/

6. CODEX Alimentarius. Standard for infant formula and formulas for special medical purposes intended for infants. CODEX STAN 72-1981. p. 1–21.

7. CODEX Alimentarius. CODEX Standard for follow-up formula CODEX STAN 156-1987. p. 1–9.

8. FAO/WHO. Enterobacter sakazakii (Cronobacter spp.) in powdered follow-up formulae: Meeting Report. Rome, Italy; 2008 p. 33–43.

9. Edited by: Lennox A, Sommerville J, Ong K, Henderson H, Allen R. Diet and nutrition survey of infants and young children, 2011. UK; 2011 p. 1–107.

10. Kersting M, Dulon M. Assessment of breast-feeding promotion in hospitals and follow-up survey of mother-infant pairs in Germany: the SuSe Study. Public Health Nutrition. 2002;5(4):547–52.

11. Hörnell A, Hofvander Y, Kylberg E. Introduction of solids and formula to breastfed infants: a longitudinal prospective study in Uppsala, Sweden. Acta Paediatrica. 2001;90(5):477–82.

12. Turberg-Romain C, Lelievre B, Le Heuzey M-F. Evolution of feeding behaviour in mothers of infants and young children from 1 to 36 months old in France. Archives de Pediatrie. 2008;15:3–16.

13. Norway. Information from the dietary assessment "Spedkost 6 måneder" relevant to the revision of Codex standard for follow-up formulae. Document provided by Ministry for Primary Industries;

14. Persson L, Samuelson G. From breastmilk to family food. Acta Paediatrica Scandinavica. 1984;73:685–92.

15. United Kingdom and Germany data. Document provided by Ministry for Primary Industries;

16. Irish Universities Nutrition Alliance. National pre-school nutrition survey [Internet]. Available from: http://www.iuna.net/?p=169

17. Norway. Information from the dietary assessment “Spedkost 12 måneder” relevant to the revision of Codex standard for follow-up formulae. Document provided by Ministry for Primary Industries;

18. Norway. Information from the dietary assessment “Spedkost 24måneder” relevant to the revision of Codex standard for follow-up formulae. Document provided by Ministry for Primary Industries; 2009 p. 92.

19. Switzerland, Ireland, and Canada data. Document provided by Ministry for Primary Industries;

20. Szymlek-Gay E, Ferguson E, Heath A-L, Fleming E. Quantities of foods consumed by 12- to 24-month-old New Zealand children. Nutrition & Dietetics. 2010;67:244–50.

21. Morton S, Carr P, Grant C, Lee A, Bandara D, Mohal J, et al. Growing up in New Zealand: A longitudinal study of New Zealand children and their families. Report 2: Now we are born. 2012.

22. Ireland data. Document provided by Ministry for Primary Industries;

23. Alexy U, Kersting M. Time trends in the consumption of dairy foods in German children and adolescents. European Journal of Clinical Nutrition. 2003;57(10):1331–7.

24. Fantino M. Contribution of specific baby foods to nutritional intake. Archives de Pediatrie. 2008;15:48–63.

25. Siega-Riz AM, Deming DM, Reidy KC, Fox MK, Condon E, Briefel RR. Food consumption patterns of infants and toddlers: where are we now? Journal of the American Dietetic Association. 2010;110:S38–51.

26. Bramhagen a C, Axelsson I. Iron status of children in southern Sweden: effects of cow’s milk and follow-on formula. Acta Paediatrica. 1999;88:1333–7.

27. Ghisolfi J, Fantino M, Turck D, de Courcy GP, Vidailhet M. Nutrient intakes of children aged 1-2 years as a function of milk consumption, cows’ milk or growing-up milk. Public Health Nutrition. 2013;16(3):524–34.

28. Koletzko B, Bhutta ZA, Cai W, Cruchet S, El Guindi M, Fuchs GJ, et al. Compositional requirements of follow-up formula for use in infancy: recommendations of an international expert group coordinated by the early nutrition academy. Annals of Nutrition & Metabolism. 2013;62(1):44–54.

29. New Zealand Istitute for Crop & Food Research. FOODfiles: Data files of the New Zealand Food Composition Database. Palmerston North: New Zealand Institute for Crop & Food Research; 2010.

30. Semba RD, Moench-Pfanner R, Sun K, Pee S De, Akhter N, Rah JH, et al. Iron-fortified milk and noodle consumption is associated with lower risk of anemia among children aged 6 – 59 mo in Indonesia. American Journal of Clinical Nutrition. 2010;92:170–6.

31. McAndrew F, Thompson J, Fellows L, Large A, Speed M, Renfrew M. Infant Feeding Survey 2010: Chapter 5. The use of milk other than breastmilk. UK; 2010 p. 1–15.

32. Le Heuzey M-F, Turberg-Romain C, Lelievre B. Feeding behaviour of infants and young children from 0 to 36 months old: comparison of mothers' habits. Archives de Pediatrie. 2007;15:17–31.

33. Poon WB, Ho WLC, Yeo CL. Survey on parenting practices among Chinese in Singapore. Singapore Medical Journal. 2007;48(11):1006–11.

34. Williams J, Wolff A, Daly A, Macdonald A, Aukett A, Booth IW. Iron supplemented formula milk related to reduction in psychomotor decline in infants from inner city areas: randomised study. BMJ. 1999;318:693–8.

35. Williams PL, Innis SM, Vogel a M, Stephen LJ. Factors influencing infant feeding practices of mothers in Vancouver. Canadian Journal of Public Health. 1999;90(2):114–9.

36. Malaysia and Philippines data. Document provided by Ministry for Primary Industries;

37. Yee CF, Chin R. Parental perception and attitudes on infant feeding practices and baby milk formula in East Malaysia. International Journal of Consumer Studies. 2007;31(4):363–70.

38. Roy S, de Groot S, Shafique S, Afroz A. Perceptions of mothers and use of breastmilk substitutes in Dhaka, Bagladesh. Journal of Health, Population and Nutrition. 2002;20(3):264–70.

39. Sobel HL, Iellamo A, Raya RR, Padilla A a, Olivé J-M, Nyunt-U S. Is unimpeded marketing for breast milk substitutes responsible for the decline in breastfeeding in the Philippines? An exploratory survey and focus group analysis. Social Science & Medicine. 2011;73(10):1445–8.

40. Bradley C, Cordano A, Hillman L, Rothman A, Leedy D. Evaluation of two iron-fortified, milk based formulas during infancy. Pediatrics. 1993;91:908–14.

41. Daly A, MacDonald A, Aukett A, Williams J, Wolf A, Davidson J, et al. Prevention of anaemia in inner city toddlers by an iron supplemented cows' milk formula. Archives of Disease in Childhood. 1996;75(1):9–16.

42. Gill DG, Vincent S, Segal DS. Follow-on formula in the prevention of iron deficiency: a multicentre study. Acta Paediatrica. 1997;86(7):683–9.

43. Haschke F, Vanura H, Male C, Owen G, Pietschnig B, Schuster E, et al. Iron nutrition and growth of breast- and formula-fed infants during the first 9 months of life. Journal of Pediatric Gastroenterology and Nutrition. 1993;16:151–6.

44. Morley R, Abbott R, Fairweather-Tait S, MacFadyen U, Stephenson T, Lucas A. Iron fortified follow on formula from 9 to 18 months improves iron status but not development or growth: a randomised trial. Archives of Disease in Childhood. 1999;81(3):247–52.

45. Singhal A, Morley R, Abbott R, Fairweather-Tait S, Stephenson T, Lucas A. Clinical safety of iron-fortified formulas. Pediatrics. 2000;105(3):E38.

46. Szymlek-gay EA, Ferguson EL, Heath A-L, Gray AR, Gibson RS. Food-based strategies improve iron status in toddlers : a randomized
Clinical Nutrition. 2009;90:1541–51.

47. Virtanen M, Svahn C, Viinikka L, Räihä N, Siimes M, Axelsson I. Iron-fortified and unfortified cow's milk: Effects on iron intakes and iron status in young children. Acta Paediatrica. 2001;90:724–31.

48. Wall CR, Grant CC, Taua N, Wilson C, Thompson JMD. Milk versus medicine for the treatment of iron deficiency anaemia in hospitalised infants. Archives of Disease in Childhood. 2005;90(10):1033–8.

49. Rivera JA, Shamah T, Villalpando S, Monterrubio E. Effectiveness of a large-scale iron-fortified milk distribution program on anemia and iron deficiency in low-income young children in Mexico. American Journal of Clinical Nutrition. 2010;91:431–9.

50. Walter T, Pino P, Pizarro F, Lozoff B. Prevention of iron-deficiency anemia: Comparison of high- and low-iron formulas in term healthy infants after six months of life. Journal of Pediatrics. 1998;132:635–40.

51. Villalpando S, Shamah T, Rivera J a, Lara Y, Monterrubio E. Fortifying milk with ferrous gluconate and zinc oxide in a public nutrition program reduced the prevalence of anemia in toddlers. The Journal of Nutrition. 2006;136(10):2633–7.

52. Sazawal S, Dhingra U, Dhingra P, Hiremath G, Sarkar A, Dutta A, et al. Micronutrient fortified milk improves iron status, anemia and growth among children 1-4 years: a double masked, randomized, controlled trial. PloS one. 2010;5(8):e1-8.

53. Gibson RS. Principles of Nutritional Assessment. Second Ed. New York: Oxford University Press; 2005.

54. Assessing the iron status of populations : including literat
Joint World Health Organization/Centers for Disease Control and Prevention Technical Consultation on the Assessment of Iron Status at the Population Level. 2nd ed. Geneva, Switzerland; 2004. p. 1–108.

55. Thurnham DI, Mccabe LD, Haldar S, Wieringa FT, Northrop-Clewes CA, Mccabe GP. Adjusting plasma ferritin concentrations to remove the effects of subclinical inflammation in the assessment of iron deficiency: a meta-analysis. American Journal of Cinical Nutrition. 2010;92:546–55.

56. Cook JD, Flowers CH, Skikne BS. The quantitative assessment of body iron. Blood. 2003;101(9):3359–64.

57. Cogswell ME, Looker AC, Pfeiffer CM, Cook JD, Lacher DA, Beard JL, et al. Assessment of iron deficiency in US preschool children and nonpregnant females of childbearing age: National Health and Nutrition Examination Survey 2003 – 2006. American Journal of Cinical Nutrition. 2009;89:1334–42.

58. Cross A, Heath A-L, Ferguson E, Gray A, Szymlek-Gay E. Rates of common communicable illnesses in non-anaemic 12-24 month old South Island, New Zealand children. The New Zealand Medical Journal. 2009;122(1290):24–35.

59. Soh P, Ferguson E, McKenzie J, Homs M, Gibson R. Iron deficiency and risk factors for lower iron stores in 6-24-month-old New Zealanders. European Journal of Cllinical Nutrition. 2004;58(1):71–9.

60. Morgan E, Heath A-L, Szymlek-gay E, Gibson R, Gray A, Bailey K, et al. Red meat and a fortified manufactured toddler milk drink increase dietary zinc intakes without affecting zinc satus of New Zealand toddlers. Journal of Nutrition. 2010;140:2221–6.

61. Salmenpera L, Perheentupa J, Pakarinen P, Siimes M. Zinc supplementation of infant formula. American Journal of Clinical Nutrition. 1994;59:985–9.

62. De Benoist B, Darnton-Hill I, Davidsson L, Fontaine O, Hotz C. Conclusions of the Joint WHO/UNICEF/IAEA/IZiNCG Interagency Meeting on Zinc Status Indicators. Food and nutrition bulletin. 2007;28:S480–4.

63. King JC. Zinc: an essential but elusive nutrient. American Journal of Cinical Nutrition. 2011;94:679–84.

64. Sazawal S, Dhingra U, Dhingra P, Hiremath G, Kumar J, Sarkar A, et al. Effects of fortified milk on morbidity in young children in north India: community based, randomised, double masked placebo controlled trial. BMJ. 2007;334(7585):140.

65. Nutrient Reference Values for Australia and New Zealand [Internet]. National Health and Medical Research Council, Australian Government Ministry of Health, New Zealand Government. Available from: http://www.nrv.gov.au/nutrients/zinc.htm

66. Serra-Majem L, Nissensohn M, Øverby NC, Fekete K. Dietary methods and biomarkers of omega 3 fatty acids: a systematic review. The British Journal of Nutrition. 2012;107:S64–76.

67. Makrides M, Neumann M, Simmer K, Gibson R. A critical appraisal of the role of dietary long-chain polyunsaturated fatty acids on neural indices of term infants: A randomized, controlled trial. Pediatrics. 2000;105(1):32–8.

68. Auestad N, Halter R, Hall R, Blatter M, Bogle M, Burks W, et al. Growth and development in term infants fed long-chain polyunsaturated fatty acids: a double-masked, randomised, parallel, prospective, multivariate study. Pediatrics. 2001;108:372–81.

69. Auestad N, Scott DT, Janowsky JS, Jacobsen C, Carroll RE, Montalto MB, et al. Visual, cognitive, and language assessments at 39 months: A follow-up study of children fed formulas containing long-chain polyunsaturated fatty acids to 1 year of age. Pediatrics. 2003;112:e177–183.

70. Birch EE, Carlson SE, Hoffman DR, Fitzgerald-Gustafson KM, Fu VLN, Drover JR, et al. The DIAMOND (DHA Intake And Measurement Of Neural Development) Study: a double-masked, randomized controlled clinical trial of the maturation of infant visual acuity as a function of the dietary level of docosahexaenoic acid. American Journal of Cinical Nutrition. 2010;91:848–59.

71. Birch EE, Castañeda YS, Wheaton DH, Birch DG, Uauy RD, Hoffman DR. Visual maturation of term infants fed long-chain polyunsaturated fatty acid-supplemented or control formula for 12 mo. American Journal of Cinical Nutrition. 2005;81(4):871–9.

72. Birch EE, Hoffman DR, Castañeda YS, Fawcett SL, Birch DG, Uauy RD. A randomized controlled trial of long-chain polyunsaturated fatty acid supplementation of formula in term infants after weaning at 6 wk of age. American Journal of Cinical Nutrition. 2002;75(3):570–80.

73. Hoffman D, Birch E, Castaneda Y, Fawcett S, Wheaton D, Birch D, et al. Visual function in breast-fed term infants weaned to formula with or without long-chain polyunsaturateds at 4 to 6 months: a randomized clinical trial. Journal of Pediatrics. 2003;142:669–77.

74. Auestad N, Montalto M, Hall R, Fitzgerald K, Wheller R, Connor W, et al. Visual acuity, erythrocyte fatty acid composition, and growth in term infants fed formulas with long chain polyunsaturated fatty acids for one year. Pediatric Research. 1997;41:1–10.

75. Makrides M, Neumann M, Simmer K, Gibson R. Dietary long-chain polyunsaturated fatty acids do not influence growth of term infants: a randomized clinical trial. Pediatrics. 1999;104(3):468–75.

76. Salmenpera L, Siimes M, Nanto V, Perheentupa J. Copper supplementation: failure to increase plasma copper and ceruloplasmin concentrations in healthy infants. American Journal of Clinical Nutrition. 1989;50:843–7.

77. Szymlek-Gay E, Skeaff S, Zhao Y, Gray A, Ferguson E, Heath A-L. Iodine-fortifed milk improves iodine status in toddlers. 14th International symposium on trace elements in man and animals. Enshi, Hubei, China; 2011. p. 220.

78. Kumpulainen J, Salmenpera L, Siimes MA, Koivistoinen P, Lehto J, Perheentupa J. Formula feeding results in lower selenium status than breast feeding or selenium supplemented formula feeding: a longitudinal study. American Journal of Clinical Nutrition. 1987;45:49–53.

79. Houghton LLA, Gray AAR, Szymlek-gay EEA, Heath A-LAM, Ferguson EL. Vitamin D-fortified milk achieves the targeted serum 25-hydroxyvitamin D concentration without affecting that of parathyroid hormone in New Zealand toddlers. Journal of Nutrition. 2011;141:1840–6.

80. Lopez-Teros V, Quihui-Cota L, Rosa O, Grijalva-Haro M, Valencia M, Green M, et al. Vitamin A-fortified milk increases total body vitamin A stores in Mexican preschoolers. The Journal of Nutrition. 2013;143:221–6.

81. McNaughton S, Marks G. Selenium Content of Australian Foods: A Review of Literature Values. Journal of Food Composition and Analysis. 2002;15(2):169–82.

82. Dorea JG. Selenium and breast-feeding. The British Journal of Nutrition. 2002;88(5):443–61.

83. Akeson P, Axelsson I, Raiha N. Growth and nutrient intake in three- to twelve-month-old infants fed human milk or formulas with varying protein concentrations. Journal of Pediatric Gastroenterology & Nutrition. 1998;26:1–8.

84. Koletzko B, Kries V, Closa R, Escribano J, Scaglioni S, Giovannini M, et al. Lower protein in infant formula is associated with lower weight up to age 2 y: a randomized clinical trial. American Journal of Clinical Nutrition. 2009;89:1836–45.

85. Lönnerdal B, Chen CL. Effects of formula protein level and ratio on infant growth, plasma amino acids and serum trace elements. II. Follow-up formula. Acta Paediatrica Scandinavica. 1990;79(3):266–73.

86. Brenna JT, Varamini B, Jensen RG, Diersen-Schade DA, Boettcher JA, Arterburn LM. Docosahexaenoic and arachidonic acid concentrations in human breast milk worldwide. American Journal of Clinical Nutrition. 2007;85(6):1457–64.

87. Sachdev H, Gera T, Nestel P. Effect of iron supplementation on physical growth in children: systematic review of randomised controlled trials. Public Health Nutrition. 2007;9(07):904–20.

88. Baird J, Fisher D, Lucas P, Kleijen J, Roberts H, Law C. Being big or growing fast: systematic review of size and growth in infancy and later obesity. BMJ. 2005;331:929–31.

89. Monteiro P, Victoria C. Rapid growth in infancy and childhood and obesity in later life - a systematic review. Obesity Revue. 2005;6:143–54.

90. Scott D, Janowsky J, Carroll R, Taylor J, Auestad N, Montalto M. Formula supplementation with long-chain polyunsaturated fatty acids: are there developmental benefits? Pediatrics. 1998;102(5):E59.

91. Colombo J, Carlson SE, Cheatham CL, Fitzgerald-Gustafson KM, Kepler A, Doty T. Long-chain polyunsaturated fatty acid supplementation in infancy reduces heart rate and positively affects distribution of attention. Pediatric Research. 2011;70(4):406–10.

92. Drover JR, Felius J, Hoffman DR, Castañeda YS, Garfield S, Wheaton DH, et al. A randomized trial of DHA intake during infancy: school readiness and receptive vocabulary at 2-3.5 years of age. Early Human Development. 2012;88(11):885–91.

93. Prager TC, Zou YL, Jensen CL, Fraley JK, Anderson RE, Heird WC. Evaluation of methods for assessing visual function of infants. Journal of American Association for Pediatric Ophthalmology and Strabismus. 1999;3(5):275–82.

94. Morale SE, Hoffman DR, Castañeda YS, Wheaton DH, Burns RA, Birch EE. Duration of long-chain polyunsaturated fatty acids availability in the diet and visual acuity. Early Human Development. 2005;81(2):197–203.

95. Birch E, Hoffman D, Uauy R, Birch D, Prestidge C. Visual acuity and the essentiality of docosahexaenoic acid in the diet of term infants. Pediatric Nutrition. 1998;44(2):201.

96. Cattaneo A, Timmer A, Bomestar T, Bua J, Kumar S, Tamburlini G. Child nutrition in countries of the Commonwealth of Independent States: time to redirect strategies? Public Health Nutrition. 2008;11(12):1209–19.

97. Fantino M, Gourmet E. Energy consumption and nutritional intake in non breast-fed children under 36 months old. Archives de Pediatrie. 2008;15:32–47.

98. Hay G, Johnston C, Whitelaw A, Trygg K, Refsum H. Folate and cobalamin status in relation to breastfeeding and weaning in healthy infants. American Journal of Clinical Nutrition. 2008;88(1):105–14.

99. Schwartz J, Dube K, Alexy U, Kalhoff H, Kersting M. PUFA and LC-PUFA intake during the first year of life: can dietary practice achieve a guidline diet? European Journal of Clinical Nutrition. 2010;64:124–30.

100. Thorsdottir I, Gunnarsson BS. Symposium on “Nutrition and health in children and adolescents” Session 2: Dietary quality and dietary recommendations in children and adolescents Dietary quality and adequacy of micronutrient intakes in children. Proceedings of the Nutrition Society. 2007;65(04):366–75.

101. Barbarich BN, Willows ND, Wang L, Clandinin MT. Polyunsaturated fatty acids and anthropometric indices of children in rural China. European Journal of Clinical Nutrition. 2006;60(9):1100–7.

102. Castro TG, Baraldi LG, Muniz PT, Cardoso M a. Dietary practices and nutritional status of 0-24-month-old children from Brazilian Amazonia. Public Health Nutrition. 2009;12(12):2335–42.

103. Faber M. Complementary foods consumed by 6 – 12-month-old rural infants in South Africa are inadequate in micronutrients. Public Health Nutrition. 2007;8(04):373–81.

104. Hotz C, Gibson R. Complementary feeding practices and dietary intakes from complementary foods amongst weanlings in rural Malawi. European Journal of Public Health. 2001;55:841–9.

105. Kumar D, Goel NK, Mittal PC, Misra P. Influence of infant-feeding practices on nutritional status of under-five children. Indian Journal of Pediatrics. 2006;73(5):417–21.

106. Labadarios D, Steyn N, Maunder E, MacIntryre U, Gericke G, Swart R, et al. The National Food Consumption Survey (NFCS): South Africa, 1999. Public Health Nutrition. 2007;8(05):533–43.

107. Pasricha S-R, Shet A, Black J, Sudarshan H, Prashanth N, Biggs B-A. Vitamin B-12, folate, iron, and vitamin A concentrations in rural Indian children are associated with continued breastfeeding, complementary diet, and maternal nutrition. American Journal of Cinical Nutrition. 2011;94:1358–70.

108. Garriguet D. Beverage consumption of children and teens. Statistics Canada. 2008;19(4):17–22.

109. Coleman BL. Early introduction of non-formula cow's milk to southern Ontario infants. Canadian Journal of Public Health. 2006;97(3):187–90.

110. Krebs NF, Westcott JE, Butler N, Robinson C, Bell M, Hambidge KM. Meat as a first complementary food for breastfed infants: feasibility and impact on zinc intake and status. Journal of Pediatric Gastroenterology and Nutrition. 2006;42(2):207–14.

111. Translation of BfR original document (Germany). Nutrients in toddler milk. Document provided by Ministry for Primary Industries; 2011 p. 1–10.

112. Webb K, Rutishauser I, Knezevic N. Foods, nutrients and portions consumed by a sample of Australian children aged 16-24 months. Nutrition & Dietetics. 2008;65(1):56–65.

113. Uauy R, Hoffman DR, Mena P, Llanos A, Birch EE. Term infant studies of DHA and ARA supplementation on neurodevelopment: results of randomized controlled trials. Journal of Pediatrics. 2003;143:S17–25.